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Case Report
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Multiple endocrine neoplasia similar to human subtype 2A in a dog: Medullary thyroid carcinoma, bilateral pheochromocytoma and parathyroid adenoma

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Abstract
Human multiple endocrine neoplasia subtype 2A (MEN 2A) is characterized by medullary thyroid carcinoma, pheochromocytoma and parathyroid hyperplasia or adenoma in the same individual. In this report, a case of a female Rottweiler with medullary thyroid carcinoma, bilateral pheochromocytoma and parathyroid adenoma was described. Clinical manifestations of muscle weakness, polydipsia, polyuria, diarrhea and weight loss were observed. Two adrenal neoplasms were identified incidentally by ultrasonography, and tumor in the left thyroid lobe was identified by palpation. Primary hyperparathyroidism was diagnosed by biochemical testing. Histopathology report was consistent with diagnosis of bilateral pheochromocytoma and parathyroid adenoma. Immunohistochemical staining was positive for calcitonin and synaptophysin, and negative for thyroglobulin, which confirmed medullary thyroid carcinoma. This case in a dog is presenting neoplastic characteristics similar to human MEN 2A and emphasizing the importance of using immunohistochemistry for confirmation.
Keywords: Calcitonin, Immunohistochemistry, MEN 2A, Synaptophysin, Thyroglobulin.

Introduction

Human multiple endocrine neoplasia (MEN) is characterized by the development of two or more tumors in specific endocrine glands in the same individual. Two major forms are recognized: MEN 1 and MEN 2 (Table 1). MEN 2 is caused by a dominant autosomal mutation within the RET proto-oncogene (Brandi *et al*., 2001). MEN 2 is further divided into MEN 2A, MEN 2B and familial medullary thyroid carcinoma (FMTC). MEN 2A, or Sipple syndrome, is characterized by a phenotypic association that comprises medullary thyroid carcinoma (MTC), unilateral or bilateral pheochromocytoma (PCC) and parathyroid adenoma (PA) or parathyroid hyperplasia (Brandi *et al*., 2001). In dogs, several neoplastic associations have been described in endocrine organs (Reusch, 2015), but those types of neoplasia have seldom been found in the specific association described for human MEN. To the best of the authors' knowledge, only one case of neoplasic associations specific of human MEN 2A has been reported in the literature, in a 15-year old Fox Terrier diagnosed with MTC, unilateral PCC and hyperplasia in two parathyroid glands (Peterson *et al*., 1982). The purpose of this report is to analyze the neoplastic association that corresponds to human subtype MEN 2A in a dog and to emphasize the importance of immunohistochemistry (IHC) as a powerful tool for the diagnosis of this syndrome.

Case Details

A 32 kg, 11-year old neutered female Rottweiler was referred to the Endocrinology Unit at the hospital of Veterinary Medicine of the University of Buenos Aires. This case was referred due to the incidental finding of bilateral adrenal gland tumor during ultrasound examination and normal adrenocortical function (Table 2). The haematological investigation and biochemical tests results were within reference ranges, with the exception of total calcium (Ca) levels, 3.55 mmol/l (reference ranges: 2.25-3 mmol/l), phosphatemia (Pi), 0.56 mmol/l (reference ranges: 0.71-1.13 nmol/l) and serum alkaline phosphatase (SAP), 470 U/l (reference value: up to 250U/l). Few months before, the owner had taken the dog to the hospital with the complaint of chronic diarrhea and mild weight loss, progressive weakness, polyuria and polydipsia, which they were still present. On physical examination, a mass was palpated on the left side of the neck, consistent with anatomic location of the thyroid gland.

From the clinical signs and biochemical profiles, a presumptive diagnosis of primary

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Table 1. Classification of Human Multiple Endocrine Neoplasia.

Neoplastic association	*MEN 1*	*MEN 2*		
		MEN 2A	*MEN 2B*	*FMTC*
Medullary thyroid carcinoma	---	100%	100%	100%
Pheochromocytoma	---	50%	50%	---
Adenoma/Parathyroid hyperplasia	Yes	20-30%	---	---
Pituitary adenoma	Yes	---	---	---
Pancreatic tumors	Yes	---	---	---
Mutated gene	MEN gene	RET gene		

Table 2. Endocrine biochemical tests results.

