UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

CANINE PRIMARY HYPERPARATHYROIDISM: CLINICAL APPROACH TO HYPERCALCAEMIA

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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Aos meus pais.

Acknowledgement

Abstract

Canine primary hyperparathyroidism: clinical approach to hypercalcaemia

Canine primary hyperparathyroidism (PHPTH) is an endocrine disorder, where one or more parathyroid glands autonomously produce and secrete parathyroid hormone (PTH), which results in hypercalcaemia (Feldman, 2010).

Diagnosis of PHPTH is achieved when there are inappropriate PTH concentrations (normal or increased) in the presence of elevated ionised calcium (iCa) concentration with no other identifiable cause (Skelly, 2012). iCa is the only active fraction of calcium and does not always correlate to total calcium, which is why iCa should be used to assess serum calcium status (Schenck & Chew, 2008).

PHPTH is usually diagnosed after detection of hypercalcaemia in a blood analysis performed for unrelated reasons, as clinical signs are often not perceived by the owners (Feldman, Hoar, Pollard, & Nelson, 2005; Feldman, 2015a).

Treatment by parathyroidectomy, percutaneous ultrasound-guided ethanol ablation or percutaneous ultrasound-guided heat ablation is curative and prognosis is excellent for treated dogs, but hypocalcaemia is a frequent postoperative complication (Caplan, 2013; Feldman, 2015a; Flanders, 2003; Nelson, 2009; Rasor, Pollard, & Feldman, 2007; Séguin & Brownlee, 2012).

The retrospective study had the objective of characterising a sample of six dogs diagnosed with PHPTH at Anderson Moores Veterinary Specialists and analyse the procedures and tests conducted in the clinical approach to previously identified hypercalcaemia.

Key words: primary hyperparathyroidism, parathyroid hormone, hypercalcaemia, ionised calcium

Resumo

Hiperparatiroidismo primário canino: abordagem clínica à hipercalcémia

O hiperparatiroidismo primário canino (PHPTH) é uma doença endócrina, na qual uma ou mais glândulas paratiróides produzem e secretam hormona paratiroideia ou paratormona (PTH) autonomamente, o que resulta em hipercalcémia (Feldman, 2010).

O diagnóstico de PHPTH é efectuado quando existem concentrações de PTH inapropriadas (normais ou aumentadas) na presença de concentrações aumentadas de cálcio ionizado (iCa) sem outra causa identificável (Skelly, 2012). O iCa é a única fracção activa do cálcio e nem sempre se correlaciona com o cálcio total, motivo pelo qual o iCa deve ser utilizado para avaliar o cálcio em circulação (Schenck & Chew, 2008)

O PHPTH é normalmente diagnosticado após a detecção de hipercalcémia numa análise sanguínea efectuada por motivos não relacionados, uma vez que os sinais clínicos normalmente não são identificados pelos donos (Feldman, Hoar, Pollard, & Nelson, 2005; Feldman, 2015a)..

O tratamento com paratiroidectomia, ablação percutânea com etanol guiada por ultra-som ou ablação percutânea com calor guiada por ultra-som é curativo e tem um prognóstico excelente para animais tratados, embora a hipocalcémia seja uma complicação pós-cirurgica frequente (Caplan, 2013; Feldman, 2015a; Flanders, 2003; Nelson, 2009; Rasor, Pollard, & Feldman, 2007; Séguin & Brownlee, 2012).

O estudo retrospectivo deste trabalho teve como objectivo a caracterização de uma amostra de seis cães diagnosticados com PHPTH na Anderson Moores Veterinary Specialists e analizar os procedimentos e testes efectuados na abordagem clínica à hipercalcémia previamente identificada.

Palavras-chave: hiperparatiroidismo primário, hormona paratiroideia, paratormona, hipercalcémia, cálcio ionizado

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Abstract

ACTH – adrenocorticotropic hormone

ADH – antidiuretic hormone

AKI – acute kidney injury

ALP – alkaline phosphatase

ALT – alanine aminotransferase

ARF – acute renal failure

BARF – biologically appropriate raw food

BID – twice daily (*bis in die*)

BUN – blood urea nitrogen

cAMP - cyclic adenosine monophosphate

CaR – calcium-sensing receptor

CBC – complete blood count

CKD – chronic kidney disease

CO_2 – carbon dioxide

CRF – chronic renal failure

CT – computed tomography

ECF – extracellular fluid

ECG – electrocardiogram

EDTA – ethylenediaminetetraacetic acid

FGF-23 – fibroblast growth factor-23

FNA – fine needle aspirate

GFR – glomerular filtration rate

GH – growth hormone

GPCR – G protein-coupled receptor

HHM – humoral hypercalcaemia of malignancy

HPTH – hyperparathyroidism

iCa – ionised calcium

IRIS – International Renal Interest Society

IRMA – immunoradiometric assays

IV – intravenous administration

MEN – multiple endocrine neoplasia

PHPTH – primary hyperparathyroidism

PO – oral administration (*per os*)

PTH – parathyroid hormone

PTHrP – parathyroid hormone-related protein

RIA – radioimmunoassay

SC – subcutaneous administration

SID – once daily (*semel in die*)

tCa – total calcium

TID – thrice daily (*ter in die*)

US – ultrasonography

USG – urine specific gravity

UTI – urinary tract infection

VDR – vitamin D receptor

Placement

The student's curricular placement was in Anderson Moores Veterinary Specialists in Winchester, United Kingdom for a 6 months externship from October 2nd 2015 to March 18th 2016.

A total of 990 hours were completed during the placement on a schedule basis from Monday to Friday, with 9-hour days from 8.30am to 17.30pm.

The first and last half hour of each day was spent in ward rounds where the medics presented their cases and gave relevant information to both the on call medic and nurses.

The student had a rotation between internal medicine consults, imaging, medicine procedures and wards, with one out of four weeks spent in wards. The student had the opportunity to follow the cases from the first consult to the complete investigation by imaging, medical procedures and access to laboratory results.

A total of 204 hours were spent in internal medicine consults, 272 hours in imaging and 170 hours in medicine procedures.

Of the 272 hours completed in imaging included mostly ultrasound exams, ultrasound-guided procedures, radiography but also an adding total of approximately 68 hours in computed tomography (CT) scans and fluoroscopy (swallow studies and cystography). Ultrasound-guided procedures included cystocentesis, fine needle aspirates, tru-cut biopsies (mainly liver, kidney and miscellaneous tumours), abdominal and thoracic fluid sampling, bile sampling and chest drainage.

The medicine procedures included gastrointestinal, respiratory and urinary endoscopy, foreign body endoscopic removal, percutaneous endoscopic gastrostomy, oesophagostomy feeding tubes, cerebrospinal fluid taps, joint taps, bronchoalveolar lavage, bone marrow biopsy and aspiration,

Approximately 34 hours were spent in assisting to miscellaneous activities including: magnetic resonance imaging scans, wound management, chest drains placement, skin biopsies, muscle biopsies, echocardiography, electrocardiography and electromyography.

1. Primary Hyperparathyroidism

1.1.   Parathyroids

1.1.1.   Anatomy

The parathyroid glands are small ellipsoid purplish endocrine organs, closely related to the thyroid gland, which is located on the external surface of the trachea. These glands generally measure 2-5mm in diameter and 0,5-1mm in width, with no demonstrated correlation to body weight according to a study by Mulligan and Francis in 1951[1] (Hullinger, 2013; Rosol & Capen, 1997).

There are usually four structurally independent glands, one embedded (external parathyroid) and one on the surface (internal parathyroid) of each of the thyroid glands (Figure 1). The external parathyroid, also known as parathyroid gland III, is frequently located on the surface of the thyroid gland on its cranial dorsolateral edge. The internal parathyroid, or parathyroid gland IV, is usually found at various depths of thyroid tissue, in the caudal portion of the thyroid. In 1951, Mulligan and Francis also reported common variations in number and location of the parathyroid glands. Marine (1914)[2] and Reed et al. (1928)[3] reported an incidence of accessory parathyroid tissue in 3 to 6% of dogs (Hullinger, 2013).

Figure 1 - Internal and external parathyroid glands (modified from Bonczynski, 2007).
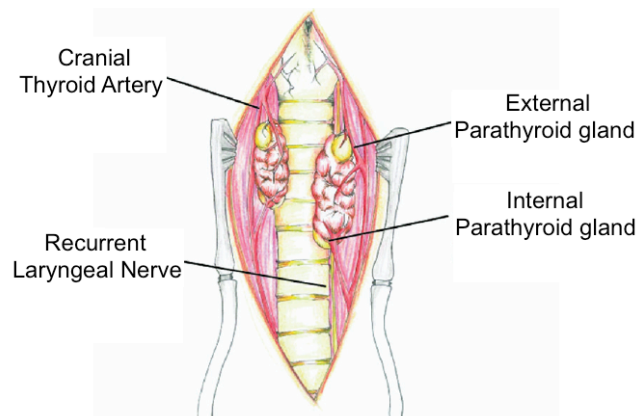
The blood supply is related to the supply from the thyroid glands, with the external parathyroid being vascularised by small branches of the cranial thyroid artery and the internal parathyroid by vessels surrounding the thyroid parenchyma. The venous and lymphatic

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[1] Mulligan, R.M., Francis, K.C. (1951). Weights of thyroid and parathyroid glands of normal male dogs. *The Anatomical Record* 110:139–143.
[2] Marine, D. (1914). Observations on tetany in dogs. *Journal of Experimental Medicine* 19:89–105.
[3] Reed, C.I., Lackey, R.W., Payte, J.I. (1928) Observations on parathyroidectomized dogs, with particular attention to the regional incidence of tetany and to the blood mineral changes in this condition. *American Journal of Physiology* 84:176–188.

drainage are also the same as the thyroid glands and it is likely that the innervation of the parathyroids is too (Figure 2) (Hullinger, 2013; Rosol & Capen, 1997).

Figure 2 - Parathyroid glands and surrounding structures (modified from Caplan, 2013).
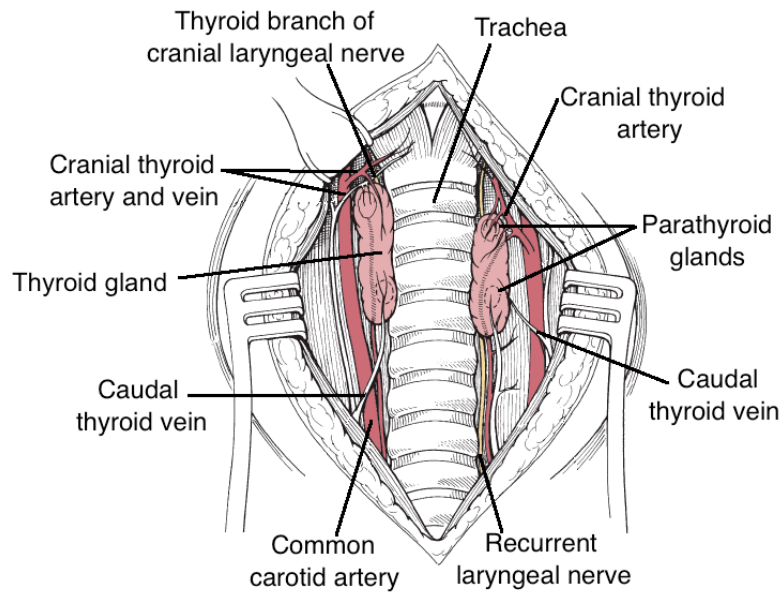
1.1.2. Histology

The parathyroid tissue is composed by principal or chief cells, small polygonal cells with round nuclei and slightly acidophilic pale staining cytoplasm (Figure 3), that produce and secrete parathyroid hormone (PTH). PTH is stored in granules of irregular shape in the cytoplasm. Oxyphil cells are also present, usually in clusters of cells. These cells are larger than chief cells with very acidophilic cytoplasm, but their function is not fully understood (Mescher, 2013).

Figure 3 - Parathyroid chief cells organised in chords around capillaries. Haematoxylin and eosin, 200x (modified from Mescher, 2013).
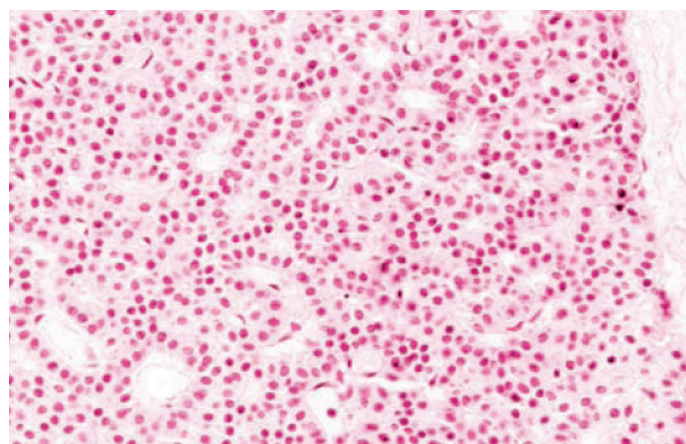
1.1.3. Calcium physiology

1.1.3.1. Function and distribution

Calcium is an inorganic element essential to survival, included in the macrominerals group (Goff, 2015). Calcium has two primary functions: (1) to provide skeletal support and (2) to participate in biochemical intracellular and extracellular functions (Feldman, 2015a; Schenck, Chew, Nagode & Rosol, 2012).

Ionised calcium (iCa or Ca^{2+}) is necessary for several body functions including enzymatic reactions, membrane transport and stability, blood coagulation, nerve conduction, neuromuscular transmission, muscle contraction, vascular smooth muscle tone, hormone secretion, bone formation and resorption, control of hepatic glycogen metabolism, cell growth and division (Schenck et al., 2012).

The vast majority of calcium (99%) is found in bone, primarily as hydroxyapatite; 0.9% is intracellular; and 0.1% is extracellular. Most of the intracellular calcium is contained in mitochondria, granules of the endoplasmic reticulum or bound to proteins, with only a very low concentration of calcium present in the cytosol (Cunningham & Klein, 2007; Rosol, Chew, Nagode & Capen, 1995).

There is a 10,000-fold concentration gradient of iCa between extracellular fluid and cytosol, which enables iCa to serve as a messenger to activate intracellular processes (Rosol et al., 1995) through a rise and fall during brief cellular responses or during initial phases of sustained responses (Rasmussen, 1989). The iCa role is carried at very low and tightly controlled concentrations, because excess in cytosolic iCa causes toxicity and may lead to cellular death (Rasmussen, Barrett, Smallwood, Bollag & Isales, 1990; Rasmussen, 1989; Schenck et al., 2012).

The calcium in the extracellular fluid (ECF), including intestinal and blood calcium, exists in three fractions: iCa (56%), protein-bound (34%) and complexed (10%). These values refer to healthy dogs (Schenck & Chew, 2012). The variation between ionised and protein-bound calcium is dependent on serum pH, with a rise in acidity causing increased competition between hydrogen ions and iCa for the negative-charged sites in the proteins, resulting in higher concentrations of iCa (Rosol et al., 1995).

The biologically active and actively regulated fraction of calcium is iCa (Feldman, 2015a; Rosol et al., 1995). The protein-bound calcium is mainly bound to albumin, and in a smaller amount to globulins, while complexed calcium is bound to phosphate, bicarbonate, sulfate, citrate and lactate (Goff, 2015; Rosol et al., 1995; Schenck et al., 2012).

1.1.3.2. Calcium homeostasis

Normal homeostasis of serum calcium maintains the concentration within a narrow range, making adjustments within a 5% deviation of the normal values (Cunningham & Klein, 2007). The normal serum concentrations of calcium in dogs are 9.0 - 11.5mg/dL or 2.2 - 3.8mmol/l for total calcium (tCa) and 5.0 - 6.0mg/dl or 1.2 - 1.5mmol/l for iCa (Schenck et al., 2012), though each laboratory has its own reference range.

The calcium serum concentration is mainly regulated by: (1) PTH; (2) calcitriol, the active form of vitamin D; and (3) calcitonin. Under specific conditions other hormones, including adrenal corticosteroids, oestrogens, thyroxine, growth hormone (GH), glucagon and parathyroid hormone-related protein (PTHrP), may also affect the calcium control (Rosol & Capen, 1997).

In fetus the calcium homeostasis is maintained by PTHrP, as low levels of PTH are present (MacIsaac, Caple, et al., 1991; MacIsaac, Heath, et al., 1991).

The calcium regulating hormones act on three target organs: (1) the intestine, the absorption site; (2) the kidneys, the excretion site; and (3) the skeleton, the largest storage site in the body (Favus, Bushinsky & Lemann, 2006; Rosol & Capen, 1996). In normal adult animals the amount of calcium absorbed in the intestine matches the amount excreted by urine and the small losses through sweat and intestinal secretions (Rosol et al., 1995).

Intestinal absorption of calcium can occur in two mechanisms: a passive, nonsaturable, paracellular diffusion or an active, saturable, cell-mediated transport. The passive absorption depends on electrochemical gradients, requiring high concentrations of calcium in the intestinal lumen. This diffusional process depends on the permeability of each intestinal segment, which is highest in the duodenum, jejunum and ileum, intermediate in the colon and lowest in the cecum (Favus et al., 2006). The active transport absorbs calcium down a concentration gradient, from the intestinal lumen to the interstitial fluid. This process is facilitated by transporting proteins including: a calcium channel protein that allows iCa to cross to the cytosol, a vitamin D-dependent calcium-binding protein that transports calcium from the apical surface of cells, which is then pumped by the plasma membrane Ca^{2+} ATPase pump protein to the extracellular space on the basolateral side of the cell. All three of these proteins are dependent on calcitriol (Goff, 2015).

The amount of calcium absorbed by each mechanism depends on the calcium content of the diet. High calcium intakes result in larger amounts being absorbed passively, which in turn trigger lower concentrations of calcitriol, the hormone responsible for stimulating active absorption. On the other hand, lower calcium dietary content stimulates calcitriol secretion and, consequently, increases active absorption on a longer-term basis. Calcitriol stimulation of the intestinal epithelial vitamin D receptor (VDR) will contribute to the production of proteins that partake in cell-mediated transport (Favus et al., 2006).

The absorption in the intestines depends on: the acidity, the presence of other dietary components or disease in the small intestine, the integrity of the villi and on calcitriol stimulation (Schenck et al., 2012).

The non-protein bound calcium, including ionised and complexed calcium, is the ultrafilterable fraction of calcium, which composes the glomerular filtrate. To maintain calcium homeostasis, the kidney must reabsorb 98% or more of the filtered calcium. The majority of calcium is reabsorbed in the proximal convoluted tubules (70%), mainly by passive mechanisms, and in the thick ascending loop of Henle (20%). Approximately 8% of calcium is actively absorbed in the distal convoluted tubules, the main site for physiologic regulation of its excretion (Figure 4) (Favus et al., 2006; Rosol et al., 1995).

Figure 4 - Sites of calcium resorption in the nephron. Passive reabsorption occurs in the proximal convoluted tubule and PTH regulation occurs in the distal convoluted tubule (from Rosol et al., 1995).
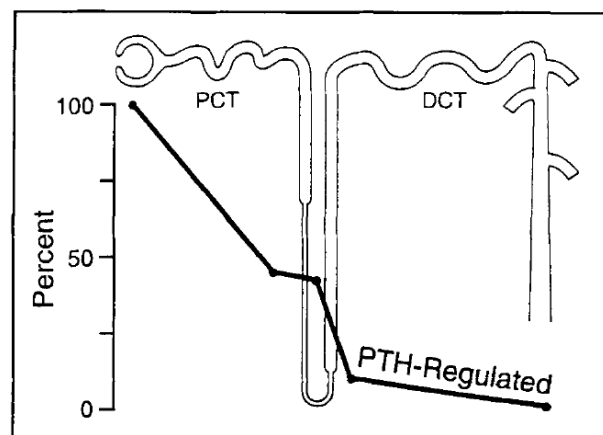
Legend: PCT - proximal convoluted tubule; DCT - distal convoluted tubule

The bone osteocytes are surrounded by lacunae, which are connected by canaliculi. The fluid in the lacunae and canaliculi is rich in calcium, which can be rapidly transported to the ECF when stimulated by PTH (Goff, 2015).

The calcium in this fluid is present as amorphous crystals or in solution and comprises 0.5% of the bone calcium (Cunningham & Klein, 2007). When larger amounts of calcium are required osteoclasts reabsorb solid bone, dissolving the mineralised matrix in calcium and phosphorus, which can then be mobilised to the ECF (Schenck et al., 2012).

Hypocalcaemia results in the secretion of PTH by the parathyroid glands within seconds. The presence of increased PTH secretion will: increase the renal tubule reabsorption within minutes, decreasing the urinary losses; mobilise bone calcium stores, both osteocytic and osteoclastic-mediated within minutes to hours; indirectly enhance intestinal absorption of calcium by stimulating the synthesis of calcitriol within 24 hours (Favus et al., 2006; Goff, 2015).

Hypercalcaemia, a less frequent occurrence, results in the suppression of PTH secretion and consequently decreases tubular reabsorption and increases renal excretion of calcium. This decline in PTH along with higher iCa concentrations decreases calcitriol synthesis (Schenck et al., 2012). Increased calcitonin secretion by the thyroid, also inhibits renal reabsorption and bone calcium resorption, decreasing the mobilisation of calcium to the ECF (Goff, 2015). Serum phosphate or inorganic phosphorus is also affected by calcium regulating hormones: PTH reduces phosphate renal reabsorption and stimulates phosphate release from the bone; and calcitriol increases phosphate intestinal absorption (Brown & Juppner, 2006; Rosol & Capen, 1996).

1.1.3.3. Parathyroid hormone

PTH is an 84-amino acid straight chain polypeptide produced and secreted by chief cells of the parathyroid glands. The intact PTH (1-84) is the biologically active form of the hormone, which has two functional domains: the N-terminal (PTH 1-34), which has the major biological activity, and the C-terminal (Rosol & Capen, 1996; Rosol et al., 1995).

The ribosomes of the chief cells synthesise the precursor of PTH, preproparathyroid hormone (preproPTH), composed by 115 amino acids (a.a.). PreproPTH penetrates the cisternal space of the rough endoplasmic reticulum where, within a minute, a 25 a.a sequence is cleaved, resulting in proparathyroid hormone (proPTH). Then proPTH, composed by 90 a.a, goes to the Golgi apparatus where enzymes cleavage a 6 a.a. sequence concluding the synthesis of mature PTH (Figure 5) (Rosol & Capen, 1997).