Biochemistry	*Results*	*Reference ranges*	*Method*
PTH 1-84	6.42	0.6-3.55 pmol/l	CL
Ca	3.55	2.25-3 mmol/l	SP
ICa	2.13	1.1-1.4 mmol/l	ISE
Pi	0.56	0.71-1.13 nmol/l	SP
SAP	470	Up to 250 U/l	SP
UC:Cr	7.0	< 10×10-6	CL/SP
Post-Dx UC:Cr	3.1	<50% of UC:Cr	CL/SP
Co	68.9	13.79-165.4 nmol/l	CL
Post-ACTH Co	344.8	165.4-441.4 nmol/l	CL
VMA	19	12.0 mg/l*	HPLC
Glucose	5.32	3.92-6.16 mmol/l	SP
Potassium	3.9	3.6-5.8 mmol/l	ISE
Sodium	142	140-155 mmol/l	ISE
TSH	0.19	0.03-0.35 ng/ml	CL
FT4	15.44	7.72-20.59 pmol/l	CL
TT4	28.3	12.87-38.1 nmol/l	CL

PTH: Parathyroid hormone; Ca: Total calcium; iCa: Ionized calcium; Pi: Inorganic phosphate; SAP: Serum alkaline phosphatase; UC:Cr: Urine cortisol-creatinine ratio; Post Dx UC:Cr: Post-dexamethasone urine cortisol-creatinine ratio; Co: Plasmatic cortisol; Post-ACTH Co: Post-ACTH (adrenocorticotrophic hormone) cortisol; VMA: vanillin-mandelic acid (*values obtained in the central laboratory of School Animals Veterinary Hospital, Faculty of Vet. Sciences from 8 normal dogs, unpublished data). TSH: Thyroid-stimulating hormone; FT4: Free thyroxine; TT4: Total thyroxine; CL: Chemiluminescence; HPLC: High-performance liquid chromatography; SP: Spectrophotometry. ISE: Ion-selective electrode.

hyperparathyroidism (PHPTH) was suspected. On the other hand, after hypercortisolism was discarded, suspected PCC as an option of bilateral adrenal neoplasia. Accordingly, following laboratory serum test were ordered: Parathyroid hormone (PTH) and ionized calcium (iCa) to confirm PHPTH; thyroid-stimulating hormone (TSH), total thyroxine (T4) and free canine T4 to evaluate functionality of thyroid gland; 24-hour urine test: Vanillin-mandelic acid (VMA, catecholamines' degradation product) to reach the diagnose of PCC (Gilson *et al*., 1994). For this test, 24 hours urine was collected in a recipient with 6N hydrochloric acid (HCL6N); the animal was hospitalized and through a urinary catheter, urine sample was collected. Blood pressure was measured to complement the possible diagnosis of PCC. Ultrasound of thyroid, parathyroid and adrenal glands was also carried out.

Results showed increased levels of PTH, iCa and VMA (Table 2). Three serial blood pressure measurements were taken at three different time points using impulse oscillometry (petMAP classic, Ramsey Medical, Inc-Tampa, USA) showed a hypertensive state with an average systolic pressure 205 mm Hg (considered normal less than 150 mmHg) and an average diastolic pressure 95 mm Hg (considered normal less than 95 mm Hg) (Reusch, 2015).

By ultrasound, a neoplasm was observed in the left thyroid lobe with rounded shape (2 cm x 1.8 cm), and the right parathyroid gland was bigger (1.2 cm in diameter) and hypoechoic.

The ultrasound (prior to consultation) and Doppler ultrasound, performed in the hospital, have revealed an altered shape and size of the left adrenal gland (4.3 cm long x 2.2 cm wide); enlarged right adrenal gland (10.3 cm long x 5.4 cm wide) in proximity to caudal vena cava (CVC). Additionally, turbulent blood flow in the CVC due to neoplastic invasion intraluminal was present.

Elevated PTH and iCa confirmed the diagnosis of PHPTH; whereas, high blood pressure and VMA data were strongly compatible with PCC. Imaging studies supported the diagnosis of PHPTH possibly associated to bilateral PCC and thyroid neoplasic. Once diagnosis was established, owner did not return to the hospital, but two months later, the patient was admitted to the hospital service emergency with the complaint of tachypnea, tachycardia, hyperglycemia, fever and hypertension. The owner opted for humane euthanasia and authorized necropsy.

In this report, the case was diagnosed with medullary thyroid carcinoma (Fig. 1), bilateral pheochromocytoma (Fig. 2) and parathyroid adenoma (Fig. 3).

A 2 cm hard mass was noted with peripheral vascularization in the middle of the left thyroid lobe (Fig. 1A). The right adrenal gland was very large with irregular shape (10.3 cm long x 5.4 cm wide). The CVC was found displaced medially, while the right kidney was found displaced laterally. Abdominal phrenic vein (APV) and the CVC were being invaded by the neoplasia (Fig. 2A). Upon dissection, it appeared firm and hemorrhagic, and adrenal cortex area was not recognizable macroscopically (Fig. 2B).

The left adrenal gland was altered in shape and size (4.3 cm long and 2.2 wide), with APV invasion (Fig. 2C). Macroscopically, within the adrenal gland, noticeable hemorrhagic areas and thinning of the adrenal cortex were observed (Fig. 2D).

The right parathyroid gland, in cranial position, was larger than the rest (1.2 cm in diameter) with cystic appearance inside (Fig. 3A,B).

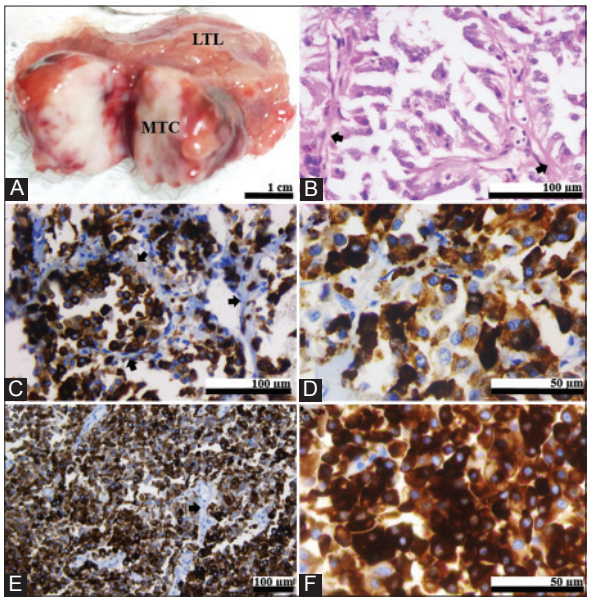

Fig. 1. (A-F) Medullary thyroid carcinoma. (A) Medullary thyroid carcinoma (MTC), macroscopic appearance. LTL: Left thyroid lobe (Bar = 1cm). (B) Structures of papillary appearance, formed by cubic and cylindrical cells with intensely acidophilic cytoplasm arranged over a fibrovascular stroma (arrows) (HE. Bar = 100µm). (C) Positive reaction of neoplastic cells with anti-calcitonin antibody, arranged in nests delimited by fibrovascular tissue septa (arrows) (Bar = 100µm). (D) Positive cytoplasmic reaction with anti-calcitonin antibody. Rounded nuclei and apparent nucleoli (Bar = 50µm). (E) Positive cytoplasmic reaction in neoplastic cells with anti-synaptophysin antibody. A fibrovascular tissue septum can be observed (arrow) (Bar = 100µm). (F) Positive cytoplasmic reaction of neoplastic cells with anti-synaptophysin antibody (Bar = 50 µm).

To perform IHC and histopathological procedures, samples of all the lesions were fixed in 10% buffered formalin and embedded in paraffin. Then, 3µm sections were cut, fixed on slides and stained with hematoxylin-eosin (HE) and appropriate antibodies.

IHC procedures were performed using the avidin-biotin complex (ABC) and the immunoperoxidase detection system (Millipore IHC Select®), while the chromogen 3.3'-diaminobenzidine (DAB) was used for development. Antibodies used are shown in Table 3.

Images were captured on a Leica DC160 digital camera connected to a trinocular microscope (Leica DM4000B led).