Figure 5 - Synthesis and secretion of PTH and the regulation sites of PTH biosynthesis by iCa or calcitriol (1,25 Vit D_3) (from Schenck et al., 2012).
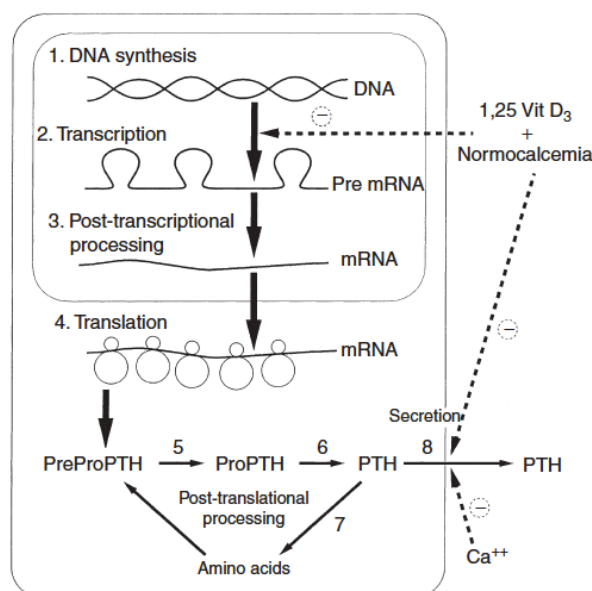
The parathyroid glands' function is regulated through the stimulation or inhibition of the secretion and synthesis of PTH and parathyroid cellular proliferation. The main regulators are iCa, via extracellular calcium-sensing receptor (CaR), and calcitriol, via the VDR, with iCa being the most important regulator in a minute-to-minute basis (Feldman, 2015a; Schenck et al., 2012). Low iCa concentration stimulates PTH secretion, PTH gene expression and parathyroid cellular proliferation, whilst high iCa concentration inhibits those actions. Calcitriol inhibits PTH gene expression and may reduce the secretion of PTH and parathyroid cellular proliferation. According to Slatopolsky et al., 1996[1], phosphate also directly stimulates PTH gene expression and is thought to also indirectly stimulate PTH secretion and parathyroid cellular proliferation (Brown & Juppner, 2006).

The CaR is a G protein-coupled receptor (GPCR) expressed in the parathyroid cells, where it inhibits PTH secretion when calcium binds to it. CaR is also present in the kidneys, where it enhances or reduces the calcium excretion in urine depending on the iCa concentration (Feldman, 2015a).

The different responses to increase PTH secretion depend on how long hypocalcaemia lasts. The initial response is to secrete the PTH contained in the secretory vesicles in the chief cells, which happens over seconds to a few minutes. If the stimulus is maintained for longer periods of time, the following actions take place: (1) the intracellular degradation of PTH is reduced (resulting in more biologically active PTH available for secretion), from minutes to around an hour; (2) there is an increase in the PTH gene expression and consequently an increase in the synthesised PTH, from several hours to days; (3) there is proliferation of parathyroid cells, from days to weeks or longer, eventually leading to enlarged glands (Brown & Juppner, 2006). The amount of PTH available for secretion depends on the synthesised amount and the amount that suffers intracellular degradation (Schenck et al., 2012).

The rate of PTH secretion has a steep inverse sigmoidal relation with iCa, in which small variations in iCa result in marked changes in PTH secretion. This is important to maintain a precise control in the concentrations of iCa (Rosol et al., 1995). According to Brown, 1991[2], the set point for PTH secretion is the iCa concentration that occurs at serum PTH concentration midway between maximal an minimal values of PTH obtained experimentally (Figure 6). Normal iCa concentrations are slightly higher than the set point (Schenck et al., 2012).

PTH has a short half-life (less than 5 minutes) before it is metabolised peripherically and excreted, mainly by the liver and kidneys. In the liver PTH is metabolised and degraded by Kupffer cells and renal clearance occurs through glomerular filtration (Kronenberg, Bringhurst, Segre & Potts, 2001; Rosol et al., 1995).

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[1] Slatopolsky, E., Finch, J., Denda, M., Ritter, C., Zhong, M., Dusso, A., Mac-Donald, P., Brown (1996). Phosphorus restriction prevents parathyroid gland growth. High phosphorus directly stimulates PTH secretion in vitro. *Journal Clinical Investigation* 97: 2534–2540.
[2] Brown, E.M (1991). Extracellular Ca^{2+} sensing, regulation of parathyroid cell function, and role of Ca^{2+} and other ions as extracellular (first) messengers. *Physiology Reviews* 71:371–411.

Figure 6 - Serum calcium "set point", the concentration of serum iCa at which PTH concentration is half maximal (from Rosol, 1995).
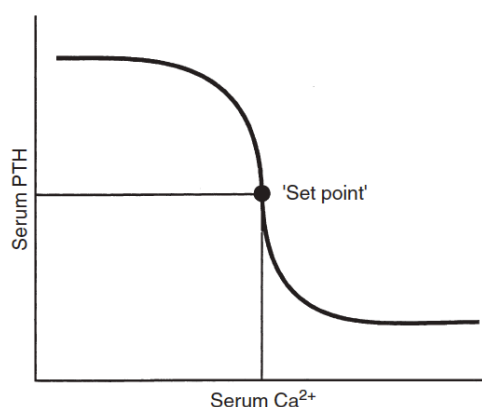
The PTH1 receptor or PTH/PTHrP receptor is the main receptor mediating PTH action, binding the N-terminal of PTH and PTHrP equally. It is mostly expressed in renal epithelial cells and osteoblasts, though it is also present in other cells. PTH2 receptor, on the other hand, binds PTH but not PTHrP (Schenck et al., 2012).

PTH is the main hormone controlling iCa on a minute-to-minute basis, with the kidneys and bones as target organs. PTH actions in the kidney are: to increase the reabsorption of calcium, to inhibit phosphate reabsorption and to stimulate the synthesis of calcitriol. In the bones, PTH causes mobilisation of calcium and phosphate from the rapid turning-over pool and after a few hours from an additional slower pool to the blood. Chronically, PTH leads to an increase in the number and on the activity of osteoclast cells and if intermittently administrated enhances the formation of trabecular bone. The overall effect of PTH is to increase iCa concentration, decrease phosphate concentration and increase renal synthesis of calcitriol (Brown & Juppner, 2006).

1.1.3.4. Parathyroid hormone-related protein

PTHrP is a protein composed of 139, 141 or 173 a.a. considered a polyhormone with multiple biologically active regions (Rosol & Capen, 1997; Rosol et al., 1992). The N-terminal region of the three forms of PTHrP (1-34) has significant homology with the N-terminal region of PTH (Broadus & Nissenson, 2006; Philbrick, 2001). This homology explains how PTHrP is able to bind and activate the PTH1 receptors in the kidney and bone with an affinity similar to PTH (Philbrick, 2001; Rosol et al., 1992). PTHrP secreted by tumours can stimulate this receptor and mimic PTH action, causing the paraneoplasic effects on the metabolism of calcium and phosphate that occur in humoral hypercalcaemia of malignancy (HHM) syndrome (Philbrick, 2001; Wysolmerski, 2012).

PTHrP is also produced by several normal tissues including: stratified squamous epithelium, adrenal cortex and medulla, fetal and adult parathyroid glands, adenohypophysis, thyroid, skeletal and smooth muscle, kidney, bone, lactating mammary gland, brain, pancreas, ovary,

testicle, myometrium and placenta. The expression of PTHrP gene in a large number of tissues accounts for the variety of types of neoplasms that can cause HHM (Rosol et al., 1992).

The different actions of PTHrP can be divided in three groups: (1) normal endocrine, (2) normal paracrine and (3) abnormal endocrine in HHM. In most normal tissues PTHrP carries a paracrine action. PTHrP acts and possibly is also metabolised and degraded locally, which explains its low circulating concentrations (Rosol et al., 1992).

Studies in sheep and mice have showed that in fetus PTHrP has an endocrine activity, where the midregion is responsible for the transport of calcium through the placenta, necessary for the fetus to maintain the required calcium concentration and also to keep the fetal-maternal calcium gradient, with the fetus having the highest concentration (Care et al., 1990; Kovacs et al., 1996).

1.1.3.5. Calcitriol

In domestic animals the active metabolites derived from Vitamin D_2 and Vitamin D_3 have equal bioactivity, so the generic terms 1,25-dihydroxyvitamin D or $1,25(OH)_2D$ and calcitriol are used to refer to the metabolites from both Vitamin D_2 and Vitamin D_3 (Schenck et al., 2012).

Dogs and cats are dependent on dietary intake of Vitamin D, as they are unable to adequately synthesise Vitamin D in the skin like herbivores and omnivores (How, Hazewinkel & Mol, 1994).

Vitamin D requires metabolic activation to be physiologically functional. The metabolism of vitamin D starts in the liver, where it is hydroxylation into 25-hydroxyvitamin D occurs. This metabolite is then transported to the kidney, where in the renal tubules 1α-hydroxylase produces 1,25-dihydroxyvitamin D (calcitriol) (Bonczynski, 2007; Holick & Garabedian, 2006). The second step in this metabolic activation is closely controlled by ionic and hormonal mechanisms, with serum concentrations of PTH, calcitriol, phosphate and calcium being the main regulators. Renal 1α-hydroxylase is inhibited by hypercalcaemia, hyperphosphataemia, excess of calcitriol and absence of PTH (Bonczynski, 2007; Pike, Meyer & Lee, 2011; Schenck et al., 2012). GH, oestrogen and prolactin are also important in increasing renal synthesis of calcitriol during growth, pregnancy and lactation, respectively (Rosol et al., 1995).

The main function of calcitriol is to maintain calcium and phosphorus homeostasis, in order to sustain adequate levels for bone matrix mineralisation (Pike et al., 2011; Rosol & Capen, 1997). Calcitriol maintains calcium levels predominantly through: (1) stimulation of intestinal calcium absorption, by activating several proteins in the small intestine to facilitate the movement of calcium into the circulation; (2) osteoclastic bone resorption by inducing pre-osteoclasts to become mature osteoclasts, which dissolve bone mineral and matrix releasing

calcium into the extracellular space; (3) inhibition of PTH synthesis; and (4) negative feedback on calcitriol formation in the kidney (Holick & Garabedian, 2006; Rosol et al., 1995). Calcitriol also carries functions not related to calcium homeostasis, including inhibition of cell growth and stimulation of cell differentiation (Rosol & Capen, 1997).

Calcitriol acts on the VDR that is present in larger quantities on its target tissues: bone, kidney, intestine and parathyroid glands; but it is also present in many other tissues (Schenck et al., 2012).

1.1.3.6. Calcitonin

Calcitonin is a 32 amino acid polypeptide synthesised in the thyroid by parafollicular or C cells (Cunningham & Klein, 2007).

This hormone leads to the decrease of serum iCa following rapid rises. Calcitonin is considered an "emergency hormone" to protect against hypercalcaemia, not being one of the primary minute-to-minute regulators of iCa (Rosol & Capen, 1997; Rosol et al., 1995).

The regulation of the secretion by C cells is mediated by CaR, the same receptor as the chief cells in the parathyroid glands. The secretion is continuous in normocalcaemia and markedly increased when iCa rises. Hyperphosphataemia also serves as a stimulant for calcitonin secretion (Rosol & Capen, 1997)

Calcitonin decreases iCa by inhibiting osteoclastic osteolysis and decreasing osteoclasts numbers, inhibiting bone resorption and decreasing calcium movement from the bone to the plasma (Rosol & Capen, 1997). This inhibition, however, is only temporary and chronic hypercalcaemia will result in high concentrations of calcitonin and C cell hyperplasia, having limited biological significance in iCa concentrations (Rosol et al., 1995).

Calcitonin also decreases phosphate reabsorption in renal tubules, stimulates diuresis of sodium, chloride and calcium, and leads to short term increases in bone formation rate (Rosol & Capen, 1997).

1.2. Hyperparathyroidism

Hyperparathyroidism (HPTH) is a condition caused by excessive levels of PTH in the body (Venes, 2013). This condition can be divided in primary and secondary HPTH, with two distinctive forms of secondary hyperparathyroidism: renal secondary hyperparathyroidism and nutritional secondary hyperparathyroidism (Polzin, 2010).

In primary hyperparathyroidism (PHPTH), there are inappropriate high or high-normal levels of PTH despite elevated concentrations of serum calcium, with no other identifiable cause (Skelly, 2012). On the other hand, in secondary hyperparathyroidism, PTH concentrations rise in response to low serum calcium or high serum phosphate levels (Venes, 2013).

1.2.1. Renal secondary hyperparathyroidism

Renal secondary hyperparathyroidism is a common complication of chronic kidney disease (CKD), in which impaired renal function ultimately results in excessive PTH secretion (Polzin, 2010; Rosol & Capen, 1997). The overall frequency of dogs with CKD that have renal HPTH is 76%, with 36% in International Renal Interest Society (IRIS) stage 1, 50% in IRIS stage 2, 96% in IRIS stage 3 and 100% in IRIS stage 4 (Cortadellas, Palacio, Talavera & Bayón, 2010).

The pathophysiology of this disease is complex and multifactorial in its origin (Polzin, 2010). The loss of nephrons due to CKD results in a decrease in the glomerular filtration rate (GFR), which in turn causes phosphate retention (Chew, Dibartola & Schenck, 2011; Cortadellas et al., 2010). This increase in serum phosphate causes a reduction in iCa concentration, through the formation of complexes, and also inhibits renal 1α-hydroxylase activity, which reduces the production of calcitriol (Cortadellas et al., 2010; Polzin, Ross, & Osborne, 2009; Polzin, 2010).

Fibroblast growth factor-23 (FGF-23) is a protein that maintains phosphate homeostasis. When phosphate levels are increased, as it occurs in CKD, FGF-23 causes an increase in its renal excretion and also inhibits renal 1α-hydroxylase, reducing calcitriol production and consequently decreasing the intestinal absorption of phosphate (Brito Galvão, Nagode, Schenck, & Chew, 2013).

The decreased concentrations of calcitriol occur not only because of 1α-hydroxylase inhibition but also, in the later stages of CKD, due to the damage in renal tubular cells, which limits the synthetic capacity of calcitriol (Cortadellas et al., 2010; Polzin, 2010; Rosol & Capen, 1997; Skelly, 2012). The concentrations of iCa are decreased because of the relative calcitriol deficiency, the formation of complexes with phosphate and skeletal resistance to PTH action (Skelly, 2012).

The secondary HPTH is a product of the reduction in calcitriol and iCa and the increase in phosphate, because these changes stimulate the parathyroid glands to increase PTH secretion (Chew et al., 2011; Cortadellas et al., 2010; Grauer, 2009; Polzin et al., 2009; Polzin, 2010; Rosol & Capen, 1997).

Secondary HPTH in the first stages of CKD is often responsible for maintaining normal concentrations of calcitriol (Chew et al., 2011; Cortadellas et al., 2010; Rosol & Capen, 1997), as PTH stimulates renal 1α-hydroxylase and the synthesis of calcitriol (Polzin, 2010). PTH also increases iCa through its direct action in the kidneys and bone and indirectly by increasing intestinal absorption of calcium, which leads to more calcium being mobilised to the blood and also improves the renal excretion of phosphate (Grauer, 2009). Animals with early CKD, usually have normal phosphate and iCa because PTH compensates the hyperphosphataemia and mild hypocalcaemia, though both can be present (Chew et al., 2011; Rosol & Capen, 1997).

Renal secondary hyperparathyroidism is beneficial in short-term, where it maintains phosphate within its normal concentration. In the long-term, however, PTH seizes to be able to prevent hyperphosphataemia and the consequences overweight the benefits (Chew et al., 2011; Polzin et al., 2009).

Decreased concentrations of calcitriol cause skeletal resistance to PTH and elevate the set-point for calcium-induced suppression of PTH secretion, which allows for hyperparathyroidism to persist even with normal or increased iCa concentrations (Polzin et al., 2009; Polzin, 2010). The absence of calcitriol as a parathyroid cell inhibitor, also allows for parathyroid gland hyperplasia to occur (Canalejo et al., 2003; Polzin, 2010; Rosol & Capen, 1997; Skelly, 2012).

Secondary HPTH causes damage to several organs, including: bones, kidneys, brain, heart, smooth muscle, lungs, erythrocytes, lymphocytes, pancreas, adrenal glands and testes (Bro & Olgaard, 1997; Cortadellas et al., 2010; Polzin, 2010). PTH toxicity seems to increase the entry of calcium into cells with PTH1 and/or PTH2 receptors, which can promote cellular death (Polzin, 2010).

PTH stimulates osteoclastic resorption, which chronically results in fibrous osteodystrophy (rubber jaw), and may also cause soft tissue mineralisation (Chew et al., 2011; Grauer, 2009; Polzin et al., 2009). High levels of PTH may cause the progressive loss of renal function by promotion of nephrocalcinosis (Polzin, 2010). PTH is also a uremic toxin that is thought to contribute to nonregenerative anaemia through impairment of erythropoiesis and decrease in the life span of red blood cells (Chew et al., 2011; Grauer, 2009). Other consequences include: carbohydrate intolerance, platelet dysfunction, impaired cardiac and skeletal muscle function, altered B cell proliferation, synaptosome and T cell dysfunction and defective fatty acid metabolism (Polzin, 2010).

The high frequency of dogs with CKD and renal HPTH, along with the facts that PTH is a uremic toxin and both HPTH and hyperphosphataemia correlate with progression of CKD and are associated with a decreased survival time, emphasises the importance of managing both HPTH and hyperphosphataemia (Chew et al., 2011; Cortadellas et al., 2010; Grauer, 2009; Rosol & Capen, 1997; Roudebush, Polzin, Adams, Towell, & Forrester, 2010). The aims of managing ion disturbances and HPTH are: (1) to reduce hyperphosphataemia, (2) to restore normal concentrations of calcitriol and (3) to maintain normal calcium concentrations (Polzin et al., 2009).

The management of renal HPTH comprises phosphate restriction, which includes dietary phosphate restriction and intestinal phosphate binding-agents, and calcitriol administration (Skelly, 2012). These measures are referred as renoprotective therapies, as they slow the progression of CKD, being especially important in IRIS stages 2 and 3 (Brown, 2007).

Phosphate restriction is the most important therapeutic measure in dogs with stable compensated CKD, preventing the progression of the disease by blunting renal HPTH, though PTH may not return to normal (Chew et al., 2011). The aim of phosphate restriction is to maintain a plasma concentration below 4.6mg/dl in IRIS stage 2, below 5.0mg/dl in IRIS stage 3 and below 6.0mg/dl in IRIS stage 4 (IRIS, 2015).

Dietary phosphate restriction can be achieved by feeding clinical renal diet therapy. If phosphate concentrations persist higher than the recommended values for the respective IRIS stage, intestinal phosphate binding-agents such as calcium acetate, calcium carbonate, aluminium hydroxide, aluminium carbonate or lanthanum carbonate can be used (Brown, 2007; Chew et al., 2011; Grauer, 2009; IRIS, 2015).

Calcitriol therapy in patients with controlled phosphate concentrations has been shown to prolong survival in dogs with CKD when administered in stage 3 and stage 4. PTH and iCa must be monitored to prevent iatrogenic complications (IRIS, 2015; Polzin et al., 2009; Roudebush et al., 2010).

1.2.2. Nutritional secondary hyperparathyroidism

Nutritional secondary hyperparathyroidism is a compensatory response to imbalances of calcium and phosphate of dietary origin. This mineral imbalance occurs when dogs are fed a diet with low levels of calcium or vitamin D, or diets with high phosphate content and normal or low levels of calcium (Feldman, 2015a). Frequent sources of mineral imbalances, as the ones referred, are diets composed predominantly of meat (Rosol & Capen, 1997; Skelly, 2012).

The low levels of calcium and high levels of phosphate in the diet cause insufficient absorption of calcium and excessive absorption of phosphate. Phosphate also forms complexes with calcium reducing the amount of this mineral available for intestinal absorption (Rosol & Capen, 1997).

These imbalances result in hypocalcaemia, which stimulates PTH secretion and leads to HPTH and both hypertrophy and hyperplasia of all parathyroid glands (Rosol & Capen, 1997). The rise in PTH secretion causes an increase in iCa concentration, partly due to bone resorption, which returns iCa concentration to normal and lowers serum phosphate (Schenck et al., 2012).

Chronic ingestion of mineral imbalanced diets causes chronic PTH elevations, which leads to the development of metabolic bone disease, that unlike renal HPTH, tends to cause osteopaenia of the long bones and vertebrae (Rosol & Capen, 1997; Schenck et al., 2012). As a result, pathologic fractures can occur and owners commonly observe the development of acute lameness (Feldman, 2015a). After correction of the diet, there is usually resolution of the bone abnormalities but residual deformities may persist after pathologic fractures (Barr, 2006).

According to Kallfelz, 1990[1], the incidence of nutritional HPTH has decreased substantially since the feeding of nutritionally complete and balanced diets has increased. On the other hand, biologically appropriate raw food (BARF) and homemade diets are more likely to cause nutritional HPTH (Schenck et al., 2012; Skelly, 2012).

Young dogs are more susceptible but nutritional HPTH can affect dogs of any age that are fed imbalanced diets (Schenck et al., 2012).

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[1] Kallfelz, F.A. (1990). Nutritional supplements in small animal practice: boon or bane? In: *Proceedings of the 8th American College Veterinary Internal Medicine Forum*. Washington, DC.

1.3. Primary Hyperparathyroidism

1.3.1. Definition and aetiology

As previously referred, PHPTH is defined as inappropriate high or high-normal levels of PTH despite elevated concentrations of serum calcium with no other identifiable cause (Skelly, 2012).

The cause for excessive inappropriate secretion of PTH is the presence of one or more abnormal parathyroid glands functioning autonomously. Usually the cause is a solitary parathyroid adenoma, though it may also be due to adenomas in more than one gland, adenomatous hyperplasia of one or more parathyroid glands or parathyroid carcinoma (Feldman, 2010).