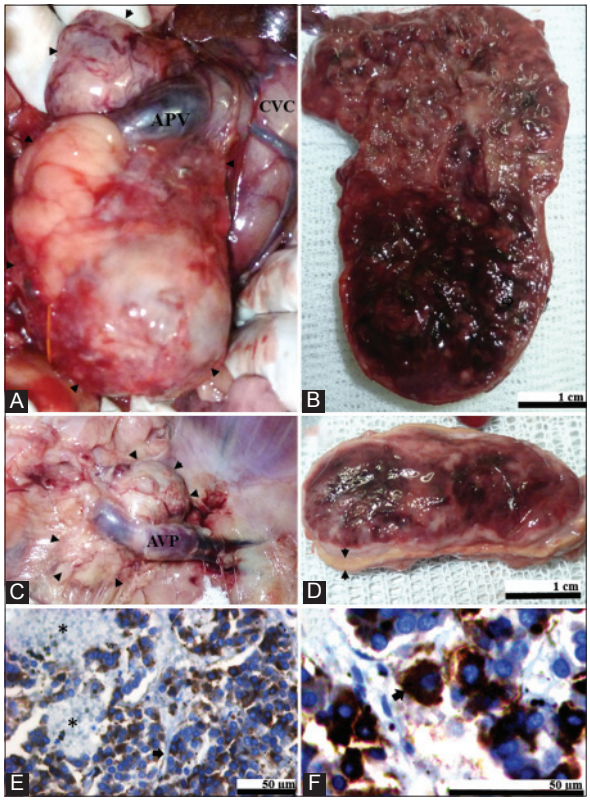

Fig. 2. (A-F) Pheochromocytoma. (A) Right adrenal gland, macroscopic appearance with abdominal phrenic vein (APV) and caudal vena cava (CVC) tumor invasion (B) Right adrenal gland, longitudinal section, neoplasia with congestive, hemorrhagic appearance. No cortical tissue observed macroscopically (Bar = 1 cm). (C) Left adrenal gland, macroscopic appearance with APV invasion (D) Left adrenal gland (macroscopic appearance) longitudinal section. Please note the hemorrhagic appearance of the neoplasia and thinning of the adrenal cortex (arrows) (Bar = 1 cm). (E) Positive cytoplasmic reaction of neoplastic cells with anti-synaptophysin antibody. Cells form nests delimited by fibrovascular septa (arrow), with vascular congestion (*) (Bar = 50 µm). (F) Polyhedral cells, cytoplasm reactive to anti-synaptophysin antibody (arrow). Cells with rounded nuclei, dispersed chromatin and small nucleoli (Bar = 50 µm).

Quantification of the IHC staining was performed, semi-quantitatively, through the percentage of tumor cells stained positively/cells per field. Staining intensity was subjectively classified as mild, moderate and intense.

In the thyroid tissue we observed cubic and cylindrical cells proliferation, arranged over a delicate fibrovascular stroma; it also showed a pseudostratified lining with branching papillae. Morphologically, these cells were characterized by oval or rounded nuclei, moderate anisocariosis and variable quantities of acidophilic cytoplasm, which let to high anisocytosis. Mitotic figures were also observed (Fig. 1B). More than 60% of neoplastic cells showed intense positive cytoplasmic reaction to anti-calcitonin antibody (Fig. 1C,D). Cytoplasmic staining with anti-synaptophysin antibody was intensely positive in more than 90% of neoplastic cells (Fig. 1E,F).

Neoplastic cells stained negative for thyroglobulin (TG); only the areas of normal thyroid parenchyma stained positive. Cromogranin A (CgA) staining was also negative. Morphologically and immunohistochemically, the neoplasia was consistent with an MTC.

Adrenal cortex thinning was observed, caused by proliferation of polyhedral monomorphic cells originated from adrenal medullary. Morphologically, they consisted in rounded nuclei with dispersed chromatin, though some of them were hyperchromatic, with small nucleoli and acidophilic, finely granular and scarcely apparent cytoplasm. Cells were arranged in multiple narrow nests, delimited by the fibrovascular septa of the stroma surrounding the neoplasia. The lesion also showed intense vascular congestion and some necrosis areas (Fig. 2E). Cytoplasmic staining with anti-synaptophysin antibody was moderately positive in 60% of neoplastic cells (Fig. 2F). CgA staining was negative. Morphologically and immunohistochemically, the adrenal neoplastic tissue was consistent with bilateral PCC.

The parathyroid tissue showed a proliferative process formed by irregular cords of polyhedral cells with basophilic granular cytoplasm, with large rounded nuclei with dispersed chromatin and smaller eosinophilic cells with pyknotic nucleus (Fig. 3C,D). The diagnosis was consistent with chief cells parathyroid adenoma.