In studies conducted in 130, 110 and 238 dogs (Feldman, 2014; Feldman, Hoar, Pollard & Nelson, 2005; Rasor, Pollard & Feldman, 2007) with PHPTH, approximately 90% had a solitary parathyroid gland enlarged, but in a study by Milovancev (2013) including 62 dogs, only 68% were reported to have solitary gland enlarged and 26% had two enlarged glands.

In a large series of dogs with PHPTH caused by a solitary mass, 87% were adenomas, 8% were primary hyperplasia and 5% were carcinomas (Feldman, 2015a). Similarly, Arbaugh et al. (2012) reported 94% of adenomas and 6% of hyperplasia in 17 dogs with a solitary parathyroid gland affected. Wisner et al. (1997), however, reported around 20% of hyperplasia and 20% of adenocarcinomas, values much higher than other studies presented before and after.

Parathyroid carcinomas are more rare, affecting from 5 to 10% of dogs with PHPTH (Berger & Feldman, 1987; Gear et al., 2005).

According to DeVries et al. (1993)[1], multiple gland involvement can have any combination of adenoma, carcinoma or hyperplasia and the histopathology of recurrent PHPTH masses has the same likelihood of being the same as the previous excised or ablated gland as being different (Feldman, 2015a).

Histopathological classification of parathyroid tissue is complicated and inconsistent. Within 17 glands examined by three clinical pathologists there was disagreement on their classification in 35% (Ham et al., 2009). It has been suggested by van Vonderen, Kooistra, Peeters, Rijnberk, & van den Ingh (2003) that adenomatous and hyperplasic tissue have no functional difference and may be a single entity with a continuum of morphologic structures.

The histological classification may have no clinical significance other than confirmation of excision of autonomous parathyroid tissue. Its clinical relevance is limited as all tumours act

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[1] DeVries, S.E., et al. (1993). Primary parathyroid gland hyperplasia in dogs: six cases (1982-1991), *Journal American Veterinary Medicine Association* 202:1132.

biologically similar and local invasion and distant metastasis are not a common component (Feldman, 2014, 2015a; Ham et al., 2009).

1.3.2. Pathophysiology

PTH causes an increase in renal synthesis of calcitriol, by stimulation of renal 1α-hydroxylase and a decrease in phosphate concentrations, by inhibition of phosphate renal tubular reabsorption and increased phosphaturia. PTH also increases iCa concentrations, through stimulation of bone resorption, stimulation of renal tubular reabsorption of calcium and indirectly by stimulating calcitriol production, which increases intestinal absorption of calcium (Brown & Juppner, 2006).

In PHPTH there is autonomous secretion of PTH that results in a loss of homeostatic control from its normal suppressors, namely increased iCa, calcitriol and phosphate concentrations. PTH continues to be produced and secreted without responding to the negative feedback by those factors, ultimately leading to hypercalcaemia, hypophosphataemia and hyperphosphaturia (Brown & Juppner, 2006; Feldman, 2010; Klausner, Fernandez, O'Leary, Johnston & Osborne, 1986). The normal mechanism to correct hypercalcaemia is the suppression of PTH secretion, which is not possible in the case of autonomous secretion by the parathyroid glands, so increased PTH secretion and hypercalcaemia persist (Feldman, 2010).

As bone resorption is continuously stimulated, chronically cancellous and cortical bone thinning occur and eventually fibrous osteodystrophy develops (Klausner et al., 1986). Stiff gait and fractures can occur but are uncommon. Only one dog in the 360 from several studies presented with pathologic fractures (Arbaugh, Smeak & Monnet, 2012; Feldman et al., 2005; Gear, Neiger, Skelly & Herrtage, 2005; Ham et al., 2009; Milovancev & Schmiedt, 2013; Pollard, Long, Nelson, Hornof & Feldman, 2001; Sawyer et al., 2011).

According to Arnaud and Strewler (1981)[1], PTH initially reduces the renal excretion of calcium, but as iCa concentrations increase, renal tubular resorption is surpassed and hypercalciuria develops. As PTH reduces the reabsorption of bicarbonate in the proximal convoluted tubules, this loss may lead to hyperchloraemic metabolic acidosis. The combination of hypercalciuria, increased urine pH and excretion of substances that promote precipitation of calcium (e.g. phosphate and oxalate) facilitates the urolith formation in this disease (Klausner et al., 1986; Skelly, 2012). Additionally, uroliths predispose urinary tract infection (UTI) occurrence (Feldman, 2015a).

The resulting hypercalcaemia is responsible for the development of a reversible form of nephrogenic diabetes insipidus. Calcium acts as an antagonist of antidiuretic hormone (ADH)

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[1] Arnaud, C. D., and Strewler, G. J.(1981). Primary hyperparathyroidism. *Seminars in Nephrology*, 1:376-393.

in renal collecting tubules, disrupting the urine-concentrating mechanism, causing polyuria and consequent compensatory polydipsia (Feldman, 2010; Skelly, 2012).

It is not fully understood if mild PHPTH directly impairs renal function, but in the majority of human patients renal function remains stable (Wysolmerski & Insogna, 2012). In a reduced number of dogs the disease seems to affect renal function, but not all cases with severe kidney disease could be justified by hydronephrosis and function loss caused by uroliths or nephroliths obstruction (Feldman, 2015a).

Hypercalcaemia tends to hyperpolarise membranes, which can cause muscular, neuromuscular and neurological signs (e.g. decreased activity, weakness, shivering, trembling or stiff gait). Increased calcium can also affect gastrointestinal motility by reducing the excitability of smooth muscle, leading to gastrointestinal signs (Feldman, 2015a).

1.3.3. Signalment

PHPTH affects older dogs, with more than 95% being 7 years or older (Feldman, 2010). In a study with 210 dogs the mean age at diagnosis was 11.2 years, with a range from 6 to 17 years, and in a series of 335 dogs it was 10.7 years (Feldman et al., 2005; Feldman, 2014).

The mean body weight reported at diagnosis was 22.2kg, ranging from 2.6 to 58.8kg, and 24kg in a study with 210 dogs and in a series of 335 dogs, respectively (Feldman et al., 2005; Feldman, 2014).

There seems to be no apparent gender predisposition for this disease, and Feldman et al. (2005) reported 54% of the dogs were males and 46% were females.

In a study by Feldman et al. (2005) including 210 dogs, 20% were Keeshonds, 14% were mixed breed dogs, 9% were Labrador retrievers, 6% were German Shepherd dogs and 6% were Golden retrievers, with the remaining being from 39 different breeds. It was referred that Keeshonds are not a breed commonly seen in their hospital, which underlines the relevance of PHPTH in this breed. In studies by Gear et al. (2005) and Rasor et al. (2007) which included 110 and 29 dogs with PHPTH, 19% and 14%, respectively were Keeshonds. In a more recent publication, Feldman reported that in a series of 335 dogs, 14% of the dogs were Keeshonds, though a large number of breeds were represented (Feldman, 2014). PHPTH in Keeshonds is heritable and seems to have an autosomal dominant mode of inheritance with a possible age-dependent penetrance (Goldstein et al., 2007).

In a study by Refsal et al. (2001)[1] it was found that Keeshonds have an odds ratio of 50.7 for PHPTH and are the breed most likely to have the disease, with Golden retrievers presenting an odds ratio of 1.6 and Dachshunds with an odds ratio of 2.0 (Goldstein et al., 2007).

There has been one report for neonatal HPTH in German shepherd dogs by Thompson et al. (1984)[1], but no other case has been reported since (Skelly, 2012).

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[1] Refsal, K.R., Provencher-Bolliger, A.L., Graham, P.A., et al. (2001). Update on the diagnosis and treatment of disorders of calcium regulation. *Veterinary Clinics of North America: Small Animal Practice*; 31:1043–1062.

1.3.4. Anamnesis - clinical signs

The clinical signs in PHPTH result from the actions of excessive PTH in the body, not the space the tumour itself occupies (Nelson, 2009).

In 20 to 50% of PHPTH cases, the owners do not notice any clinical signs even after being informed of which signs to expect (Feldman, 2015a). In the study by Feldman et al. (2005) conducted in 210 dogs with PHPTH, in 42% of the cases hypercalcaemia was identified in laboratory testing done for reasons unrelated to PHPTH, more commonly for routine screening in geriatric dogs or routine preanaesthetic evaluation for dentistry procedures.

When clinical signs are present, they tend to be mild, insidious and nonspecific. Some owners only realise which clinical signs the dog had retrospectively after treatment for PHPTH (Feldman, 2015a). As PHPTH affects older dogs, many owners ascribe clinical signs to the ageing process (Skelly, 2012). As a general rule, dogs with PHPTH present without being ill or not as ill as dogs affected by other diseases that cause hypercalcaemia (Feldman, 2014).

In the study by Feldman et al. (2005), the remaining 58% dogs presented for abnormalities that may be associated with hypercalcaemia or PHPTH. The most observed signs affected 50% of all dogs and were those consistent with urolithiasis or UTI (i.e. stranguria, pollakiuria, and/or haematuria). Other signs reported were: polyuria and polydipsia (48%), weakness (46%), decreased activity (43%), decreased appetite (37%), weight loss or muscle wasting (18%), vomiting (13%) and shivering or trembling (10%) (Feldman et al., 2005). In another study with 110 dogs (Rasor et al., 2007) the following clinical signs were reported: polyuria and polydipsia (43%), weakness (42%), lethargy (38%), decreased appetite (32%), weight loss or muscle wasting (19%), vomiting (10%) and shivering and trembling (6%).

The less frequent signs are constipation, diarrhoea and stiff or painful gait. Central nervous system signs can occur, including mental dullness and in seldom cases obtundation, seizures, collapse or coma (Feldman, 2015a).

In dogs with parathyroid carcinoma the clinical signs and their prevalence is similar to those previously referred. The difference is weakness was the most common sign whereas polyuria and polydipsia are more common in cases of adenoma or hyperplasia (Sawyer et al., 2011).

The time between the onset of clinical signs and diagnosis ranged from 0 days (dogs with no clinical signs) to 2.5 years, with a mean of 5 months (Feldman et al., 2005).

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[1] Thompson, K.G., Jones, L.P., Smylie, W.A. *et al.,* (1984) Primary hyperparathyroidism in German shepherd dogs: a disorder of probable genetic origin. *Veterinary Pathology* 21, 370- 376.

1.3.5. Physical examination

Physical examination is generally unremarkable in dogs with PHPTH. In the study conducted in 210 dogs, 71% had no abnormalities detected on their physical exam (Feldman et al., 2005) and in a series of 335 dogs, 76% also had unremarkable physical exams (Feldman, 2014).

In the study by Feldman et al. (2005), the abnormalities detected were muscle wasting, slow to rise or apparent weakness, obesity and thin body condition, each of they occurring in less than 10% of dogs (Feldman et al., 2005).

Bone deformities are extremely rare, but have been reported by Capen and Martin (1983)[1] and Gear et al. (2005) (Feldman, 2015a).

Cervical palpation of enlarged parathyroid glands is extremely uncommon in dogs and in total of 320 dogs with PHPTH reported in different studies, only one dog with a parathyroid carcinoma had a palpable mass (Arbaugh et al., 2012; Feldman et al., 2005; Ham et al., 2009; Milovancev & Schmiedt, 2013; Sawyer et al., 2011). The masses are difficult to palpate because of their location dorsolaterally to the trachea, their small diameter of 4 to 8mm and being covered by layers of muscles (Feldman, 2015a).

Though physical examination is generally unremarkable, it is very important that it is done thoroughly in order to rule out other differential diagnosis for hypercalcaemia (e.g. hypercalcaemia of malignancy, hypoadrenocorticism, toxicosis, CKD or acute kidney injury [AKI]) (Feldman, 2010, 2015a).

1.3.6. Clinical pathology

As each laboratory has its own reference range the referred values are those used by the cited authors or the values generally used.

A summary of clinical pathology results in dogs with PHPTH can be found in Table 1.

1.3.6.1. Haemogram

Complete blood count (CBC) in dogs with PHPTH is usually unremarkable, as well as bone marrow aspirates and peripheral blood smears (Feldman, 2010, 2015a). Feldman et al. (2005) reported that there are no consistent abnormalities detected (Feldman et al., 2005).

1.3.6.2. Biochemical profile

1.3.6.2.1.   tCa and iCa in PHPTH

Hypercalcaemia (i.e. excessive amount of calcium in the blood), in dogs is usually defined as fasting serum tCa concentrations higher than 12mg/dl with a normal reference range of 9.9 to

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[1] Capen, C.C., Martin, S.L. (1983). Calcium-regulating hormones and diseases of the parathyroid glands. In Ettinger SJ, editor: *Textbook of veterinary internal medicine*, ed 2, Philadelphia, WB Saunders, p 1550.

11.6mg/dl. Ionised hypercalcaemia is present when iCa concentration is higher than 1.5mmol/l (reference range from 1.12 to 1.41mmol/l) (Feldman, 2014; Schenck et al., 2012; Venes, 2013).

In a study including 210 dogs with PHPTH by Feldman et al. (2005), all had tCa in concentrations above 12mg/dl, as it was a requirement for inclusion in the study. The mean tCa was 14.5mg/dl, ranging from 12.1 to 23.4mg/dl, with 52% of them presenting concentrations between 12 and 14mg/dl, 30% with tCa concentrations between 14 and 16mg/dl, 12% with concentrations between 16 and 18mg/dl and 6% had concentrations above 18mg/dl.

In the same study (Feldman et al., 2005), 91% of the dogs had increased iCa and 9% had iCa within reference range. The authors argue that those 9% were false values, altered by variables such as the collection method or the time between collection and the time when the assay was done, as iCa is more easily altered by those variables than tCa. The mean iCa concentration was 1.71mmol/l, ranging from 1.22 to 2.41mmol/l: 27% had concentrations between 1.42 and 1.65mmol/l, 48% had concentrations between 1.66 and 1.90mmol/l and 16% had concentrations above 1.90mmol/l.

Hypercalcaemia is the hallmark of PHPTH, and in 5 different studies the tCa mean concentrations were between 13.6mg/dl and 14.3mg/dl with a range of 12.1 to 23.4mg/dl (Feldman et al., 2005; Gear et al., 2005; Ham et al., 2009; Milovancev & Schmiedt, 2013; Pollard et al., 2001). The iCa mean concentration in those same studies was between 1.67 and 1.90mmol/l, ranging from 1.43 to 2.55mmol/l except in Feldman et al. (2005) where some dogs had iCa within range, as it has been previously justified (Feldman et al., 2005; Feldman, 2015a; Gear et al., 2005; Ham et al., 2009; Milovancev & Schmiedt, 2013; Pollard et al., 2001).

The magnitude of hypercalcaemia does not help differentiating the cause or the extent of the disease (Schenck & Chew, 2012), though Feldman and Skelly, suggest that calcium progressively increases throughout the course of the disease (Feldman, 2015a; Skelly, 2012).

1.3.6.2.2.   Measurement of tCa and iCa

Calcium status is usually assessed using tCa concentration, despite iCa being the only active fraction, because tCa measurement is more readily available. iCa does not correlate directly to tCa and inferring so can lead to errors in the interpretation of the results (Schenck & Chew, 2008). In a study by Schenck and Chew (2005) with 1633 dogs, 27% had diagnosis discordance when tCa was used to predict iCa, and in dogs with chronic renal failure (CRF) the discordance was of 36%. tCa concentration overestimated normocalcaemia and underestimated hypocalcaemia. Adjustment equations were developed in an attempt to improve the correlation between tCa and iCa using total protein and albumin, but these were not verified using iCa. In the same study the diagnosis discordance using adjustment

equations was in 37% for all dogs and of 54% in dogs with CRF. So neither tCa alone nor tCa adjustment equations can accurately predict iCa, and a direct measurement of iCa is required for its accurate assessment (Schenck & Chew, 2005, 2008). tCa can give an indication of calcium status but iCa is a more pertinent and useful (Skelly, 2012).

Serum calcium concentrations can be affected by certain factors. The factors that can falsely increase calcium when assessed in some analysers are: marked lipaemia, haemolysis and contamination by chalkboards in the laboratory and rarely postprandial samples. Usage of glassware and plastic containers washed with detergents for storage of samples can cause false increases or decreases on calcium levels. Haemoconcentration by dehydration rarely causes mild increases in calcium and prolonged storage can cause an artifactual decrease in calcium concentrations (Feldman, 2010, 2015a).

Additionally dogs younger than 3 months have slightly higher concentrations of calcium than dogs over a year old (Schenck & Chew, 2012).

iCa concentration depends of pH, where a more acid pH increases the amount of iCa because the dissociation of calcium bound to proteins is favoured, whilst a more alkaline pH causes decreases in iCa. In samples collected and processed aerobically there is loss of carbon dioxide (CO_2), which increases the pH of the sample, favouring protein and calcium binding and decreasing iCa (Schenck et al., 2012). Anaerobic collection and processing are more precise than those done aerobically, but require more complicated techniques. To surpass this difficulty, mathematical formulae were developed for specific species to correct iCa to a pH of 7.4 in samples collected aerobically, that correlate very well to iCa in anaerobic samples (Schenck et al., 2012; Schenck & Chew, 2008, 2012).

1.3.6.2.3.   Phosphorous

Dogs with PHPTH usually present with normophosphataemia or hypophosphataemia, with concentrations below 4mg/dl because of PTH induced urinary loss of phosphorous (Bonczynski, 2007; Feldman, 2015a). If phosphate concentrations are not at the low end of the reference range or below, renal failure could be developing (Skelly, 2012).

Dogs younger than 1 year old have higher normal phosphate concentrations (Feldman, 2015).

In a study with 210 dogs (Feldman et al., 2005), the mean phosphate concentration was 2.8mg/dl ranging from 1.3 to 6.1mg/dl (reference range 3.0 - 6.2mg/dl). In these dogs, 13% had a phosphate concentration below 2.0mg/dl, 52% had a concentration between 2.0 and 2.9mg/dl, 28% had a concentration between 3.0 and 3.9mg/dl, 5% had a concentration between 4.0 and 4.9mg/dl and 2% had a concentration above 4.9mg/dl. The dogs with phosphate concentrations above 4.9mg/dl also had concomitant increased blood urea nitrogen (BUN) and creatinine concentrations. The mean serum phosphate concentration in the dogs from a control group with similar ages was 4.6mg/dl, higher than in dogs with

PHPTH. Feldman (2014) in a series of 335 dogs, reported a mean serum phosphate concentration of 2.7mg/dl.

The mean serum phosphate concentrations in 3 other studies were 2.91mg/dl[1], 2.86mg/dl and 2.4mg/dl (Gear et al., 2005; Milovancev & Schmiedt, 2013; Pollard et al., 2001).

Feldman (2010) recommends evaluation of phosphorous whenever serum calcium concentrations are assessed.

1.3.6.2.4. BUN and creatinine

In the study by Feldman et al. (2005), including 210 dogs with PHPTH, the BUN mean concentration was 16.9mg/dl, ranging from 5 to 92mg/dl (reference range 18-30mg/dl) and the creatinine mean concentration was 0.8mg/dl, ranging from 0.4 to 4.1mg/dl (reference range 0.5-1.5mg/dl). Relatively to BUN concentrations, 3% of dogs had values below 10mg/dl, 60% had values between 10 and 17mg/dl, 23% had values between 18 and 22mg/dl, 10% had values between 23 and 28mg/dl and 3% had values above 30mg/dl. The distributions of creatinine concentrations were: 60% with a concentration or 1.0mg/dl or below, 37% with a concentration between 1.0 and 1.5mg/dl, 2% with a concentration between 1.6 and 2.0mg/dl and 1% with a concentration above 2.1mg/dl. In the 200 dogs from the control group, BUN and creatinine mean concentrations were 24mg/dl and 1.2mg/dl, respectively. These values were considerably higher than those from dogs with PHPTH.

In another report on 29 dogs with PHPTH (Gear et al., 2005), 13 dogs had increased BUN and 10 dogs had increased creatinine. However, in these 29 dogs there were more dogs with high renal parameters than in other reports with over 300 dogs with PHPTH where renal failure was rare (Arbaugh et al., 2012; Feldman et al., 2005; Ham et al., 2009; Milovancev & Schmiedt, 2013; Sawyer et al., 2011).

1.3.6.2.5. ALT and ALP

In the study by Feldman et al. (2005), 40% of dogs with PHPTH had an increase in alkaline phosphatase (ALP) levels, with a mean concentration of 241U/l, ranging from 12 to 4431U/l (reference range 5-92U/l). These values are possibly a result from increased osteoblastic activity in bone (Feldman, 2015a). In the study by Gear et al., (2005) 13 out of 27 dogs (48%) were also found to have increased ALP, but two had been treated with glucocorticoids and one was diagnosed with hyperadrenocorticism (HAC).

Alanine aminotransferase (ALT) concentrations are usually normal but can have mild increases (Feldman, 2015a).

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[1] Value converted from mmol/l with 1mmol/l of phosphate = 3.1mg/dl of phosphate (in Rosol, 1996).

1.3.6.3. Urinalysis

Dogs with hypercalcaemia have diluted urine resulting from the antagonistic action from calcium on ADH, regardless of aetiology (Feldman, 2015a). Mean urine specific gravity (USG) in the study by Feldman et al. (2005) with 210 dogs with PHPTH was 1.012 ranging from 1.004 to 1.037, with 24% of dogs with an USG below 1.008, 36% with an USG between 1.008 and 1.012, 33% with an USG 1.013 and 1.020, 4% with an USG between 1.021 and 1.030 and 3% with an USG above 1.030. In the control group the mean USG was 1.025, ranging from 1.004 to 1.052, considerably higher than in dogs with PHPTH.

Haematuria, pyuria, bacteriuria and crystalluria may be present in the sediment in cases when urolithiasis and/or UTI exist (Nelson, 2009).

In the study performed by Feldman et al. (2005), 29% of dogs had an UTI at time of diagnosis and one third of those 29% also had concomitant cystic calculi. Cystic calculi were present in 31% of these 210 dogs around the time PHPTH was diagnosed. In this study the calculi were constituted by calcium phosphate or calcium oxalate but a mixture of both can also be found (Feldman et al., 2005; Nelson, 2009).

In the study by Sawyer et al. (2011) in 19 dogs with PHPTH, 26% were found to have cystic calculi and in the study by Gear et al., (2005) with 11 dogs, 45% had UTI.

Table 1 – Summary of clinical pathology results in dogs with PHPTH.

Haemogram	Normal
Biochemical profile	
- tCa	↑
- iCa	↑
- Phosphate	↓/Normal
- BUN	↓/Normal
- Creatinine	↓/Normal
- ALP	Normal/↑
- ALT	Normal
Urinalysis	
- USG	often <1.020
- Sediment	haematuria, pyuria, bacteriuria, crystalluria

Legend: iCa - ionised calcium; tCa - total calcium; BUN - blood urea nitrogen; ALP - alkaline phosphatase; ALT - alanine aminotransferase; USG - urine specific gravity; ↑ - increased; ↓ - decreased.

1.3.7. Assays

1.3.7.1. Measurement of PTH and PTHrP

PTH can be measured by two-site immunoradiometric assays (IRMA) that bind antibodies to specific segments of the hormone. The assay has two different antibodies, a capture antibody and a detection antibody, the first binding the hormone to the tube or plate and the second, which is labelled, allowing a means of detection of the hormone. In 1989, Torrance and Nachreiner, validated the first IRMA for intact human PTH, that uses a capture antibody against the C-terminal of PTH and a detection antibody against PTH (1-34) (Refsal & Nachreiner, 2012; Torrance & Nachreiner, 1989a).

It was later discovered this assay measures not only the active PTH (1-84) but also large non-(1-84) inactive fragments, the majority of which are probably PTH (7-84) fragments (Brossard et al., 1996, 2000; Lepage et al., 1998). A new IRMA was then developed by Gao et al. (2001) with a detection antibody for PTH (1-4) and a capture antibody for PTH (39-84). This new assay does not have any cross-reaction with non-(1-84) PTH fragments measuring only whole PTH (1-84), the only active form of the hormone. There is high similarity between human and dog PTH sequence, which would suggest the whole PTH human assay could also be used in dogs. The whole PTH assay could be particularly valuable in diagnosis in dogs comparing to intact PTH assays, because the ratio of whole PTH assay and intact PTH assay is higher in dogs than in humans, suggesting a larger percentage of PTH (7-84) fragments are present in dogs (Estepa et al., 2003).

The relevance of the PTH whole assay may be more significant in presence of renal secondary hyperparathyroidism, where PTH (7-84) fragments are increased (Miwa et al., 2003; Schenck et al., 2012) because these are more dependent on GFR than the active PTH (1-84) is (Torrance & Nachreiner, 1989b). Whole PTH assay gives a better estimate of the active hormone present, but makes it harder to differentiate increases from baseline values (Refsal & Nachreiner, 2012).

PTH values depend greatly on the method used. Intact PTH assay and whole PTH assay have a positive correlation but intact PTH assay has higher values, probably because of the cross-reaction of inactive fragments (Estepa et al., 2003; Refsal & Nachreiner, 2012).

A study compared intact PTH measured with IRMA and a chemiluminiscent assay, a rapid test that takes around 20 minutes to get a result, unlike the IRMA that takes a few days. There was good correlation between the two, but IRMA usually had higher values than the rapid test. This test could in the future be a good option for diagnosis of calcium disorders with quick and accurate results (Ham et al., 2009).

Ideally the samples used for this assessment should be shipped and stored frozen to prevent degradation of PTH and collected with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, as it provides the best stability (Schenck et al., 2012).

PThrP in dogs can be measured using two-site IRMA and N-terminal radioimmunoassay (RIA) developed for humans, because of the high similarity between the two species (Schenck et al., 2012; Skelly, 2012). Several forms of PThrP have biological activity, including PThrP (1-36), PThrP (1-86) and PThrP (1-141), all of which can be measured with N-terminal RIAs but only the last two are measured by two-site IRMAs (Schenck et al., 2012). PThrP should also be measured in plasma with EDTA as an anticoagulant, either fresh or frozen. The reference interval for PThrP in dogs is below 0.5pmol/l (Schenck et al., 2012; Skelly, 2012).

Feldman et al. (1997)[1] compared serum PTH assays results from the two jugular veins and a cephalic vein in each dog, trying to localise functioning parathyroid gland masses. The theory was that the sample from the side with the mass would have a higher concentration of PTH than the opposite side. The results, however, concluded that it was not a reliable method for localising the side of the mass, with 11 out of 12 dogs showing similar concentrations between the two jugular veins and the cephalic vein (Feldman, 2010; Wisner et al., 1997).

1.3.7.2. PTH and PThrP in PHPTH

In PHPTH, PTH concentrations are normal to increased, PThrP should be undetectable and calcitriol should be normal to increased. Interpretation of PTH concentrations should always be done relatively to tCa concentrations or preferably with iCa concentrations, from the same sample (Feldman, 2015a; Nelson, 2009).

In 185 randomly selected dogs with PHPTH, the use of a two-site IRMA measuring intact PTH, detected a mean serum concentration of 11.3pmol/l, ranging from 2.3 to 121pmol/l (reference range 2 to 13pmol/l). In 73% of dogs, PTH was within reference values, 45% with concentrations between 2.3 and 7.9pmol/l and 28% with concentrations between 8.0 and 13.0pmol/l. The PTH concentration was between 13 and 20pmol/l in 11% and over 20pmol/l in the remaining 16% (Feldman et al., 2005). In Feldman (2014), in a series of 335 dogs, 60% had concentrations within reference range. Similar data has been reported in other studies, with a mean PTH concentration of 28.1pmol/l, 19.7pmol/l and 17.7pmol/l, respectively, a range between 4.7 and 173.4pmol/l, and a reference interval of 2 to 13pmol/l for the first and 3 to 17 for the last two (Ham et al., 2009; Pollard et al., 2001; Sawyer et al., 2011).

In dogs with hypercalcaemia PTH should be undetectable, so a value over the reference range or a value within reference range are both considered inappropriate considering the calcium concentrations (Feldman et al., 2005; Feldman, 2014), which are consistent with an autonomous secretion of PTH (Feldman, 2015a).

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[1] Feldman, E.C, Wisner, E.R., Nelson, R.W., Feldman, M.S., Kennedy, P.C. (1997). Comparison of results of hormonal analysis of samples obtained from selected venous sites versus cervical ultrasonography for localizing parathyroid masses in dogs, *Journal of American Veterinary Medical Association* 211:54.

In a hypercalcaemic dog without renal failure, normal to increased concentrations of PTH confirm the diagnosis of PHPTH (Feldman, 2014).

1.3.8. Imaging

1.3.8.1. Radiography and abdominal ultrasonography

Thoracic and abdominal radiography, and/or abdominal ultrasonography are an important part of the diagnostic approach of the hypercalcaemic dog. In dogs with PHPTH these exams are generally unremarkable (Feldman et al., 2005; Feldman, 2015a; Nelson, 2009). The exception is the previously mentioned presence of uroliths, with cystic calculi reported in 31% of dogs in one study (Feldman et al., 2005). Uroliths are more often present in the bladder but may more seldom be present in the kidneys, ureters and urethra (Feldman, 2015a).

The previously mentioned fibrous osteodystrophy, though classic in human PHPTH, is rare in dogs. Other rare radiographic changes in dogs with PHPTH include loss of lamina dura, fractures of long bones and vertebrae and soft tissue calcification (Feldman, 2015a).

1.3.8.2. Cervical Ultrasonography

Ultrasonography (US) of parathyroid glands requires a high frequency transducer (7.5 to 10MHz), because a high frequency is required to attain enough resolution for assessment of such small structures (Wisner & Nyland, 1998). US of normal and abnormal parathyroid glands is difficult because of their small size and superficial location, but most parathyroid glands in dogs can be identified via US (Liles, Linder, Cain, & Pease, 2010).

The size of parathyroid glands on US and their gross size have no statistical difference and the discrepancy between the two may be an artefact of US measurement. The smallest parathyroid gland detected on US in a study was 2.1mm in length (Liles et al., 2010).

In US, normal parathyroid glands present as discrete, well marginated structures, round to oval and hypoechoic to anechoic relatively to the surrounding thyroid parenchyma, some with distal enhancement (Reusch et al., 2000; Wisner et al., 1997). These glands are small, with median diameter of 3.3mm (Reusch et al., 2000) and a length under 2mm, not always being visible on cervical US (Wisner et al., 1997). In recent study the mean parathyroid glands length was 3.4mm, similarly to the previous study, but normal parathyroid glands with an US measurement up 7.6mm have been reported (Liles et al., 2010). The location is variable, with most being localised in the cranial or caudal pole of a thyroid lobe, but some are present in the mid-body of a thyroid lobe (Wisner et al., 1997). Ectopic and supernumerary parathyroid glands can occur, making it important to examine the whole ventral cervical region (Wisner & Nyland, 1998).

Abnormal parathyroid glands also present as round or oval, well marginated structures, hypoechoic to anechoic relatively to the surrounding thyroid gland parenchyma, with mild to

moderate distal enhancement but, with larger dimensions (Wisner, Nyland, Feldman, Nelson, & Griffey, 1993). In PHPTH the lesion is often solitary (90%), however, occasionally multiple glands are identified, with two glands being more common and 3 or 4 more rare, unlike secondary hyperplasia where usually all parathyroid glands are affected. Ectopic tumours have been reported in humans, but not in dogs (Feldman et al., 2005; Feldman, 2014, 2015a; Taeymans, 2011).

In a study where 142 masses were identified via cervical US, the median diameter was 6mm, ranging from 3 to 23mm. The diameter of the masses was 4 to 6mm (≈60%), 7 to 10mm (≈24%), 11 to 15mm (≈10%) and 16 to 23mm (≈6%) (Feldman et al., 2005).

There is a statistical difference in lesion size between adenomas or adenocarcinomas and primary or secondary hyperplasic parathyroid glands, with a mean of 7.5mm and 2.9mm and a range of 4 to 20mm and 2 to 6mm, respectively. In the study by Wisner et al. (1997), all but one of the hyperplasic cases had a size under 4mm, whilst all of the neoplasic cases had sizes over 4mm, so a presumptive diagnosis can be made based on size (i.e. hyperplasia in glands smaller than 4mm and neoplasia in glands over 4mm), but because of some overlap in both size and appearance, histopathology is necessary for a definitive diagnosis (Taeymans, 2011).

In PHPTH at least one abnormal parathyroid gland should be identified, but failure to do so does not necessarily exclude it, but it is a reason to reconsider the diagnosis (Feldman, 2014, 2015a; Nelson, 2009).

The percentages of agreement between cervical US results and surgically identified masses were of 99%, 95%, 93%, 63%, 76% and 100%, respectively (Feldman et al., 2005; Feldman, 2014; Gear et al., 2005; Ham et al., 2009; Milovancev & Schmiedt, 2013; Sawyer et al., 2011). Feldman et al. (2005), referred that the high percentage may not be real as some cases had previous cervical US but only the last US result was taken into consideration, so possibly some exams were negative for masses in the past, which were later correctly identified. The large difference between results underlines the subjectivity of US results and how important the operator's skill and experience are (Feldman et al., 2005; Feldman, 2014; Milovancev & Schmiedt, 2013).

False positive results are rare, but false negative results are frequent. Incorrect identification could be justified by variability in location and number of parathyroid glands, the complexity of the anatomy in the cervical region and also because this technique is particularly operator and equipment dependent (Wisner & Nyland, 1998).

Cervical US is a valuable tool and has become a routine exam in the diagnostic approach of a hypercalcaemic dog, especially when a parathyroid gland lesion is suspected (Feldman, 2014, 2015a; Wisner et al., 1997). US allows parathyroid glands lesions identification and location, being recommend for both diagnostic purposes and presurgical planning (Sawyer et al., 2011; Schenck et al., 2012; Wisner et al., 1993).

The advantages of preoperative location are: facilitated surgical exploration, reduced anaesthesia time and increased surgical success rate (Attie et al., 1988).

Although cervical US is an important tool, surgery exploration can be done without imaging results. The ventral cervical approach is done in a manner that each parathyroid gland is assessed and when lesions are not, or are inaccurately identified previous to surgery, they should be correctly identified during surgery (Milovancev & Schmiedt, 2013).

1.3.8.3. Scintigraphy

The first studies of double-phase scintigraphy of parathyroid glands using Technetium-99M-Sestamibi (Matwichuk et al., 1996; Wright et al., 1995) seemed promising in identifying parathyroid tumours in two dogs. A later study with 15 dogs, however, had very low sensitivity (11%) and specificity (50%) detecting and localising hyperfunctioning parathyroid tissue, which was not considered acceptable and so usage of scintigraphy is not recommended for this purpose (Matwichuk et al., 2000).

1.3.9.   Differential diagnosis

Differential diagnosis of hypercalcaemia include: non-pathologic conditions (samples from young growing dogs, non-fasting samples, laboratory error, lipaemia or contamination with detergent), transient results (haemoconcentration, hyperproteinaemia, hypoadrenocorticism or severe hypothermia) and pathologic (as PHPTH, hypercalcaemia of malignancy, CRF or acute renal failure [ARF], hypervitaminosis D, granulomatous disease, non-malignant skeletal lesions, excessive phosphate binders ingestion, excessive calcium supplementation, hypervitaminosis A or grape toxicity). The most common causes are hypercalcaemia of malignancy caused by lymphosarcoma, hypoadrenocorticism, PHPTH and CKD (Feldman, 2014, 2015a; Rosol & Capen, 1988; Schenck et al., 2012).

These differential diagnosis will be approached with more detail in the clinical approach to hypercalcaemia section.

1.3.10. Pretreatment considerations

The pillar of preanaesthesia preparation in PHPTH is evaluation of calcium concentrations and treatment of symptomatic patients. Treatment of hypercalcaemia is rarely required as most dogs are asymptomatic and only have mild to moderate hypercalcaemia. Acute hypercalcaemia treatment should be started 12 to 24 hours before anaesthesia in cases where tCa is higher than 14mg/dl, the calcium-phosphorus ratio is higher than 70, when calcium-associated cardiac arrhythmias or neurologic signs are present and in the presence of azotaemia (could be concealed by hypercalcaemia induced diuresis) (Adams, Figueiredo, & Graves, 2015; Bonczynski, 2007; Séguin & Brownlee, 2012). If tCa concentration is higher

than 15-16mg/dl, a concentration high enough to cause clinical signs in most dogs, anaesthesia should be delayed (Adams et al., 2015).

Significant hypercalcaemia can promote bradycardia, peripheral vasoconstriction, hypertension and cardiac arrhythmias, but during anaesthesia relaxation of peripheral vascular tone can cause hypotension. Electrocardiogram (ECG) changes can include bradycardia, prolonged PR interval, widened QRS complex and shortened QT interval in severe cases. Blood-gas analysis is recommended to monitor electrolytes and acid-base status as respiratory and metabolic acidosis increases the iCa fraction which can worsen hypertension and bradycardia (Adams et al., 2015; Caplan, 2013).

There are no specific drugs or techniques warranted in PHPTH cases but non-depolarising muscle relaxants (e.g. d-tubocurarine and atracurium) should be avoided as hypercalcaemia can antagonise their effects (Adams et al., 2015).

1.3.11. Treatment

Many dogs are referred based on concern that hypercalcaemia puts dogs with PHPTH at risk of developing renal failure. This is based on a false premise, as it will be discussed later on. Renal failure is rare in dogs with PHPTH and it is not a reason for treating the disease. On the other hand, uroliths and UTI are common and a reason to advise treatment (Feldman et al., 2005; Feldman, 2014; Guttin, Knox IV, & Diroff, 2015).

Even though many animals present asymptomatic, the fact that many owners only appreciate that clinical signs were present upon their resolution, underlines the importance of treating affected dogs regardless of clinical signs as it was suggested in human medicine (Feldman, 2015a; Utiger, 1999).

There are three treatment options for PHPTH in dogs: surgical (i.e. parathyroidectomy), percutaneous ultrasound-guided ethanol ablation and percutaneous ultrasound-guided heat ablation (Feldman, 2014, 2015a; Rasor et al., 2007; Séguin & Brownlee, 2012).

1.3.11.1. Surgical

Parathyroidectomy is an effective treatment for PHPTH in dogs, consisting on removal of the abnormal parathyroid gland or glands, and according to some authors is the treatment of choice (Caplan, 2013; Nelson, 2009; Rasor et al., 2007).

With the patient on dorsal recumbency, the parathyroid glands are exposed via a ventral midline cervical approach, from the larynx to the manubrium. The thyroid glands, located on the medial surface of the thyrohyoid muscles and caudolateral to the larynx, are exposed. All four parathyroid glands should be inspected before the excision of any gland (Flanders, 2003).

When the cause of PHPTH is an external parathyroid gland, the affected gland presents firm, off-colour, enlarged (0.5 to 1.0cm) and somewhat spherical, as does an affected internal parathyroid gland that can be palpated and visualised through the ventral or dorsal aspect of the thyroid parenchyma. Presurgical US identification can be helpful in locating an internal abnormal gland. It is essential that both surfaces of the thyroid glands are inspected, in order no to miss any abnormal parathyroid tissue (Flanders, 2003; Skelly, 2012). Correct identification can sometimes be challenging when abnormal tissue presents with subtle or inapparent changes (Feldman, 2015a; Liles et al., 2010).

Parathyroidectomy of an external parathyroid gland can be done without removal of thyroid tissue, because adenomas do not extend beyond the capsule of the parathyroid gland and sharp dissection between the two glands is enough to remove it (Caplan, 2013; Flanders, 2003). If there is suspicion that not all abnormal tissue was removed, partial thyroidectomy can be performed en bloc with the parathyroidectomy, removing the thyroid gland parenchyma surrounding the affected parathyroid gland. When partial thyroidectomy is executed, it is vital to assure the blood supply of the remaining tissue is not compromised (Flanders, 2003; Séguin & Brownlee, 2012).

In the case of internal parathyroid glands, thyroidectomy should be performed and the ipsilateral external gland should be preserved (Caplan, 2013).

If carcinoma is suspected based on apparent invasiveness of the surrounding tissues, complete thyroidectomy with removal of draining lymph nodes is recommended (Caplan, 2013).

Although a solitary abnormal parathyroid gland is usually present in dogs with PHPTH, multiple glands can be affected (Feldman, 2014; Feldman, Hoar, Pollard & Nelson, 2005; Rasor, Pollard & Feldman, 2007) and in the same surgery up to three glands can be removed. Dogs can function normally with only one parathyroid gland, so at least one gland should be spared to ensure the animal can maintain calcium homeostasis and permanent hypoparathyroidism is avoided (Flanders, 2003; Skelly, 2012). Even though the animal is able to function normally with only one parathyroid gland, the procedure should be done to spare as much normal tissue as possible to reduce postsurgical transient hypoparathyroidism (Flanders, 2003).

The non-affected glands have a normal or smaller size, due to the suppression caused by increased PTH levels (Flanders, 2003; Schenck et al., 2012). If all glands are uniformly enlarged, secondary hyperparathyroidism should be considered, in which case the removal of one to three glands may reduce hypercalcaemia (Caplan, 2013; Flanders, 2003; Skelly, 2012).

If no abnormal gland is identified after carefully exploring the cervical area and there is a strong suspicion of PHPTH, possible reasons include the presence of an ectopic tumour or all glands are affected uniformly by primary hyperplasia and there is no normal tissue for

comparison (Caplan, 2013; Flanders, 2003). A possible course of action is removing a parathyroid gland or a thyroid/parathyroid complex to investigate if histopathology is compatible with parathyroid hyperplasia or neoplasia (Feldman, 2014; Flanders, 2003).

In cases where tumours are not readily identifiable (e.g. if embedded in fat, if present in an internal parathyroid gland or rarely if caused by an ectopic tumour) methylene blue infusion could be an effective tool (Schenck et al., 2012). Methylene blue was first used in surgery for identification of parathyroid glands by Dudley (1971), where it was found useful in cases where identification was problematic. The normal parathyroid glands stain dusky blue and abnormal glands stain dark blue to purple. This method helped reducing surgical time and could reduce the need for total thyroidectomy and postsurgical hypoparathyroidism from the removal of excessive parathyroid tissue. Severe side effects including haemolytic anaemia and ARF were reported in dogs by Fingeroth and Smeak (1988)[1], which supports reserving methylene blue infusion for cases where a tumour is strongly suspected and not readily identified in surgery (Schenck et al., 2012). A reduction in the dose of methylene blue could possibly reduce side effects (Bewick & Pfleiderer, 2014).

A rapid chemiluminescent PTH assay has been validated to evaluate perioperative PTH levels. After parathyroidectomy of all abnormal tissue in one or more glands, the surgical incision was closed and a blood sample collected 30 to 45 minutes after the last parathyroid gland was removed. The results were available 10 to 20 minutes after sampling and were then compared with a preoperative sample. A decrease over 50% in PTH concentration was consistent with resolution of hypercalcaemia for at least 6 months in 92% of dogs, meaning this pre and postoperative PTH results allow the surgeon to confirm when the autonomous functioning parathyroid tissue has been removed (Ham et al., 2009). This statistically significant decrease was also verified in a more recent study, with a median decrease of 84.9% (Graham, Wilkinson, Culvenor, Dhand, & Churcher, 2012). This assay can be helpful in providing a confirmation of cure within the same day, rather than having to wait days or weeks for calcium levels to decline and is not influenced by parathyroidectomy postsurgical management (Graham et al., 2012; Ham et al., 2009).

In the study by Rasor et al. (2007) comparing treatment options for PHPTH parathyroidectomy was performed on 47 dogs. The success rate in this study was of 94% with hypercalcaemia controlled for a median of 561 days. Hypercalcaemia resolution occurred between 1 and 6 days after surgery: 65.9% within 48 hours, 29.5% in 2 to 4 days and 4.5% in 4 to 6 days. The only complication of parathyroidectomy reported in these 47 dogs was postsurgical hypocalcaemia, with laboratory hypocalcaemia affecting 38% but only 11% actually having clinical signs. The success rate of surgery in another study was 92% (Ham et al., 2009).