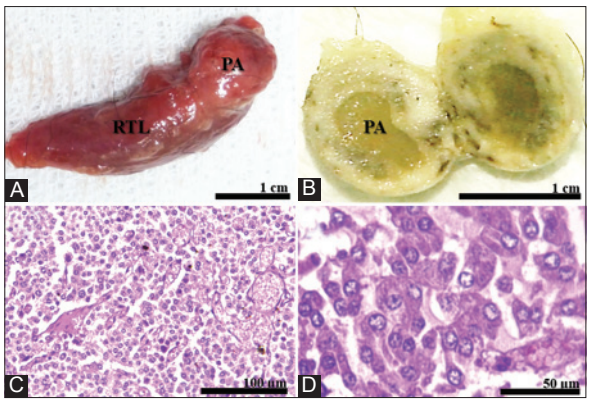

Fig. 3. (A-D) Parathyroid adenoma. (A) Parathyroid adenoma (PA) macroscopic appearance, next to the right thyroid lobe (RTL). (B) Parathyroid adenoma was dissected in half and fixed in 10% buffered formalin. (C) Chief cell adenoma parathyroid (HE. Bar = 100µm). (D) Irregular cords of polyhedral cells with basophilic granular cytoplasm, with large rounded nuclei with dispersed chromatin and smaller eosinophilic cells with pyknotic nucleus (HE. Bar = 50µm).

Discussion

This case report describes three types of neoplasia, each of which is, in itself, rare in dogs. The neoplastic association (phenotypic expression of RET proto-oncogen mutation) similar to MEN 2A in human, has only been reported in a 15-year old male neutered Fox Terrier (Peterson *et al*., 1982) diagnosed with MTC, unilateral PCC and chief cells hyperplasia in both parathyroid glands. Additionally, two previous reports with similar cases to subtype MEN 2 described an association of PA and PCC in a 13-year old Yorkshire Terrier (Wright *et al*., 1995) and FMTC in four mixed Alaskan Malamutes (Lee *et al*., 2006). In our case, the RET proto-oncogene mutation could not be analyzed (not available in Argentina), but the phenotypic expression (neoplastic association) was accurate to the MEN 2A in humans.

MTC is a neoplasia which originates from the C cells (or parafollicular cells) of the thyroid gland; in humans, it is first expressed in MEN 2A, because of its high penetrance 90%, followed by PCC 50% and, lastly, parathyroid gland lesions 20-30% (Brandi *et al*., 2001). Both in ours and Peterson's report (Peterson *et al*., 1982), thyroid function was preserved, the size and the macroscopic appearance of the MTC were similar, but the histopathological appearance was different.

Table 3. Antibodies used for immunohistochemical staining of medullary thyroid carcinomas and pheochromocytoma, and the canine tissues used as positive controls.

Primary antibody	*Type of antibody*	*Dilution*	*Positive control*
Thyroglobulin	Monoclonal mouse*	1:50	Thyroid (follicular cells)
Calcitonin	Polyclonal rabbit**	1:200	Thyroid (parafollicular cells)
Chromogranin A	Polyclonal goat*	1:50	Adrenal (medullar cells)
Synaptophysin	Monoclonal mouse*	1:50	Adrenal (medullar cells)

Negative control was performed by removing primary antibody; Santa Cruz Biotechnology*; Roche Laboratory**.

Although the MTC has certain histopathological features that make it recognizable (polygonal cells arranged in nests delimited by fibrovascular septa), some cases have shown an architectural pattern similar to that of papillary or follicular carcinomas (Hedinger *et al*., 1989). In our case, histopathological findings indicated the presence of follicular thyroid carcinoma, but IHC procedures were done when we suspected MEN 2A. The fact that the thyroid lesion stained positive for calcitonin (CT) and synaptophysin (SYN) antibodies supported the diagnosis of MTC, while the negative immunoreactivity for TG ruled out follicular thyroid carcinoma. In the immunohistochemical diagnosis of MTC, CT is the most specific and reliable marker for this type of tumor (Patnaik and Lieberman, 1991; Rigopoulou *et al*., 2008), while SYN, in determining its neuroendocrine origin, is the perfect complement (Gould *et al*., 1987). In this case, the combination of the three markers confirmed the diagnosis of MTC.

In human medicine, plasma calcitonin is used as a diagnostic marker of MTC (Toledo *et al*., 2009). In Peterson's case (Peterson *et al*., 1982), increased levels, compared to human normal reference values, contributed to the diagnosis of MTC; in the current case study their determination was not considered due to lack of reference values for dogs and association studies linking it with MTC.