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[1] Fingeroth, J.M., Smeak, D.D. (1988). Intravenous methylene blue infusion for intraoperative identification of parathyroid gland tumors in dogs. Part III: Clinical trials and results in three dogs. *Journal American Animal Hospital Association*;24:673–8.

Parathyroidectomy may fail to cure PHPHT in cases of inability to locate the abnormal parathyroid tissue, multiple parathyroid glands being affected, incomplete excision of all abnormal tissue, ectopic autonomously functioning tissue (rare) and malignant functioning distant metastasis (rare) (Feldman, 2015a; Ham et al., 2009; Rasor et al., 2007).

1.3.11.2. Percutaneous

1.3.11.2.1. Percutaneous ultrasound-guided ethanol ablation

Percutaneous ultrasound-guided ethanol ablation was first evaluated for the treatment of PHPTH in 8 dogs by Long, Goldstein, Hornof, Feldman, & Nyland (1999). After US identification of a parathyroid mass, with the dog under general anaesthesia and the ventral cervical region clipped and aseptically prepared, a 27-gauge needle attached to a syringe with ethanol (96%) were injected under US guidance in the parathyroid mass.

Ethanol is a caustic substance that causes coagulation necrosis and vascular thrombosis. The injection of ethanol is done slowly and the tip of the needle repositioned attempting to expose all of the parenchyma to the chemical. Ethanol is hyperechoic and can be easily visualised with US (Feldman, 2015a).

In 27 cases Guttin et al. (2015) used ethanol (95%) with the product of length, height and width as the injected volume, which was considered equivalent of the mass volume. The volume of injection in the 27 procedures was between 0.02 and 2.0ml.

In the study by Long et al. (1999) a total of 9 procedures were performed in 8 dogs, 7 of which were successful and tCa and iCa concentrations were within reference range within 24 hours. Transient hypocalcaemia was present in 4 dogs, but only one required treatment. In this study, six months after the treatment the 6 dogs assessed remained normocalcaemic. This procedure was considered safe and effective by the authors and an alternative to parathyroidectomy in dogs with PHPTH (Rasor et al., 2007).

In the study by Gear et al. (2005) , ethanol ablation was attempted in 5 dogs, with only 2 showing partial response to treatment but their iCa never returned to normal values. The success rate in this case was considered to be of 0%, which the authors suggested could be due to the smaller size of the glands where the procedure was attempted compared to previous reports of percutaneous treatments by Long (1999) and Pollard (2001), or due to the inexperience of the operator.

In the study by Rasor et al. (2007) comparing the treatment methods in 18 procedures of ethanol ablation, the successful rate was of 72% with a control of hypercalcaemia for a median of 540 days. In the successful cases hypercalcaemia resolved between 1 and 4 days, the majority of which (83%) resolved within the first 48 hours. A more recent study by Guttin et al. (2015) using a larger sample of 27 cases had a success rate of 85%. In the cases where treatment was effective, hypercalcaemia resolved within 72 hours after treatment, 79,2% of which was in the first 24 hours and 87% in the first 48 hours.

Long et al. (1999) reported a mean anaesthesia time of 38 minutes (Feldman, 2015a) and Guttin et al. (2015) reported a median time of 30 minutes, ranging from 15 to 45 minutes.

Reported complications from several studies for this method include: hypocalcaemia (mild and transient or with clinical signs), change in bark (in some cases transient), cough, owner reported dysphagia and hypersalivation and a cyst on the neck (possibly not related). Laboratory hypocalcaemia was present in 40% of dogs with successful treatment, but only 10% of the successfully treated dogs developed clinical signs of hypocalcaemia (Guttin et al., 2015; Long et al., 1999; Rasor et al., 2007).

According to Feldman (2014), this treatment method is no longer recommended because the leakage of the chemical causes damage to the tissues surrounding the parathyroid gland and can possibly lead to nerve damage and laryngeal paralysis. Gear et al. (2005) also questioned the utility of this method in dogs, because unlike in humans a general anaesthesia is still required, if it fails another anaesthesia is necessary and lacks the advantages of examination of both cervical sides and the possibility to send tissue for histopathology. However, the study by Guttin et al., (2015) with 27 cases suggests this method is effective, with minimal complications, low risk of hypocalcaemia, short duration of anaesthesia and is more affordable than surgery, which could justify its preference in some patients. The price of ethanol ablation in the same referral hospital was a third of the price of parathyroidectomy.

1.3.11.2.2. Percutaneous ultrasound-guided heat ablation

The first percutaneous ultrasound-guided radiofrequency heat ablation to treat PHPTH in dogs was reported by Pollard et al. (2001). Previously this method had been used to treat small hepatic masses in humans, where it was considered an effective treatment option (Jiao et al., 1999; Livraghi et al., 1999). Radiofrequency heat ablation was also more effective and required fewer treatments than ethanol in human small hepatocellular carcinomas (Livraghi et al., 1999).

Radiofrequency causes thermal necrosis at the tip of the needle, which has the advantage of damaging only a small area of tissue and not causing damage to the regional vasculature. A disadvantage of this method is the price of the equipment (Pollard et al., 2001).

The patient is anaesthetised, positioned on dorsal recumbency, the ventral cervical region is clipped and aseptically prepared. A small part of the ventral abdomen is also clipped to place a disposable cautery ground pad. A 20-gauge over-the-needle intravenous (IV) catheter without the plastic hub is used. An insulated wire connects the radiofrequency unit to the catheter stylet, where at the tip radiofrequency waves are naturally converted into heat. The catheter sleeve is used as an insulator to the surrounding normal tissues. The catheter (with stylet) is US guided into the parathyroid mass and redirected multiple times to ablate the entire mass. Radiofrequency energy of 10 to 20W is applied for 30 to 90 seconds, until an

US change in the tissue is observed. The gland becomes hyperechoic in comparison to the original echogenicity and echogenic bubbles are visible by the tip of the needle (Feldman, 2014; Pollard et al., 2001).

In this study (Pollard et al., 2001), anaesthesia duration was from 20 to 60 minutes, mostly spent guaranteeing correct placement of the needle and it was referred that as experience increased procedure time decreased. Heat was applied three or four times in each dog.

In 11 dogs included in the study by Pollard et al. (2001), 73% were successfully treated using heat ablation with hypercalcaemia resolving within 5 days. In two of the three dogs where treatment failed, heat ablation was not performed because the needle could not be satisfactorily placed. Both dogs had small masses (under 5mm in diameter), which led the authors to suggest small masses could be poor candidates for this procedure. Hypocalcaemia treatment was required in 63% of the successfully treated dogs. The only other complication was transient bark change in one dog.

In the study by Rasor et al. (2007) comparing the three treatments heat ablation was performed in 48 dogs with a treatment success rate of 90% and a control of hypercalcaemia for a median of 581 days. Hypercalcaemia resolved between 1 and 6 days: 72.7% within 48 hours, 13.6% between 2 and 4 days and 13.6% between 4 and 6 days.

In dogs with ipsilateral masses, both were ablated at the same time, but in contralateral masses there was 30 days period between procedures to reduce risk of bilateral laryngeal paralysis. Laboratory hypocalcaemia was present in 36% of successfully treated dogs, with 11% having clinical signs. Other complications present in this study included cough, change in bark and Horner's syndrome and occurred in 2%, 4% and 2% of treated dogs, respectively (Rasor et al., 2007).

1.3.11.3.   Treatment methods comparison

In the study by Rasor et al. (2007), 110 dogs were included in the study comparing the three treatment options, with success rates of parathyroidectomy, ultrasound-guided heat ablation and ultrasound-guided ethanol ablation of 94%, 90% and 72% respectively.

The cases where surgical treatment failed to control hypercalcaemia were a consequence of the inability to locate the abnormal parathyroid tissue. It was suggested that a learning curve is present in the last two treatment options and that the lower number of dogs treated with ethanol ablation could justify the lower success rate of this method (Rasor et al., 2007).

In dogs where treatment was successful, hypercalcaemia resolution was attained within 6 days, the majority of which within 48 hours of the procedure, similar to previously reported PHPTH treatments (Gear et al., 2005; Long et al., 1999; Pollard et al., 2001). Heat ablation took a significant longer period of time compared to the other two procedures, probably because of the physiopathology of the process.

Percutaneous treatment can cause complications by damaging surrounding tissues and structures by leakage of ethanol or by extension of thermal necrosis. Structures that can be affected include the laryngeal nerve and the vagosympathetic trunk (Pollard et al., 2001; Rasor et al., 2007). The only complication of parathyroidectomy in 47 dogs was postsurgical laboratorial or clinical hypocalcaemia (Rasor et al., 2007).

The treatment choice is dependent on certain factors and surgery should be recommended in the following cases: if besides a parathyroid mass a thyroid mass is present; if the mass is over 12 or 15mm; if no mass is identified in US; if calculi are present in the urinary tract (specially in males), so that their removal can be done in the same anaesthesia; and if contralateral masses are present. If the mass is too small (under 4mm) or closely associated to the carotid arteries, percutaneous treatment is not an alternative (Feldman, 2014; Nelson, 2009; Pollard et al., 2001). Percutaneous treatment can be an option in contralateral masses if treatment is done in a phased manner, at least 30 days apart to avoid iatrogenic laryngeal paralysis. Dogs with more than one parathyroid mass have increased risk of adverse effects, which supports the usage of a staged approach. These dogs also have higher risk of treatment failure (Feldman, 2014; Guttin et al., 2015).

Advantages of percutaneous treatment methods compared to surgery are the reduction of anaesthesia time, the absence of incisions or problems related to wound healing, and reduced cost of these methods (Feldman, 2014; Nelson, 2009; Pollard et al., 2001). The shorter time of anaesthesia may be an important advantage of percutaneous methods because PHPTH usually affects older dogs that can have concomitant diseases (Guttin et al., 2015).

1.3.12. Posttreatment management – hypocalcaemia

Hypocalcaemia is the most common postoperative complication following treatment of PHPTH in dogs, with reported rates between 25% and 71% (Adams et al., 2015; Arbaugh et al., 2012; Berger & Feldman, 1987; Brito Galvão & Chew, 2011; Caplan, 2013; Flanders, 2003; Graham et al., 2012; Guttin et al., 2015; Milovancev & Schmiedt, 2013; Rasor et al., 2007; Sawyer et al., 2011). Hypocalcaemia usually occurs between 1 to 7 days after treatment, the majority between 3 and 6 days (Feldman, 2010, 2014, 2015a). Despite the high number of dogs treated for PHPTH that become hypocalcaemic, quite a low number of dogs actually show clinical signs, only 30 dogs in 243 treated dogs reported in several studies. More rarely hypocalcaemia can be fatal (Arbaugh et al., 2012; Berger & Feldman, 1987; Guttin et al., 2015; Milovancev & Schmiedt, 2013; Rasor et al., 2007; Sawyer et al., 2011).

The clinical signs associated with acute hypocalcaemia are: panting, nervousness, anxiety, muscle trembling or twitching, leg cramping or pain, ataxia, stiff gait, facial rubbing, seizures (focal or generalised), biting of the feet, aggressive behaviour, hypersensitivity and vocalising

(Feldman, 2010, 2015a). Development of clinical tetany usually occurs 4 to 7 days after PHPTH treatment (Feldman, 2010).

Chronic hypercalcaemia as a result of autonomous PTH secretion leads to suppression and atrophy of normal parathyroid tissue. Excision or ablation of the parathyroid tumour causes rapid decreases of PTH concentrations and consequently tCa and iCa concentrations, possibly resulting in hypocalcaemia (Adams et al., 2015; Caplan, 2013; Flanders, 2003; Nelson, 2009).

Dogs should be monitored once of twice a day for 5 to 7 days after treatment and kept in the hospital for at least 5 days. Dogs are kept in the hospital not only for monitoring reason but also to ensure the dog remains quiet, as active dogs with hypocalcaemia are more likely develop tetany. However, if a stable dog is extremely anxious in the hospital it might be more beneficial to send the dog home early and have it come to the practice if necessary (Feldman, 2014, 2015a; Nelson, 2009; Séguin & Brownlee, 2012).

Some authors defend that there is a correlation between high preoperative concentrations of calcium and the incidence of hypocalcaemia, with tCa higher than 14mg/dl or 15mg/dl or dogs with more than one affected gland having a greater incidence (Caplan, 2013; Feldman, 2014, 2015a; Flanders, 2003; Gear et al., 2005). Median preoperative tCa in dogs that became hypocalcaemic after treatment (16.8mg/dl[1] and 16.12mg/dl) was significantly higher than dogs that didn't (13.6mg/dl[1] and 13.77mg/dl) (Feldman, 2015a; Gear et al., 2005). On the other hand, two studies have concluded the opposite, that preoperative tCa is not predictive of development of postoperative hypocalcaemia (Arbaugh et al., 2012; Milovancev & Schmiedt, 2013).

Multiple affected glands, size of the affected gland and chronicity of the disease may also be related to higher occurrence of hypocalcaemia (Feldman, 2014, 2015a; Gear et al., 2005; Nelson, 2009).

According to the authors that consider preoperative calcium concentrations to be predictive of postsurgical hypocalcaemia, dogs with concentrations below 14mg/dl are at low risk of developing hypocalcaemia. Vitamin D therapy in these cases is withheld, calcium is monitored and only if tCa and iCa concentrations are lower than 9mg/dl and 0.95mmol/l, respectively or clinical signs of tetany are observed is vitamin D therapy started (Feldman, 2010, 2014, 2015a; Nelson, 2009). In dogs with tCa and iCa concentrations above 15mg/dl and 1.75mmol/l, respectively, prophylactic treatment started in the morning before treatment with calcitriol (20 to 30ng/kg/day twice daily (BID) for the first 3 to 4 days) is recommended. There is no specific recommendation for tCa concentrations between 14 and 15mg/dl (Feldman, 2010, 2014, 2015a).

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[1] Conversion from mmol/l: 1mmol/l of calcium = 4mg/dl of calcium (in Canine & Feline Endocrinology, 2015, 4th edition)

Calcitriol therapy is preferred to other vitamin D metabolites because of its activity, rapid onset of action (1 to 4 days), short circulating half-life and quicker resolution should overdose occur (Brito Galvão & Chew, 2011; Flanders, 2003). Vitamin D therapy started during or just after the treatment procedure fails to prevent development of hypocalcaemia (Schenck et al., 2012).

However, Feldman (2015a) suggested that this approach could place dogs at discomfort and risk of developing life-threatening hypocalcaemia and now recommends that all dogs receive prophylactic therapy to prevent hypocalcaemia following treatment. The recommended protocol is calcitriol (20 to 30ng/kg) in the morning before treatment and at night (10 to 15ng/kg) and continued in a dose of 10 to 15ng/kg BID for the first two days. On the third day and every four days there should be a 10% reduction of the dose for 45 to 60 days, with tCa and iCa being checked before each reduction. If at any moment tCa or iCa are below 8.5mg/dl or 0.95mmol/l, respectively, the calcitriol should be returned to the previous dose, at least temporarily. If, on the other hand, tCa or iCa are at the upper level of reference range, calcitriol dose should be reduced by 50%. If concentrations are above the reference range, calcitriol should be stopped for 48 hours and tCa and iCa rechecked, if values are still high IV therapy should be considered (Feldman, 2015a). In transient hypoparathyroidism following PHPTH treatment, the goal is to maintain a low to low-normal concentration of tCa and iCa to prevent hypocalcaemia, minimise hypercalcaemia and stimulate the activity of parathyroid glands (Feldman, 2015b; Nelson, 2009).

Calcium salts are not usually necessary as most commercial diets have enough calcium content but following severe hypocalcaemia, supplementation with oral calcium can be used in the short-term. The most common calcium supplement is calcium carbonate (25 to 50 mg/kg/day), but calcium lactate, calcium chloride and calcium gluconate are also available (Feldman, 2010; Schenck et al., 2012; Skelly, 2012).

Another prophylactic option is usage bisphosphonates, but the effect on dogs with PHPTH has not been studied yet, so little information is available (Brito Galvão & Chew, 2011).

Despite preoperative calcium and vitamin D therapy some dogs still developed hypocalcaemia, but serious hypocalcaemia and severe clinical signs were prevented (Arbaugh et al., 2012; Feldman, 2010, 2015a; Gear et al., 2005; Sawyer et al., 2011).

Treatment of acute hypocalcaemia requires an individual protocol that depends on the severity of clinical signs, magnitude of hypocalcaemia, on the rate of decline in tCa or iCa and trend of serial measurements. An aggressive approach is necessary when relevant clinical signs, severe hypocalcaemia and when calcium is steadily or rapidly declining. Therapy should be started before clinical signs of hypocalcaemia develop (Feldman, 2015b; Schenck et al., 2012). Information on drugs and their respective calcium content and dose is available in Table 2.

The first approach in the presence of hypocalcaemic tetany is immediate, but slow, IV infusion to effect of calcium salts in a dose of 5 to 15mg/kg of elemental calcium over a 10 to 20 minute period. The different calcium salts available for parental administration are: 10% calcium gluconate (9.3mg Ca/ml) IV or subcutaneous administration (SC), 10% calcium borogluconate (15mg Ca/ml) IV and calcium chloride (27.2mg Ca/ml) IV. Some authors do not recommend calcium chloride usage, as it is caustic when accidental perivascular administration occurs, causing significant tissue death. There is no difference in their effectiveness if the dose is calculated based on elemental calcium. Guideline dosages are 0.5 to 1.5ml/kg of calcium gluconate and 0.3 to 0.9ml/kg of calcium borogluconate (Feldman, 2015b; Schenck et al., 2012). Administration of calcium SC is controversial, as it can cause skin sloughing and sterile abscesses (Schenck et al., 2012; Skelly, 2012).

As the initial bolus of calcium only lasts minutes to little over an hour and oral calcium and vitamin D supplementation can take from 24 to 96 hours to be effective, multiple IV infusions of calcium (not recommended) or continuous IV infusion of calcium can be used to control acute hypocalcaemia. A continuous rate infusion of elemental calcium at a rate of 2.5 to 3.5mg/kg/hour or 60 to 90mg/kg/day should be used until oral therapy is effective. This dose can be obtained by removing 25ml of 10% calcium gluconate, diluting in 250ml of 0.9% saline and infusing at rate of 2.5ml/kg/hour (Brito Galvão & Chew, 2011; Feldman, 2015b; Schenck et al., 2012; Skelly, 2012).

During the administration of IV calcium, heart rate and ECG should be monitored, or if ECG is not available, someone should listen to the heart or keep a finger on the pulse during the infusion. In the presence of bradycardia, arrhythmias, pulse deficits, premature complexes, ST segment elevation or QT-interval shortening the infusion should be slowed or temporarily stopped as these signs may indicate cardiotoxicity caused by the calcium infusion (Feldman, 2015b; Schenck et al., 2012; Skelly, 2012).

Calculation of the required dose and dilution in 0.9% saline helps achieving a slower rate of administration. It should be noted that the recommended doses are guidelines and patient response is the definitive factor (Feldman, 2015b; Skelly, 2012). Calcium salts should not be added to fluids with lactate, acetate, bicarbonate or phosphates as precipitation can occur and alkalinising fluids should be also avoided (Schenck et al., 2012).

Therapy should be tapered down as the remaining parathyroid glands start to resume their function (Feldman, 2015b; Schenck et al., 2012).

Table 2 – Treatment of hypocalcaemia with calcium (adapted from Schenck et al., 2012).

Drug	Preparation	Calcium content	Dose	Comment
Parenteral calcium				
Calcium gluconate	10% solution	9.3mg of Ca/ml	a. slow IV to effect (0.5-1.5 ml/kg IV) b. 5-15mg/kg/hr IV c. SC diluted Ca salts*	-stop if bradycardia or shortened QT-interval -infusion to maintain normal Ca
Calcium chloride	10% solution	27.2mg of Ca/ml	5-15 mg/kg/hr IV	- only give IV. Is extremely caustic perivascularly
Oral calcium				
Calcium carbonate	many sizes	40% tablet	25-50 mg/kg/day	- most common calcium supplement
Calcium lactate	325 and 600 mg	13% tablet	25-50 mg/kg/day	
Calcium chloride	powder	27.2%	25-50 mg/kg/day	- may cause gastric irritation
Calcium gluconate	many sizes	10%	25-50 mg/kg/day	

Legend: IV - intravenous; SC - subcutaneous.

Note 1: calcium solutions cannot be mixed with bicarbonate-containing fluids, because precipitation may occur.

Note 2: SC calcium salts can cause severe skin necrosis or mineralisation and are no longer recommended.

Note 3: oral calcium dose should be calculated on elemental calcium content.

Table 3 – Treatment of hypocalcaemia with Vitamin D (adapted from Schenck et al., 2012).

Vitamin D	Initial dose	Maintenance dose	Time for maximal effect to occur	Time for toxicity effect to resolve
ergocalciferol	4000-6000 U/kg/day	1000-2000 U/kg once daily to once weekly	5 – 21 days	1 – 18 weeks
calcitriol	20 – 30 ng/kg/day for 3-4 days	5-15 ng/kg/day	1 – 4 days	2 – 24 days

1.3.13. Prognosis

As treatment is curative, prognosis for dogs with PHPTH undergoing surgery or ablation is excellent, especially if hypocalcaemia after treatment is minimised or avoided with the usage of calcitriol (Caplan, 2013; Feldman, 2015a; Flanders, 2003; Nelson, 2009; Rasor et al., 2007; Séguin & Brownlee, 2012).

With parathyroid gland carcinomas, an excellent control of the tumour and hypercalcaemia is attained with surgery alone, with metastasis and local invasion being very infrequent (Ham et al., 2009; Sawyer et al., 2011).

Dogs that present poorly and with concomitant diseases have a less favourable prognosis (Berger & Feldman, 1987; Caplan, 2013; Gear et al., 2005).

The recurrence rate (after at least 6 months of hypercalcaemia control) seemed independent of histopathology of the mass, with a reported rate under 10% (Feldman, 2010, 2014, 2015a; Rasor et al., 2007). Keeshonds have a genetic component and as parathyroid tissue is still present, a new tumour may develop (Skelly, 2012).

1.3.14. Associated diseases

Gear et al. (2005) found a predisposition for renal failure in dogs with PHPTH. In 29 dogs, 34% and 45% had increase creatinine and urea concentrations, respectively and in 19 dogs that underwent parathyroidectomy 37% were in renal failure postoperatively. However, this was not repeatable in other studies were renal failure was a rare finding in over 300 dogs (Arbaugh et al., 2012; Feldman et al., 2005; Ham et al., 2009; Milovancev & Schmiedt, 2013; Sawyer et al., 2011). Actually, some authors considered dogs with PHPTH could be protected from developing renal failure rather than being predisposed, because of the lower phosphate, BUN and creatinine concentrations compared to control groups of dogs with similar age (Feldman et al., 2005; Feldman, 2014). It seems like renal failure induced by hypercalcaemia is not as common in dogs with PHPTH as it is in dogs with other causes of hypercalcaemia (Schenck et al., 2012).

Cystic calculi and UTI are very common in dogs with PHPTH and these findings were present at the time of diagnosis in 31% and 29%, respectively (Feldman et al., 2005). Other smaller studies have reported similar values, with 26% of 19 dogs having cystic calculi (Sawyer et al., 2011) and 45% of 11 dogs having UTI (Gear et al., 2005).

Thyroid cysts, adenoma or carcinomas were present in 5% of the dogs on cervical US at the time PHPTH was diagnosed (Feldman, 2014). In another study, 15% of dogs with hypercalcaemia had at least one thyroid nodule seen on US. These nodules were incidental findings, most benign, though they could not be distinguished based only on US features. There are currently no criteria to help differentiate benign from malignant based only on US features nor to justify cytology or biopsy to be performed (Pollard, Bohannon, & Feldman, 2014).

Multiple endocrine neoplasia (MEN) are a group of syndromes in humans where two or more endocrine glands have hyperplasia or neoplasia (Feldman, 2015a). In dogs several MEN cases have been reported and frequently include hyperadrenocorticism associated with PHPTH (Feldman, 2015a; Kiupel, Mueller, Ramos Vara, Irizarry, & Lin, 2000; Thuróczy et al., 1998; Walker, Jones, Guildford, Burbidge, & Alley, 2000; Wright et al., 1995).

2. Clinical approach to hypercalcaemia

2.1. Hypercalcaemia - clinical signs

As previously mentioned, hypercalcaemia is toxic to cells. Excessive levels of calcium can affect all tissues, but major effects occur on the central nervous system, gastrointestinal tract, cardiovascular system and kidneys (Schenck et al., 2012).

Clinical signs and lesions are dependent on the magnitude of hypercalcaemia and also on the rate of its development and duration, with rapid development (e.g. vitamin D intoxication and rapid infusion with calcium containing fluids) causing more severe signs (Schenck et al., 2012).

Despite the rate of development of hypercalcaemia, serum tCa concentrations between 12 and 14mg/dl may not be accompanied by clinical signs, whilst almost all dogs with concentrations above 15mg/dl show systemic clinical signs. With tCa concentrations above 18mg/dl animals present severely ill and with concentrations over 20mg/dl are probably in a life-threatening condition (Schenck et al., 2012).

Common clinical signs of hypercalcaemia include: polyuria and polydipsia, anorexia, dehydration, lethargy, weakness and vomiting. Other clinical signs that are less commonly associated with hypercalcaemia are constipation, cardiac arrhythmia, seizures or twitching and death. Conditions that may be associated include prerenal azotaemia, CRF, ARF and calcium urolithiasis (Feldman, 2015a; Schenck et al., 2012).

Mineralisation of soft tissues, particularly heart and kidneys, is a relevant consequence and is related to phosphorus concentration. According to Chew (1982)[1] a product of calcium (mg/dl) and phosphorus (mg/dl) over 60 causes more severe mineralisation, but if there is severe hypercalcaemia it can occur independently of phosphorus concentrations (Schenck et al., 2012).

The breed most represented after mixed breeds were the Golden Retrievers (13%), which can be justified by the high risk in this breed of both lymphoma and PHPTH (Messinger, Windham, & Ward, 2009; Modiano et al., 2005).

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[1] Chew, D.J., Meuten, D.J. (1982). Disorders of calcium and phosphorus metabolism. *Veterinary Clinicis of North America Small Animal Practice*;12:411–38.

2.2. Differential diagnosis

Causes of hypercalcaemia in dogs can be divided in non-pathologic, transient or inconsequential and persistent pathological or consequential.

Non-pathologic conditions include samples from young growing dogs, non-fasting samples, laboratory error or spurious results (e.g. lipaemia or contamination with detergent).

Transient or inconsequential results can be a product of haemoconcentration, hyperproteinaemia, hypoadrenocorticism and rarely severe hypothermia (Schenck et al., 2012).

Pathologic or consequential can be subdivided into parathyroid dependent (i.e. primary hyperparathyroidism) or parathyroid independent. The last includes the following causes of hypercalcaemia in dogs: hypercalcaemia of malignancy (e.g. HHM, haematologic malignancies and metastasis of solid tumours to bone), CRF, hypervitaminosis D, granulomatous disease (e.g. blastomycosis, histoplasmosis, schistosomiasis), ARF, non-malignant skeletal lesions, excessive calcium containing intestinal phosphate binders, excessive calcium supplementation, hypervitaminosis A and raisin or grape toxicity (Feldman, 2014; Rosol & Capen, 1988; Schenck et al., 2012).

The most common causes of hypercalcaemia in dogs are hypercalcaemia of malignancy, particularly lymphosarcoma, hypoadrenocorticism, PHPTH and CKD (Feldman, 2015a).

In 109 dogs with ionised hypercalcaemia 58% had a neoplasia (78% of which had lymphosarcoma), 17% had renal failure (89% had CRF and 11% had ARF), 13% had PHPTH, 5% had hypoadrenocorticism, 4% had a non-malignant neoplasia or granulomatous disease and 3% had vitamin D toxicity (Messinger et al., 2009). Other studies also showed neoplasia with prevalence over 50%, with 58%, 86% and 67% respectively (Bienzle, Jacobs, & Lumsden, 1993; Elliott, Dobson, Dunn, Herrtage, & Jackson, 1991; Uehlinger, Glaus, Hauser, & Reusch, 1998), all with lymphosarcoma as the most frequent cause. On the other hand, other studies showed renal failure with lower prevalence and hypoadrenocorticism as the second most prevalent cause after neoplasia (Elliott et al., 1991; Uehlinger et al., 1998).

Hypercalcaemia of malignancy can develop through systemic humoral mechanisms (HHM) or through stimulation of local bone resorption induced by metastatic neoplasms. The local forms include solid tumours of multiples myeloma and haematologic tumours (Figure 7) (Rosol & Capen, 1992).

Figure 7 – Humoral and local forms of hypercalcaemia of malignancy caused by increased stimulation of osteoclastic bone resorption or increased tubular resorption of calcium (modified from Rosol and Capen, 1992).
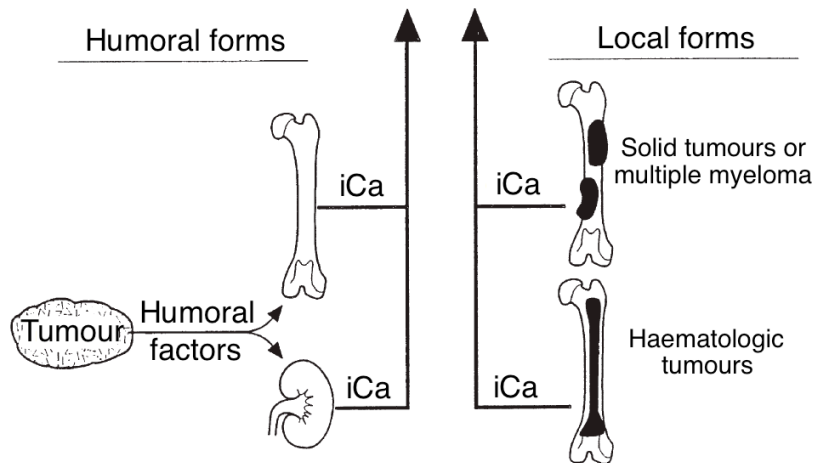
HHM is a syndrome that induces hypercalcaemia of malignancy through secretion of humoral factors acting distant from the site of the neoplasm. Metastasis of these tumours may eventually cause local bone resorption, but this is not the primary mechanism of hypercalcaemia (Rosol & Capen, 1992).

HHM is usually associated with hypercalcaemia, hypophosphataemia, hypercalciuria, hyperphosphaturia, increased cyclic adenosine monophosphate (cAMP) and increased osteoclastic bone resorption (Rosol & Capen, 1992; Schenck et al., 2012).

Humoral factors responsible for HHM include PTHrP, cytokines (e.g. IL-1, TNF-α, TGF-α and TGF-β) and calcitriol. These humoral factors can either act alone or have synergetic or additive action, but most can stimulate osteoclastic bone resorption (Figure 8) (Rosol & Capen, 1992; Schenck et al., 2012).

Hypercalcaemia in HHM develops as a result of increased osteoclastic bone resorption and increased renal calcium reabsorption. PTHrP is an important factor in HHM because it can activate PTH1 receptors in the bone and kidneys, mimicking PTH actions, causing both osteoclastic bone resorption and renal calcium reabsorption (Schenck et al., 2012). Calcitriol is not frequently associated with HHM, having low or normal concentrations in the majority of cases, but might also be increased and play a part in certain cases of HHM (Rosol et al., 1992).

Figure 8 - Humoral factors produced by tumours cause hypercalcaemia of malignancy by acting as systemic hormones which stimulate osteoclastic bone resorption of increase tubular reabsorption of calcium (modified from Rosol and Capen, 1992).

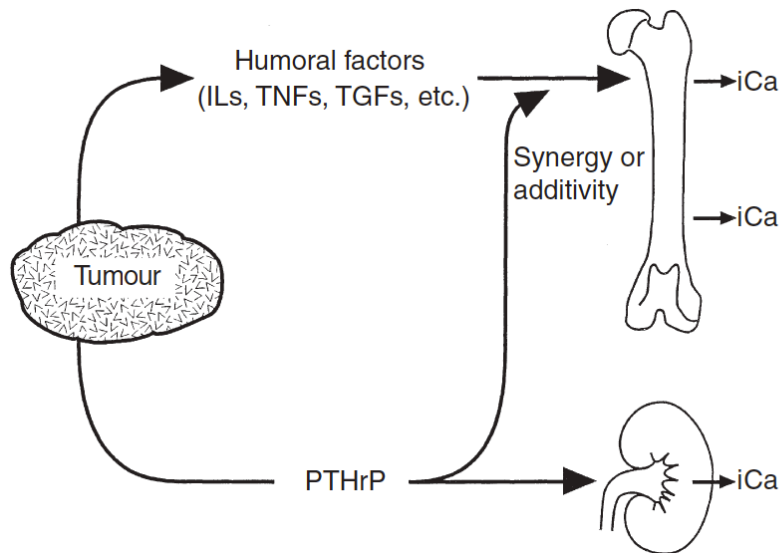Legend: IL – interleukin; TNF – tumour necrosis factor; TGF – transforming growth factor.

The most common malignancies associated with HHM are lymphoma and anal sac adenocarcinoma. Hypercalcaemia is present in lymphoma in 20 to 40% of dogs and in anal sac adenocarcinoma in 27 to 53% in recent reports (Bennett, DeNicola, Bonney, Glickman, & Knapp, 2002; Schenck et al., 2012; Williams et al., 2003). According to Weir (1988)[1] and Teske (1994)[2], dogs with lymphoma and hypercalcaemia seem to have T-cell lymphoma (Messinger et al., 2009).

More rarely, HHM has also been associated with thymoma, myeloma, melanoma and carcinomas (lung, pancreas, thyroid gland, mammary gland, nasal cavity, vaginal and testicular) (Feldman, 2014).

Haematologic malignancies may induce local bone resorption and consequent hypercalcaemia. Multiple myeloma and lymphoma are the most frequent haematologic malignancies, as they have a high incidence of bone metastasis (Rosol & Capen, 1992). According to Matus (1986)[3] 17% of dogs with multiple myeloma have hypercalcaemia (Schenck et al., 2012). Paracrine factors and cytokines (IL-1, TNF-α and TNF-β) are thought to be involved in the local bone resorption process but IL-6, TGF-α, TGF-β, PTHrP and prostaglandin E_2 may also play a role (Schenck et al., 2012).

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[1] Weir, E.C., Norrdin, R.W., Matus, R.E., et al. (1988). Humoral hypercalcemia of malignancy in canine lymphosarcoma. *Endocrinology*;122:602–608.
[2] Teske, E. (1999). Prognostic factors for malignant lymphoma in the dog: An update. Vet Q 1994;16(Suppl 1):29S–31S. 33. Kiupel M, Teske E, Bostock D. Prognostic factors for treated canine malignant lymphoma. *Veterinary Pathology*;36:292–300.
[3] Matus, R.E., Leifer, C.E., MacEwen, E.G., et al. (1986). Prognostic factors for multiple myeloma in the dog. *Journal American Veterinary Medicine Association*;188:1288–92.

Solid tumours that metastasise to bone in dogs are usually carcinomas of the mammary gland, prostate, liver and lungs, with the humerus, femur and vertebrae being the most often sites for metastasis. Local bone resorption in these tumours is thought to be also caused by IL-1, TNFs, TGF-α, TGF-β, PTHrP and prostaglandins. Usually bone involvement is restricted to the area of metastasis (Schenck et al., 2012). Primary bone tumours are rarely responsible for hypercalcaemia (Feldman, 2015a).

In the case of local bone resorption malignancies, it is not guaranteed that hypercalcaemia is not induced by production of humoral factors when HHM is concomitantly present (Rosol & Capen, 1992).

Hypoadrenocorticism can be associated with hypercalcaemia and according to Walser (1965)[1] increases in tCa may not be accompanied by increases of iCa, though recent studies have showed that iCa is frequently increased (Gow et al., 2009). The mechanism of hypercalcaemia in this disease is not fully understood but is though to be dependent on several factors, namely concentration of calcium binding proteins, increased avidity of binding proteins, reduction of renal clearance and mobilisation of calcium from the bone-blood surface (Peterson, Kintzer, & Kass, 1996). A study also concluded that hypercalcaemia in hypoadrenocorticism cases was rarely associated with increased PTH, PTHrP or calcitriol (Adler, Drobatz, & Hess, 2007; Gow et al., 2009).

In a study with 225 dogs with hypoadrenocorticism, 69 dogs (30.7%) had hypercalcaemia (Peterson et al., 1996), similarly to other reported prevalences of 28% and 36% (Haviland, Toaff-Rosenstein, Reeves, & Littman, 2016; Peterson & Feinman, 1982).

Because of the similarity of clinical signs, hypoadrenocorticism should always be a differential diagnosis of hypercalcaemia (Schenck et al., 2012).

CRF can cause hypercalcaemia that can be associated with normal or increased iCa. Hypercalcaemia with normal iCa concentrations can be a result of an increase in the complexed fraction of calcium or excessive bone resorption induced by high levels of PTH. Hypercalcaemia associated with increased iCa can be a result of a reduction in GFR and reduction of excretion of calcium. Should hyperplasia of the parathyroid glands develop, there will be excessive secretion of PTH non suppressible by higher iCa concentrations (Schenck et al., 2012).

In a study with 490 dogs with CRF, 22% had hypercalcaemia but only 9% had ionised hypercalcaemia, the only type of hypercalcaemia responsible for detrimental consequences (Schenck & Chew, 2005).

Vitamin D toxicosis is a consequence of excessive ingestion of cholecalciferol (vitamin D_3) or ergocalciferol (vitamin D_2). The toxicity can be a consequence of ingestion of creams, plants or from excessive dietary supplementation, but acute toxicosis is more frequently associated

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[1] Walser, M., Robinson, B.H.B., Duckett, J.W. (1963). The hypercalcemia of adrenal insufficiency. *Journal of Clinical Investigation*; 42:456-465

with ingestion of large amounts of cholecalciferol by ingestion of rodenticides. Hypercalcaemia and hyperphosphataemia result from increased bone resorption and gastrointestinal absorption of calcium and phosphorus (Feldman, 2015a).

With acute intoxications, clinical signs (anorexia, lethargy, polyuria, polydipsia, vomiting, diarrhoea, haematemesis, haematochezia) develop within 12 to 36 hours of ingestion and renal failure occurs within 24 to 48 hours (Groman, 2012). Rumbeiha and Murphy (2009) reported that a dose of cholecalciferol as low as 10mg/kg within a single ingestion can be lethal, despite the fact that the reported median lethal dose is 43 to 88mg/kg.

The physiopathology of PHPTH, consequence of increased PTH, has been previously developed in more detail.

It should be noted that the magnitude of ionised hypercalcaemia is not predictive of a particular condition (Messinger et al., 2009).

2.3. Diagnostic approach

Following a result with hypercalcaemia some authors defend that it is crucial to confirm if the hypercalcaemia is repeatable, particularly when increases are not substantial (Schenck et al., 2012; Schenck & Chew, 2012) whilst other authors claim results are rarely different, but rechecking is not wrong (Feldman, 2014, 2015a).

Measurement of iCa is a logical next step, to investigate the clinical relevance of the hypercalcaemia (Feldman, 2015a; Schenck & Chew, 2012). If iCa is normal or low, CRF is a differential diagnosis, but a second sample can be submitted to confirm an unexpected result (Feldman, 2015a).

It should be noted that the approach used for identifying hypercalcaemia is faulty because routinely tCa is measured and iCa measured only if tCa is increased. The problem with this approach is that there is an increased frequency of hypercalcaemia when iCa is used as a screening test compared to tCa (Schenck & Chew, 2005).

2.3.1. Signalment

Hypercalcaemia of malignancy can be present in dogs of any age, though some tumours affect predominantly a particular group. Lymphoma can affect dogs of any age and gender, but some breeds seem to be predisposed (Feldman, 2015a). Apocrine gland adenocarcinoma of the anal sac usually affects older dogs, with a reported age at presentation of 7 to 19 years and a median of 11 years, affecting dogs of almost every breed (Bennett et al., 2002).

Toxic exposure, granulomatous diseases and CRF can also occur in dogs of any age, though CRF has an increased prevalence in dogs over 5 to 6 years of age (Brown, 2007; Feldman, 2015a).

Hypoadrenocorticism is a disease usually affecting young to middle aged dogs, but can range from 2 months to 14 years of age. Standard Poodles and Bearded Collies are the most represented breeds but Nova Scotia Duck Tolling Retrievers, Leonbergers, Portuguese Water Spaniels, Great Danes, Rottweilers, Wheaten Terriers and West Highland White Terriers also seem to be predisposed (Church, 2012).

PHPTH is overrepresented in Keeshonds and usually affects dogs over 7 years old, with a mean age of 10 to 11 years (Feldman et al., 2005; Feldman, 2014).

2.3.2. History and physical examination

Information concerning possible exposure to excessive vitamin D should be obtained, particularly regarding diet, vitamin and mineral supplementation, exposure to rodenticides and houseplants (Feldman, 2015a; Schenck et al., 2012).

The presence of clinical signs such as polyuria, polydipsia, appetite, difficulty eating, activity, change in body condition, tolerance to exercise, pain, vomiting and diarrhoea should also be enquired (Feldman, 2014, 2015a).

Physical examination should be done carefully and explore possible physical signs that explain the hypercalcaemia: palpation of the peripheral lymph nodes for lymphoma, mammary chain for mammary cancer, kidneys (if possible) and spine, ribs and long bones for pain related to an osteolytic lesion; evaluation of the oral cavity for presence of "rubber jaw" and other signs of CRF and of the rectal and perineal area for signs of apocrine adenocarcinoma of the anal sac or another tumour; digital vaginal examination to evaluate the presence of vaginal tumour; presence of poor pulse and slow heart rate which is consistent with hypoadrenocorticism. It is also important to assess the hydration status and the severity of illness (Feldman, 2014, 2015a).

History may be enough to reach a presumable diagnosis, in the case of vitamin D toxicosis, or physical examination, should a mass or effusion be found (Schenck et al., 2012). The absence of relevant history and absence of clinical signs is also useful to increase suspicions of PHPTH, which is more unlikely the more ill the animal presents (Feldman, 2015a).

2.3.3. Clinical pathology

A CBC, serum biochemistry profile and urinalysis should be obtained.

A CBC with normocytic, normochromic and nonregenerative anaemia is commonly associated with CRF, hypoadrenocorticism and several neoplasias. If neutropaenia, anaemia or thrombocytopaenia is present bone marrow evaluation should be considered if a diagnosis

has not been obtained after clinical pathology and imaging tests (Feldman, 2014; Schenck et al., 2012)

The biochemistry profile should include BUN, creatinine, sodium, potassium, phosphate, total proteins and globulins (Feldman, 2014, 2015a).

Increases in BUN, creatinine and phosphate are consistent with renal failure and vitamin D toxicosis; hyperphosphataemia and normal BUN and creatinine are possibly associated with osteolysis secondary to metastasis; hyperkalaemia, hyponatraemia, hyperphosphataemia and a sodium-to-potassium ratio under 27:1 are suggestive of hypoadrenocorticism; hyperproteinaemia and hyperglobinaemia are consistent with myeloma; hypophosphataemia to normal phosphate concentrations are present in PHPTH and hypercalcaemia of malignancy (Feldman, 2014, 2015a).

A basal cortisol level should be measured to exclude the possibility of hypoadrenocorticism, but if hypoadrenocorticism is considered likely an adrenocorticotropic hormone (ACTH) stimulation test should be performed (Feldman, 2015a).

Concomitant presence of hypercalcaemia and azotaemia can be a diagnostic dilemma as hypercalcaemia can be either the cause or a consequence of renal disease. When CRF is the primary problem, phosphate concentrations are usually increased; iCa is normal to low, but was shown to be increased in 9% of dogs with CRF; nonregenerative anaemia and proteinuria are common; kidneys are small and irregular; tCa is usually below 12.5mg/dl. With PHPTH as the primary disease, iCa is increased; hypercalcaemia does not respond as well to aggressive fluid therapy and diuretics; tCa is usually above 13.0mg/dl (Feldman, 2015a; Schenck et al., 2012; Schenck & Chew, 2005).

Dogs with hypercalcaemia normally have diluted and an USG lower than 1.020 is common in dogs with renal disease, hypoadrenocorticism and PHPTH (Feldman, 2014, 2015a).

2.3.4. Imaging

Thoracic radiographs and abdominal radiographs or preferably abdominal US should be performed.

Thoracic radiographs are important to evaluate the cranial mediastinum for the presence of a mass suggestive of lymphoma but also to evaluate the perihilar area and lungs for neoplasia and systemic mycosis, presence of other soft tissue masses or calcification, osteolysis and osteoporosis and microcardia (suggestive of hypoadrenocorticism). If a mass is identified, a fine needle aspirate (FNA) or biopsy should be obtained for cytology or histology if possible. Osteolysis can suggest myeloma or osteolysis secondary to metastasis and osteoporosis, though difficult to identify on plain radiographs, can suggest PHPTH and hypercalcaemia of malignancy (Feldman, 2014, 2015a).

In abdominal US, the liver, spleen and mesenteric and sublumbar lymph nodes should be evaluated and if abnormalities are detected, FNAs or biopsies should be taken. The most

common malignancy found is lymphoma but other neoplasias may be present. The kidneys should also be evaluated, but cases with CRF should have been detected with the previous biochemistry profile (Feldman, 2014, 2015a).

Soft tissue calcification may be present in any hypercalcaemic dog with a calcium phosphorus product over 60 to 80, but is more frequent in CRF and vitamin D toxicosis. Uroliths are also a possibility in any dog with hypercalcaemia and are very common in dogs with PHPTH (Feldman et al., 2005; Feldman, 2010).

Adenocarcinoma of the anal sac may present as a mass in the pelvic canal in abdominal radiographs (Feldman, 2015a).

Radiographs of painful bones should be performed to search for osteolysis and when local lesions are present samples should be obtained to confirm if those lesions can be the cause of hypercalcaemia (Schenck et al., 2012).

Absence of abnormalities on thoracic and abdominal imaging other than the presence of uroliths is consistent with PHPTH (Feldman et al., 2005).

Cervical US can also be useful in determining if hypercalcaemia is parathyroid-dependent or parathyroid-independent. The presence of a solitary parathyroid mass in a dog without CKD is very suggestive of PHPTH (Feldman, 2015a; Schenck et al., 2012).

2.3.5. Assays

The following step is to measure calcium-regulating hormones. The objective is to determine if hypercalcaemia is dependent on the parathyroid glands or not. Increased PTH suggests that the disease is parathyroid-dependent, while decreased PTH suggests there is an independent cause for hypercalcaemia that is suppressing the parathyroid glands (Nelson, 2009; Torrance & Nachreiner, 1989b).

PTHrP is often increased in hypercalcaemia of malignancy and undetectable in PHPTH, but requires a careful evaluation of results because PTHrP is not always increased in dogs with hypercalcaemia of malignancy (Feldman, 2015a; Schenck et al., 2012).

Measurement of vitamin D analogues (25-hydroxyvitamin D and 1,25-dihydroxyvitamin D) can be relevant to determine if there was ingestion of cholecalciferol, ergocalciferol or excessive supplementation calcitriol (Schenck et al., 2012).

If after all these procedures a diagnosis has not been obtained, bone marrow and lymph node aspirates for cytological examination can be useful. Bone marrow aspirates are not recommended in dogs with normal CBC and normal physical examination (Feldman, 2010, 2015a).

It is important to always maintain lymphoma as a differential diagnosis for hypercalcaemia until definitive diagnosis is reached (Feldman, 2014).

2.4. Acute treatment

The magnitude and rate of hypercalcaemia affect the clinical signs and urgency of therapy. There are no universal guidelines and reference ranges are very dependent on the laboratory, but a general suggestion of when to approach hypercalcaemia with acute therapy may be the following: asymptomatic dogs or with mild clinical signs (usually tCa under 12mg/dl) usually do not require immediate therapy; tCa between 12 and 14mg/dl may be well tolerated if hypercalcaemia developed chronically and not require immediate treatment but in the case of acute hypercalcaemia treatment may be required; tCa between 14 and 15mg/dl requires treatment independently of the presence of clinical signs; and tCa over 15mg/dl demands an aggressive treatment (Groman, 2012).

Besides the magnitude and rate of development of hypercalcaemia, the treatment plan should also consider whether calcium concentrations are stable or increasing and if acid-base and other electrolyte disturbances are present. History, physical examination, presence of neurologic, cardiac, renal dysfunction or uroliths increase the urgency and need for aggressive treatment (Schenck et al., 2012).

The definitive treatment for hypercalcaemia is achieved by treating the cause, but this is not always possible and an acute management is necessary before the underlying cause can be addressed (Schenck & Chew, 2012). Acute treatment reduces hypercalcaemia while a definitive diagnosis is investigated or until permanent treatment resolves hypercalcaemia permanently or a chronic management of hypercalcaemia can reduce calcium levels (Schenck et al., 2012).

The goal of acute treatment of hypercalcaemia is to alleviate clinical signs, prevent soft tissue mineralisation and minimise renal damage by inhibiting bone resorption, increasing urinary calcium excretion or decreasing intestinal calcium absorption (Groman, 2012). Treatment, with its doses and indications are summarised in Table 3.

Parental fluid therapy is the most important treatment for hypercalcaemia because it corrects dehydration, which is important because haemoconcentration increases iCa concentration, and after rehydration diuresis is induced, leading to renal excretion of calcium. Fluid therapy alone may not restore normocalcaemia but is essential in the initial approach. Fluid therapy should be used carefully in the presence of congestive heart failure and hypertension (Feldman, 2015a; Groman, 2012).

Dehydration should be corrected within 4 to 6 hours with IV fluid therapy and then continued to induce volume expansion with rates between 100 and 125ml/kg/day (Schenck et al., 2012). Saline (0.9% sodium chloride) is the preferred fluid because it adequately corrects dehydration, increases GFR, does not contain calcium (unlike Ringer's Lactate or Hartmann's) and has a higher concentration of sodium, which increases calcium renal

excretion by competing with calcium for renal reabsorption (Schenck et al., 2012; Vasilopulos & Mackin, 2003).

Administration of diuretics is the second most important measure in acute hypercalcaemia. After rehydration, diuretics, namely furosemide (2-4mg/kg BID to TID (thrice daily) IV, SC or PO (oral administration)), can be used to increase calciuresis. Not all diuretics induce calciuresis and particularly thiazides should be avoided as they can aggravate hypercalcaemia (Feldman, 2015a; Schenck et al., 2012).

Glucocorticoids should be used in dogs where hypercalcaemia persists after fluid therapy and diuretics. This therapy is effective against hypercalcaemia from lymphoma, apocrine adenocarcinoma of the anal sac, multiple myeloma, thymoma, hypoadrenocorticism, hypervitaminosis D, hypervitaminosis A or granulomatous disease, but has minimal action with other causes. Glucocorticoids reduce hypercalcaemia by inducing cytolisis dogs with lymphosarcoma, reduction of bone resorption, decrease of intestinal absorption of calcium and increase of calcium renal excretion (Schenck & Chew, 2012). The glucocorticoids used are prednisone in a dose of 1 to 2.2mg/kg every 12 hours PO, SC or IV or dexamethasone in a dose of 0.1 to 0.22mg/kg every 12 hours SC or IV (Feldman, 2015a; Schenck et al., 2012). Glucocorticoid therapy should be withheld if possible until a definitive diagnosis has been obtained, because the consequent cytotoxicity can make a definitive diagnosis of lymphoma through histopathology difficult or impossible (Schenck et al., 2012).

Calcitonin should be considered in dogs instead of glucocorticoids before a definitive diagnosis is obtained and is indicated in dogs with hypervitaminosis D. Reduction of hypercalcaemia occurs from inhibition of bone resorption and decrease of renal calcium reabsorption. The recommended dose of calcitonin is between 4 and 6IU/kg SC BID to TID (Feldman, 2015a; Schenck et al., 2012). Calcitonin has a rapid onset of action but only lasting a few hours and resistance frequently develops after a few days (Groman, 2012; Schenck et al., 2012).

Bisphosphonates are used for a more chronic control of hypercalcaemia, acting by reduction of number and action of osteoclasts. Several bisphosphonates are available, but usually pamidronate is the most frequently used because it is well tolerated, is more potent, more reliable, has an earlier action onset and longer lasting and only requires an IV administration every 1 to 3 weeks. The expensive price is a disadvantage of bisphosphonates (Feldman, 2015a; Hostutler et al., 2005; Schenck & Chew, 2012). The recommended dose of pamidronate is 1.3mg/kg diluted in 150ml of 0.9% saline infused in 2 hours, which can be repeated after a week. Bisphosphonates should only be started after the correction of dehydration to reduce risk of renal damage (Schenck et al., 2012).

Sodium bicarbonate can be used in dogs with severe hypercalcaemia and metabolic acidosis. Bicarbonate is an alkalinising agent that reduces iCa by increasing the bound fraction of calcium. A dose of 1mEq/kg slow IV bolus (up to a maximum of 4mEq/Kg of total dose) is recommended, with the action lasting between 120 to 180 minutes. Sodium bicarbonate can only be given to effect for a short period of time because alkalinisation with concomitant hypercalcaemia can promote tissue mineralisation. Acid-base status should be closely monitored (Schenck et al., 2012; Vasilopulos & Mackin, 2003).

Alternative therapy includes mithramycin, EDTA, haemodialysis or peritoneal dialysis. Mithramycin can reduce hypercalcaemia but has limited use in dogs because of consequent nephrotoxicity, hepatotoxicity and thrombocytopaenia. The currently recommended dose is 25µm/kg IV in 5% dextrose given over 2 to 4 hours every 2 to 4 weeks. EDTA in doses of 25 to 27mg/kg/hour can reduce hypercalcaemia by chelating circulating calcium and the complexes are then excreted by the kidneys. EDTA should be reserved for crisis because of its nephrotoxicity. Haemodialysis and peritoneal dialysis are useful when other approaches fail and in case of intrinsic renal failure caused by hypercalcaemia, but there is limited information of its use in dogs (Schenck et al., 2012; Vasilopulos & Mackin, 2003).

In the future calcium receptor agonists, calcium channel blockers, somatostatin congeners and nonhypercalcaemic analogues of calcitriol may gain relevance in the treatment of acute hypercalcaemia (Schenck et al., 2012; Vasilopulos & Mackin, 2003).

Reductions in iCa concentrations improve clinical signs, even if normal calcium concentrations are not restored (Schenck et al., 2012).

To monitor treatment and the calcium status, iCa is better than tCa as it is the active fraction and when iCa is not available tCa should be used with caution because of the possible discordance between the two (Groman, 2012).

Table 4 – Specific treatment of hypercalcaemia (adapted from Schenck et al., 2012)

Treatment	Dose	Indications	Comments
Volume expansion			
Subcutaneous saline (0.9%)	75-100 ml/kg/day	mild hypercalcaemia	contraindicated if peripheral oedema is present
Intravenous saline (0.9%)	100-125 ml/kg/day	moderate to severe hypercalcaemia	contraindicated if CHF and hypertension; minimal decreases as single therapy when caused by severe pathologic hypercalcaemia
Diuretics			
Furosemide	2-4 mg/kg BID to TID IV, SC, PO	moderate to severe hypercalcaemia	volume expansion is necessary before usage; rapid onset of action
Alkalinising agent			
Sodium bicarbonate	1 mEq/kg IV slow bolus; up to 4mEq/kg total dose	severe hypercalcaemia	requires close monitoring; rapid onset of action
Glucocorticoids			
Prednisolone	1-2.2 mg/kg BID PO, SC, IV	moderate to severe hypercalcaemia	use of these drugs before identification of cause may make definitive diagnosis difficult or impossible
Dexamethasone	0.1-0.22 mg/kg BID IV, SC	same	
Bone resorption inhibitors			
Calcitonin	4-6 IU/kg SC BID to TID	Hypervitaminosis D	response may be short-lived; vomiting may occur; rapid onset of action
Bisphosphonates:			
- EHDP-didronel	15 mg/kg SID to BID	moderate to severe hypercalcaemia	delayed onset of action
- Clodronate	20-25 mg/kg in 4hr IV infusion	same	approved for humans in Europe
- Pamidronate	1.3 mg/kg in 150 ml 0.9% saline in 2h IV infusion, can repeat in one week	same	very expensive
Mithramycin	25µg/kg IV in 5% dextrose over 2-4h every 2-4 weeks	severe hypercalcaemia, refractory HHM	limited use in dogs; nephrotoxicity, hepatotoxicity, thrombocytopaenia
Miscellaneous			
Sodium EDTA	25-75 mg/kg/h	severe hypercalcaemia	nephrotoxicity
Peritoneal dialysis	low calcium dialysate	severe hypercalcaemia	short duration of response; use in hypercalcaemia not reported

Legend: IV, intravenous; SC, subcutaneous; PO, oral; SID, once daily; BID, twice daily; TID, thrice daily; HHM, humoral hypercalcaemia of malignancy; CHF – congestive heart failure.

Note 1: when administrating saline (0.9%), potassium supplementation is necessary (add 5-40 mEq KCl/l depending on serum potassium concentration).

2.5. Prognosis

The prognosis is very dependent on the cause of hypercalcaemia.

As previously mentioned, prognosis for PHPTH is excellent for treated dogs (Caplan, 2013; Feldman, 2015a; Flanders, 2003; Nelson, 2009; Rasor et al., 2007; Séguin & Brownlee, 2012).

Prognosis for CRF depends on the rate of progression, being poorer in animals with extensive endstage lesions, advanced osteodystrophy, progressive proteinuria, progressive loss of lean muscle mass and body weight, severe intractable anaemia, unmanageable systemic hypertension, progressive azotaemia and inability to maintain fluid and electrolyte balance despite treatment (Chew et al., 2011).

Hypoadrenocorticism is a treatable disease with excellent prognosis in dogs when life-long treatment is continued (Church, 2012).

Dogs with hypercalcaemia of malignancy have short survival times (Schenck et al., 2012). T-cell lymphoma, the type associated with hypercalcaemia, has a worse prognosis than B-cell lymphoma. The survival time in an aggressive disease has a median survival of 159 days while low-grade lymphoma as a median survival of 634 days (Avery et al., 2014; Seelig et al., 2014; Zandvliet, 2016).

In a study with 133 dogs (Williams et al., 2003) with adenocarcinoma of the anal sac the median survival for treated dogs was 544 days, with the best results in dogs that underwent surgical and adjuvant treatment. There was a high rate of metastasis reported. Dogs with pulmonary metastasis had lower survival times. A study by Bennett et al. (2002) had a significantly lower median survival time of 6 months and 79% of dogs had metastasis at the time of diagnosis.

Prognosis for vitamin D intoxication can be good in dogs that are asymptomatic and are decontaminated immediately and for dogs where hypercalcaemia is resolved before soft tissue mineralisation occurs. Prognosis for dogs with soft tissue mineralisation is less favourable, as soft tissue mineralisation is usually irreversible and can cause renal, gastrointestinal and cardiac damage. The rate and magnitude of development of hypercalcaemia and hyperphosphataemia will determine renal functional loss (Groman, 2012). The death rate in dogs with vitamin D toxicosis from ingestion of rodenticides is high, because of the rapid development and animals only presenting after significant renal damage (Rumbeiha & Murphy, 2009).

3. Retrospective study: Clinical approach to hypercalcaemia in 6 dogs with PHPTH

3.1. Objective

The objectives of the present study ate the broadening the knowledge of PHPTH in dogs and on the clinical approach to hypercalcaemia.

The proposed goals of this study are:

- the characterisation of 6 dogs diagnosed with PHPTH, after identification of hypercalcaemia.

- the analysis of the procedures and tests performed in the clinical approach

3.2. Material and methods

This study consists of a retrospective analysis of a sample of dogs clinically diagnosed with PHPTH in Anderson Moores Veterinary Specialists between the 14th of May 2015 and the 9th of September 2015.

This study analyses a sample of 6 dogs where hypercalcaemia was identified, specifically how the definitive clinical diagnosis of PHPTH was reached.

As the sample consists of a small number of individuals and any data would have limited interpretation value, no statistical analysis was performed in this study.

The cases were researched based on their presenting complain of hypercalcaemia and/or polyuria and polydipsia. Data was obtained from their clinical history and laboratory analysis from the easyVET software used at the practice.

This study included all dogs that presented between the fore mentioned timeframe with hypercalcaemia and were eventually diagnosed with PHPTH based on PTH measurements.

3.3. Results

3.3.1. Signalment

The six dogs had an age at diagnosis from 8 and 12 years of age, with a mean of 10.56 years. The sample was composed by five males and one female, all of which were neutered. The represented breeds were Basset Hound, Golden Retriever, German Shepherd cross, Shih-Tzu, Staffordshire Bull Terrier and Toy Poodle.

3.3.2. Previous history

Previous clinical history included recurrent anal sac impaction, cough, epilepsy, raisins and chocolate ingestion, pyoderma, recurrent otitis externa and periodontal disease. One of the cases had no information regarding previous history and another had recently been diagnosed with hyperadrenocorticism. The cases with epilepsy and hyperadrenocorticism were medicated with Imepitoin 100mg BID and Trilostane 30mg SID (once daily), respectively.

3.3.3. Clinical signs

The clinical signs at presentation were the following: polyuria and polydipsia, present in all of the cases, change in behaviour, hind limb weakness, weight loss, polyphagia, weight gain, exercise intolerance, each present in only one case. In the case with concomitant HAC, polyuria and polydipsia were still reported after levels of cortisol were controlled by treatment with Trilostane.

3.3.4. Physical examination

Physical examinations were unremarkable with the exception of the following findings: abdominal enlargement, tail base alopecia, tartar, grade II/VI left sided systolic cardiac murmur, hepatomegaly, alopecia and ulceration in a stifle.

3.3.5. Clinical pathology

The biochemical analysis where hypercalcaemia was identified and prompted the investigation for the cause were: investigation of polyuria and polydipsia and other reported clinical signs, monitoring resolution of raisin toxicity and routine preanaesthetic screening prior to a dental extraction procedure.

tCa and iCa concentrations were measured in the six cases prior to treatment (Table 5 and Table 6) and hypercalcaemia and ionised hypercalcaemia were present in all of the cases

with the exception of the second measurement of tCa and iCa in case 3. Note that these two measurements were not performed at the same time, and in this case the second measurement of tCa was done at the same time as the third measurement of iCa.

Table 5 – Successive concentrations of serum tCa measured in the six cases, before treatment.

	tCa		**Reference range**
Case 1	3.64 mmol/l	-	2.36-2.84 mmol/l
Case 2	3.26 mmol/l	3.54 mmol/l	2.36-2.84 mmol/l
Case 3	3.24 mmol/l	2.80 mmol/l	2.20-3.00 mmol/l
Case 4	4.07 mmol/l	3.87 mmol/l	2.20-3.00 mmol/l
Case 5	3.66 mmol/l	-	2.20-3.00 mmol/l
Case 6	3.74 mmol/l	3.29 mmol/l	2.20-3.00 mmol/l

Table 6 – Successive concentrations of serum iCa measured in the six cases, before treatment.

	iCa			**Reference range**
Case 1	1.73 mmol/l	-	-	1.12-1.4 mmol/l
Case 2	1.40 moml/l	-	-	1.12-1.4 mmol/l
Case 3	1.61 mmol/l	1.34 mmol/l	1.56 mmol/l	1.12-1.4 mmol/l
Case 4	2.07 mmol/l	1.74 mmol/l	-	1.12-1.4 mmol/l
Case 5	1.93 mmol/l	1.79 mmol/l	-	1.12-1.4 mmol/l
Case 6	1.69 mmol/l	-	-	1.12-1.4 mmol/l

Phosphate was measured in all six cases (Table 7) and though hypophosphataemia was only present in two cases, the remaining had phosphate concentrations on the lower end of the reference range, with the exception of the first measurement in case 3.

Table 7 – Successive concentrations of serum phosphate measured in the six cases, before treatment.

	Phosphate		**Reference range**
Case 1	0.41 mmol/l	-	0.80-1.60 mmol/l
Case 2	1.06 mmol/l	1.09 mmol/l	0.80-1.60 mmol/l
Case 3	1.33 mmol/l	0.80 mmol/l	0.80-1.60 mmol/l
Case 4	1.02 mmol/l	0.70 mmol/l	0.80-1.60 mmol/l
Case 5	1.05 mmol/l	-	0.81-2.00 mmol/l
Case 6	0.91 mmol/l	1.10 mmol/l	0.80-1.60 mmol/l

PTH concentrations were increased in five of the cases and within normal limits in one case. As normal concentrations of PTH are considered inappropriate in the presence of ionised hypercalcaemia, this result is also considered abnormal (Table 8).

Table 8 – Successive PTH serum concentrations measured in the six cases, before treatment.

	PTH		Reference range
Case 1	246.0 pg/ml	-	20.0-65.0 pg/ml
Case 2	212.0 pg/ml	-	20.0-65.0 pg/ml
Case 3	46.0 pg/ml	26.0 pg/ml	20.0-65.0 pg/ml
Case 4	325.0 pg/ml	-	20.0-65.0 pg/ml
Case 5	101.0 pg/ml	-	20.0-65.0 pg/ml
Case 6	132.0 pg/ml	385.0 pg/ml	20.0-65.0 pg/ml

PTHrP concentrations were measured in five of the cases. PTHrP concentrations were undetectable in four cases (< 0.1 pmol/l) and detectable in only one case, but still below the reference range (Table 9).

Table 9 – Successive PTHrP serum concentrations measured in five of the cases.

	PTHrP	Reference range
Case 1	0.1 pmol/l	< 0.5 pmol/l
Case 2	< 0.1 pmol/l	< 0.5 pmol/l
Case 3	< 0.1 pmol/l	< 0.5 pmol/l
Case 5	< 0.1 pmol/l	< 0.5 pmol/l
Case 6	< 0.1 pmol/l	< 0.5 pmol/l

Urea and creatinine concentrations were within normal limits in all of the cases (Table 10).

Table 10 – Urea and creatinine serum concentrations in the six cases.

	Urea	Reference range	Creatinine	Reference range
Case 1	6.8 mmol/l	3.1-10.1 mmol/l	107.0 µmol/l	20.0-144.5 µmol/l
Case 2	5.3 mmol/l	3.1-10.1 mmol/l	63.0 µmol/l	20.0-144.5 µmol/l
Case 3	4.71 mmol/l	1.70-7.40 mmol/l	74 µmol/l	20.0-124.0 µmol/l
Case 4	7.3 mmol/l	1.70-7.40 mmol/l	73 µmol/l	20.0-124.0 µmol/l
Case 5	6.0 mmol/l	2.5-9.0 mmol/l	103 µmol/l	44.0-159.0 µmol/l
Case 6	5.8 mmol/l	1.70-7.40 mmol/l	91 µmol/l	20.0-124.0 µmol/l

Vitamin D metabolites were only measured in one case and the results were within normal limits (Table 11).

Table 11 – Vitamin D metabolites measured in one case.

	Result	Reference range
Vitamin D	52.2 nmol/l	25-150 nmol/l
Calcitriol	54.6 pmol/l	40-150 pmol/l

Urinalysis was performed in three cases, all with no active sediment. Urine culture was only performed in one case (which also had urinalysis), with no growth detected.

USG was measured in five of the cases, with densities ranging from 1.010 to 1.018 (Table 12).

Table 12 – Successive USG in five of the cases, before treatment.

	USG		
Case 2	1.010	-	-
Case 3	1.014	1.015	1.018
Case 4	1.012	-	-
Case 5	1.014	-	-
Case 6	1.014	-	-

3.3.6. Imaging

Imaging exams were performed in five of the cases. Thoracic radiographs were taken in four cases, all of which were within normal limits. Full abdominal US exams were performed in three cases and US exam of the bladder was performed in two cases. On abdominal and bladder US the following relevant findings were present: slightly hyperechoic parenchyma and an 8mm in diameter nodule, whose samples collected by FNA were consistent with vacuolar hepatopathy and nodular hyperplasia; a 5cm solid splenic mass, whose FNA samples were consistent with lymphoid hyperplasia and extra-medullary haematopoiesis; bilateral nephropathy and nephrolithiasis; two cases with small calculus in the bladder, one measuring up to 3mm and the other measuring up to 4mm; two cases with sediment in the bladder. Only one case that had abdominal US had no uroliths present.

Cervical US exams to assess the parathyroid glands were performed in five cases, with a suspected parathyroid nodule being identified in all of the cases (Table 13). In one case a second lesion in the ipsilateral thyroid gland was identified on US.

Table 13 – Parathyroid nodule diameter measured on cervical US.

	Nodule diameter
Case 2	3.7 mm
Case 3	4.4 mm
Case 4	3.5 mm
Case 5	7.0 mm
Case 6	3.4 mm

3.3.7. Treatment

Surgery (e.g. parathyroidectomy) was performed on three of the cases. The surgery was done with the patient on dorsal recumbency, with a sandbag under the neck and with the thoracic limbs positioned caudally along the chest. The ventral neck was clipped and aseptically prepared.

In one case, after the exposure of right thyroid gland an enlarged parathyroid gland was identified, but a second lesion identified on US was not visible in surgery. So, to assure complete removal of the lesions, a complete right thyroidectomy was performed. A mass of 5mm in diameter was identified 8cm caudally to the left thyroid gland and was also removed.

In another case, both thyroid glands presented smooth with only a nodule palpated on the left cranial thyroid gland, presumably the left cranial parathyroid gland. The gland was removed with a narrow margin of thyroid tissue.

In the last case where surgery was performed, the affected parathyroid gland was difficult to discern from the thyroid gland, so part of the right thyroid was also removed. The left parathyroid and thyroid glands were within normal limits. After surgery, however, iCa did not return to normal limits and histopathology results revealed that the removed tissue was a reactive lymph node and no thyroid or parathyroid tissue was present. After repeating cervical US, which revealed unchanged appearance of the thyroid and parathyroid glands, a revision surgery was performed. A 7mm nodule was identified, but due to strong attachments to thyroid tissue and the presence of a presumed altered internal parathyroid gland, complete extracapsular right thyroidectomy was performed.

3.3.8. Posttreatment management

Postoperatively iCa and tCa returned to normal limits. As previously mentioned, in the last case the affected parathyroid gland was not removed in the first surgery and therefore only the iCa and tCa results after the second and curative surgery will be considered. On one case after an iCa measurement of 1.2 mmol/l on the day after surgery the iCa decreased to below the reference range (1.04 and 1.02 mmol/l) and dehydrotachysterol (vitamin D analogue) therapy was started at 0.01mg/kg BID, the patient showed no clinical signs of hypocalcaemia and the following monitoring of iCa were within reference range.

The second case had iCa within normal limits and was discharged with a palatable calcium-phosphorous-vitamin D preparation one tablet BID. The last case had iCa concentrations between 1.09 and 1.4 mmol/l, without clinical signs of hypocalcaemia and was discharged with no medication, but was recommended dehydrotachysterol therapy if iCa decreased to below 0.8mmol/l.

The following monitoring of iCa and tCa were within normal limits in all three cases and dehydrotachysterol therapy was tapered off in the medicated case.

3.3.9. Histopathology

All three cases had histological diagnosis of the removed tissue. Two cases had results compatible with parathyroid carcinoma and the other case had a parathyroid adenoma. The case that also had a mass identified caudally to contralateral thyroid gland was one of the two with diagnosis of carcinoma on both lesions on the ipsilateral thyroid gland and also on the contralateral mass.

3.4. Discussion

The cases previously described have a mean age at diagnosis of 10.56 years, very similar to the mean 10.7 years from Feldman (2014), even though this was quite a small sample. The male population seemed to be overrepresented, but large series show no gender predisposition (Feldman et al., 2005).

One of the analysed cases had concomitant HAC. Adrenal tumours have been reported in cases of MEN and HAC with concomitant PHPTH have also been previously reported (Feldman, 2015a; Gear et al., 2005; Kiupel et al., 2000; Thuróczy et al., 1998; Walker et al., 2000; Wright et al., 1995).

All of the owners, except for one, reported the presence of polyuria and polydipsia at some point before treatment. In this case polyuria and polydipsia were only noticed after the owner started monitoring the amount of water drank, after being advised to do so. In another case polyuria and polydipsia were described, but the owner reported resolution even before the investigations for hypercalcaemia were conducted. These could be a cases where the owners miss the detection of signs, as it commonly occurs with PHPTH (Feldman et al., 2005), because both had USG were performed and the results (between 1.012 and 1.018) were lower than the expected for a normal dog.

Clinical signs reported, other than polyuria and polydipsia, included change in behaviour, hind limb weakness and weight loss. Weight gain, polyphagia and exercise intolerance were present in the dog diagnosed with HAC. The first three clinical signs are all commonly reported in dogs with PHPTH, whilst the other three are not, but could be caused by the concomitant HAC in this case (Feldman et al., 2005; Rasor et al., 2007).

The physical examinations were unremarkable, as it is expected with this disease (Feldman et al., 2005; Feldman, 2014), with only unrelated problems detected. Abdominal enlargement and tail base alopecia were present in the patient with concomitant HAC, alopecia and ulceration in a stifle were present in the patient with previous history of pyoderma and hepatomegaly was present in the patient that was diagnosed with vacuolar hepatopathy and nodular hyperplasia. The presence of findings on physical examination could be explained by the advanced age of the patients, which makes the presence of concomitant problems more likely.

Hypercalcaemia was detected, in two of the cases, on a biochemical analysis performed for reasons unrelated to clinical signs of PHPTH. This often occurs in this disease, where owners commonly do not perceive the clinical signs and hypercalcaemia is detected on a serendipitous exam, frequently routine screening prior to surgery (Feldman et al., 2005).

In all of the cases the initial suspicion for PHPTH arose after the detection of increased tCa concentration, which as previously mentioned, is not the ideal method (Schenck & Chew, 2005, 2008). In one case the measurements of iCa revealed an elevated iCa concentration

with a concomitant normal tCa concentration. This is a good example of the importance o the measurement of iCa, as this is the only active fraction of calcium in the body and does not always correlate to tCa (Schenck & Chew, 2005, 2008).

Hypercalcaemia and ionised hypercalcaemia were present in all cases, as expected. The tCa concentrations ranged from 3.24 to 4.07mmol/l, with the exception of the previously mentioned normal tCa concentration of 2.8mmol/l. The iCa concentrations ranged from 1.4 to 2.07mmol/l. It must be noted that reference ranges vary between laboratories and not all these results were obtained from the same one.

The iCa fraction is dependent on serum pH, with increases in pH resulting in lower concentrations of iCa. In samples collected and processed aerobically, loss of CO_2 causes increases in pH and consequently causes an artifactual lower measured iCa concentration measured (Schenck et al., 2012). The physiologic serum pH in a dog is between 7.35 and 7.46 (Flaherty & Blackwood, 2007). So, when a more alkaline pH value is present, it can mean that the real iCa concentration is slightly higher than the measured value. Though the result was not always made available in all iCa measurements, some blood gas analysis cartridges also provide pH of the sample. It is possible that the case with the iCa measurement at the top of the reference range (1.4mmol/l) was considered as hypercalcaemia because of the pH of the sample was more alkaline and iCa was therefore falsely underestimated.

The magnitude of hypercalcaemia does not help differentiating the causes of hypercalcaemia, but is relevant to determine whether acute treatment is necessary or not (Feldman, 2015b; Messinger et al., 2009; Schenck et al., 2012).

Only two cases had phosphate concentration below the reference range, but the remaining had phosphate concentrations on the lower end of the reference range. The only exception was the first phosphate measurement in case 3 where phosphate was in well in within reference range, but there is no information of the level of iCa at the time, which could be normal despite elevation of tCa concentration.

PTH concentrations were measured in all of the cases, ranging from 26.0 to 385.0 pg/ml and were above the reference range in five of the cases. Only one case had a normal PTH concentration, which in the presence of increased iCa is also considered inappropriate (Feldman et al., 2005; Feldman, 2014). There is a higher proportion of cases where inappropriate concentrations of PTH were above the reference range compared to the data from Feldman et al. (2005). In this study only 23% of cases had PTH concentrations above the reference range, but it must be noted that there was a significantly wider reference range (18.2-118.3 pg/ml)[1] in comparison to the reference range used by these laboratories (20-65 pg/ml) and there is no information available at to which PTH analysis was used.

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[1] Conversion from pmol/l: 1 mmol/l of PTH = 9.1 mg/dl of calcium (in Canine & Feline Endocrinology, 2015, 4th edition)

PTHrP was measured in all but one of the cases. PTHrP concentration was undetectable (<0.1pmol/l) in four of the five cases where it was measured and of 0.1pmol/l in the remaining, which is still within normal limits (<0.5pmol/l), as expected in dogs with PHPTH.

Urea and creatinine concentrations were within reference range in all cases, as it would be expected in PHPTH. In the study by Feldman et al. (2005) the results for urea and creatinine were significantly lower than the control group and increased renal parameters were rare in over 300 dogs diagnosed with PHPTH (Arbaugh et al., 2012; Feldman et al., 2005; Ham et al., 2009; Milovancev & Schmiedt, 2013; Sawyer et al., 2011). This led to the hypothesis that PHPTH is not associated renal failure and may actually reduce the risk of its development.

PHPTH was diagnosed in all of the cases by inappropriate levels of PTH in the presence of ionised hypercalcaemia, with normal renal parameters (urea and creatinine). PTH must be measured at the same time as increased iCa level is present for the diagnosis to be confirmed (Feldman, 2015a; Nelson, 2009). In two cases, PTH was not measured at the same time as iCa, but as persistent hypercalcaemia was present, and suspected parathyroid nodules were identified in US, PHPTH was very likely and it was deemed unnecessary to repeat PTH measurements. The case with two PTH measurements was approached differently and PTH was measured again, as it had not been measured at the same time as iCa and because a nodule smaller than expected for a adenoma or adenocarcinoma was identified on US.

Vitamin D metabolites were only measured in the case that after identification of hypercalcaemia, returned to normal iCa values and had no parathyroid nodule evident on the first US exam. The other cases were not as suspicious for hypervitaminosis D because previous phosphate concentrations were on the lower end of the reference range, unlike the first measurement on this case, which was well within reference range. The second measurement of phosphate, however, made this differential diagnosis unlikely.

Urine culture was only performed in one case, with no growth detected and urinalysis was only performed on three cases. Ideally urinalysis and uroculture would have been performed on all patients, as UTI affects a large percentage of dogs with PHPTH and may be a cause of discomfort to the patient (Feldman et al., 2005).

USG was measured in five of the cases and densities ranged from 1.010 to 1.018, lower than expected for a normal dog. The USG in a study with 210 dogs showed that the majority of cases (69%) had concentrations between 1.008 and 1.020 (Feldman et al., 2005).

US exam of the bladder or urinary tract was only performed on five of the cases: three had a calculus present in the bladder, urethra and/or kidneys, one had sediment in the bladder and only one had no relevant findings. This last one also had no crystals identified in the urinalysis. US of the urinary tract is important because of the high prevalence of calculi in

dogs with PHPTH and these, along with UTI are reasons to recommend treatment (Feldman et al., 2005; Feldman, 2014; Guttin, Knox IV, & Diroff, 2015).

Only one of the six cases had no imaging performed. Imaging of the thorax and abdomen are an important part of the approach to the hypercalcaemia patient. In a dog with PHPTH these are usually unremarkable, with exception of the identification of uroliths. Nonetheless imaging is important to help exclude other differential diagnosis of hypercalcaemia and if unremarkable makes PHPTH a more likely diagnosis. Ideally these would always be performed, but as the cases presented with history and physical examinations that made other differential diagnosis less likely, performing PTH and PTHrP measurements before imaging is not incorrect. This approach, however, has the disadvantage of missing concomitant problems that could be identified by thoracic or abdominal imaging, which are relatively likely considering the advanced average age of dogs diagnosed with PHPTH.

The cases where imaging identified abnormalities in the liver and spleen, had FNA and cytology performed, which helped exclude lymphoma affecting these organs as a cause of hypercalcaemia.

Five of the studied cases had a cervical US exam to assess the parathyroid glands. A suspected parathyroid nodule was identified in all of the cases, measuring from 3.4 to 7mm in diameter. The majority of masses identified in US in a study with 142 dogs with PHPTH measured between 4 and 6mm in diameter (Feldman et al., 2005), though in these cases three of the five had diameters between 3.4 and 3.7mm. Cervical US exam is an important to part of the approach of hypercalcaemia, especially when PHPTH is suspected. This exam is also useful in aiding presurgical planning (Feldman, 2014, 2015a; Sawyer et al., 2011; Schenck et al., 2012; Wisner et al., 1997, 1993).

An interesting fact is that even though in the study by Feldman et al. (2005) cervical US exams had identification rates of parathyroid nodules near 100%, only the last US result was recorded and it is likely that in previous exams the nodule was not identified. This occurred in one of the cases, where the nodule was only identified on the second US exam.

Only the last three cases had surgical treatment, as the owners from the first three elected not to proceed with surgery. In those three cases the surgery was performed with the patient in dorsal recumbency through a ventral midline approach.

The advantage of surgery comparatively to other treatment methods was obvious in the case where a mass not visible on US was identified and removed during surgery, which would not have been possible in a percutaneous treatment.

One case had to be revised as a lymph node was initially removed instead of the parathyroid gland. One option to help localising the parathyroid gland is a blue methylene infusion, which allows readily identification of the parathyroid tissue, but only in reserved circumstances because of the possible deleterious side effects (Schenck et al., 2012). Another option is the usage of a rapid chemiluminescent PTH assay, rather than to wait for histopathology to

confirm the tumour removal or normal iCa concentrations to return to normal limits as it would provide information of remaining autonomous PTH secreting tissue (Ham et al., 2009). Postsurgical monitoring is vital, as hypocalcaemia is a common complication of parathyroidectomy and iCa or tCa monitoring allow for timely correction of calcium levels before clinical signs develop. In the majority of cases hypocalcaemia develops between 3 and 6 days after surgery and ideally the dog should be kept in the hospital for 5 to 7 days after surgery, which is not always possible because of the cost or behaviour of the dog (Feldman, 2010, 2014, 2015a; Nelson, 2009; Séguin & Brownlee, 2012). One of the cases that did not have parathyroidectomy performed was admitted for surgery but had a deterioration in behaviour and became aggressive, which complicated postsurgical management and ultimately led to the decision of not to proceed with surgical treatment. This decision was made because postsurgical hypocalcaemia can lead to serious consequences including death and neither the surgeon nor the owner were willing to risk not having postsurgical monitoring. The only option would have been to sedate the patient throughout the postsurgical period until discharge a few days later, which also carried significant risks and increased costs.

All three cases that had surgery had histopathological analysis of the removed tissue. Two cases were diagnosed with parathyroid carcinoma and one with parathyroid adenoma. Although parathyroid carcinomas are more rare than adenomas or hyperplasia and are present in approximately 5% of dogs with PHPTH (Feldman, 2015a), Wisner et al. (1997) reported a significantly higher incidence of 20%. Another justification for relatively rare tumour type to be represented in two animals, other than the small number of dogs in this sample, is the difficulty of classifying parathyroid tissue, which often results in disagreement between clinical pathologists (Ham et al., 2009). However, as adenomas and carcinomas act identically and local invasion and metastasis are rarely reported, there is limited relevance to differentiating between the two (Feldman, 2014, 2015a; Ham et al., 2009).

In one of the cases where surgery was performed, a mass caudally to the contralateral thyroid gland was identified and the histopathology diagnosis was the same as the parathyroid nodule (carcinoma). It is very unlikely that a parathyroid nodule would metastasise and cross the mid line and a second tumour could have independently originated from ectopic tissue (Feldman, 2015a).

Histopathology is still important to allow confirmation that the removed structure has parathyroid tissue that could be responsible for the autonomous secretion of PTH. The identification of the removed tissue as part of a lymph node, guided the case towards a revision surgery rather than investigating another differential diagnosis.

Prognosis for dogs with PHPTH treated surgically is excellent, but concomitant diseases result in a less favourable prognosis (Berger & Feldman, 1987; Caplan, 2013; Feldman,

2015a; Flanders, 2003; Gear et al., 2005; Nelson, 2009; Rasor et al., 2007; Séguin & Brownlee, 2012).

There is limited information of prognosis for untreated dogs with PHPTH, but if hypercalcaemia persists, it will continue carry deleterious effects on the organism. As PHPTH usually causes stable minimal to moderate hypercalcaemia, but urolithiasis, renal mineralisation and chronic renal injury can occur. As previously mentioned renal failure caused by PHPTH is rare (Arbaugh et al., 2012; Feldman et al., 2005; Ham et al., 2009; Milovancev & Schmiedt, 2013; Sawyer et al., 2011).

The clinical approach to hypercalcaemia was not the ideal in all cases, as limited funds often restrain the tests and procedures that can be performed, as was the example with the cases where imaging exams were limited. This challenges the hypercalcaemia diagnosis, but a logical approach based on history and physical examination allows to workaround the lack for some tests or procedures.

The absence of blood-gas analysers in some practices prevents the measurement of iCa in the practice, which often results in measurement of tCa alone, which as previously discussed is not always reliable.

3.5. Conclusion

PHPTH is one of the most common differential diagnosis for hypercalcaemia in the dog and a thorough physical exam and a detailed history are very helpful in increasing the index of suspicion for this disease. As the physical exam is often unremarkable, clinical signs are milder and the patient is not as ill as in the cases affected by another differential diagnosis of hypercalcaemia, the absence of these clinical findings helps identifying this disease (Feldman, 2014).

PHPTH is characterised by inappropriate PTH concentrations in the presence of ionised hypercalcaemia and normal PTH concentrations are also considered inappropriate. This is a good example of how results within reference range can be abnormal and indicate unregulated PTH production and secretion (Graves, 2011).

As PHPTH has a straightforward treatment with excellent prognosis and if left untreated hypercalcaemia can lead to several consequences, calcium status assessment should ideally be performed in elderly dogs and in those with signs of polyuria and polydipsia. Even though signs often go unnoticed, it has been reported by Feldman (2015a) that some owners realise that clinical signs were present in retrospective following treatment, which means an improvement in quality of life of the patient occurs. Treatment is therefore important, even if the problems are not obvious to the owners, as it improves quality of life and reduces the risk of development of uroliths and UTI.

It is also worth mentioning that even though some owners do notice clinical signs related to PHPTH they attribute these changes to the ageing process, which will complicate the diagnosis (Skelly, 2012).

In imaging exams sometimes findings are unrelated to the cause of hypercalcaemia. As patients with PHPTH present with advanced ages, it is more likely that other concomitant problems will be present. Knowledge of what to expect in imaging exams that can explain hypercalcaemia and obtaining a definitive diagnosis through cytology or histology is important to conclude if that finding could in fact justify the presence of hypercalcaemia or if more testing is required. The most common examples of tumours detected in imaging that can justify hypercalcaemia include lymphoma, particularly T-cell lymphoma, and anal sac adenocarcinoma (Bennett et al., 2002; Messinger et al., 2009; Schenck et al., 2012; Williams et al., 2003).

Cervical US is an important part of the investigation of hypercalcaemia, particularly if PHPTH is one of the differential diagnosis with higher index of suspicion. This exam allows the assessment of the parathyroid glands, and if a nodule is not visible in the exam, it does not necessary exclude PHPTH as the cause of hypercalcaemia, though make the diagnosis oof PHPTH less likely (Feldman, 2014, 2015a; Nelson, 2009).

This study had several limitations, including the retrospective design, the very small sample of dogs analysed and the unavailability of information regarding previous history and follow-up for some of the studied animals.

Despite the limitations it was possible to conclude that PHPTH is a disease that frequently goes unnoticed by the owners and is commonly found through biochemical analysis conducted for different health problems. The fact that often hypercalcaemia is only identified for reasons unrelated to PHPTH, the prevalence of this disease is likely underestimated.

tCa is not the ideal test to assess calcium status, as it can overestimate normocalcaemia and underestimate hypocalcaemia. iCa should be used whenever possible to obtain a more accurate assessment, as it is the only active fraction. The disadvantage of iCa is that is less readily available in many practices and when blood gas analysers are not available, an external laboratory must be submitted, which increases costs and the timeframe to obtain a result.

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