In the specific case of calcitonin, its role in inducing hypocalcemia within the PHPTH context is not well-known (Feldman, 2015), while hypocalcemia within the context of MTC is infrequent (Patnaik *et al*., 1978). In our case, hypercalcemia generated by PHPTH had a clear influence on calcium levels.

On the other hand, chronic diarrhea may be attributed to a rise in plasma calcitonin secretion, considering that in humans 30% of patients with MTC show increased levels (Moline and Eng, 2011) and in dogs the infusion of calcitonin generated diarrheal episodes due to increased motility and a decrease in intestinal water absorption (Cox *et al*., 1979).

PCC is a functional paraganglioma that originates from adrenal medullary chromaffin cells. In humans, 27% of PCC are hereditary and associated with mutations in several genes, of which RET proto-oncogene is responsible for MEN 2 (Subramaniam, 2011).

In dogs, PCC presentation is sporadic and its finding is usually incidental, as in our case (ultrasound). Reported cases of PCC with a possible hereditary component reduce to subtypes MEN 2A (Peterson *et al*., 1982) and MEN 2 (Wright *et al*., 1995) already mentioned in the MTC discussion. Other possible associations found in dogs are PCC and pituitary tumor (Von Dehn *et al*., 1995; Bennett and Norman, 1998) and PCC, adrenal cortical tumor and pituitary tumor (Thuróczy *et al*., 1998).

PCC malignancy criteria remain controversial, in human FCC malignancy is currently defined by the presence of metastasis, not local invasion; in dogs, however, either local invasion or distant metastasis usually qualifies for definition of malignancy (Reusch, 2015). Histologically, it may be difficult to distinguish benign from malignant PCC (Reusch, 2015). However, we define malignancy PCC based on local vascular invasion and through the scale PASS (Pheochromocytoma of the Adrenal Gland Scaled Score) used in human medicine; foci of necrosis, vascular invasion, monomorphic growth, nuclear hyperchromatism and cells arranged in nests (Parenti *et al*., 2012).

Clinical signs in patients with PCC are usually nonspecific, but they can be explained considering the type of catecholamine and its actions, quantities, type of secretion (continuous or episodic), location and type of receptor on which they have effect (Sako *et al*., 2001). In dogs with PCC, noradrenaline is the predominant catecholamine, and the one that appeared to increase systolic blood pressure in our case. Some dogs showed an increased secretion of adrenaline that may potentiate cardiorespiratory signs (tachycardia, tachypnea) and metabolic signs (hyperglycemia), and PCC may secrete other peptides that cause other signs, such as interleukin-6 (fever) and VIP (diarrhea). Muscle weakness, weight loss, polyuria and polydipsia are signs attributed both to PCC and PHPTH (Feldman, 2015; Reusch, 2015).

Although the dog presented a large tumor with CVC and APV invasion, no local signs (such as ascites, hind-limb edema or abdominal distension) were observed.

It was initially suspected as bilateral PCC, after discarding the hypercortisolism by assessing urinary cortisol pre- and post-oral dexamethasone, inhibition test plasma cortisol pre- and post-intravenous dexamethasone and ACTH stimulation test (Behrend, 2015). Although the determination of the relationship normetanephrine urine/urine creatinine (degradation product of noradrenaline) proved to be best method for the diagnosis of pheochromocytoma in dogs, in this case we considered the use of vanillin-mandelic acid (VMA) because in our country at the time of diagnosis, it was the only tool available for PCC diagnosis in veterinary practice. The value obtained was above reference value of the central laboratory of the Hospital School, Small Animals, Faculty of Veterinary Sciences, UBA (Unpublished data). VMA in dogs, in absence of reference values, was used, as reported by Gilson *et al*. (1994) (13.2 mg/l); similar reference value obtained in our hospital. Sustained hypertension was a fact important for suspected PCC.

Finally, histopathology and IHC confirmed the definitive diagnosis of PCC. We opted for an IHC procedure with SYN antibody (reliable marker for normal and tumoral neuroendocrine cells) (Buffa *et al*.,

1987). Though CgA is the most specific tumor marker for neuroendocrine differentiation, in our case, all types of neoplasia stained negative for CgA. In this respect, it should be noted that its sensitivity depends on granular density, which in turn depends on the degree of tissue differentiation; it is also worth mentioning that in this case the contents of presynaptic vesicles (they contain CgA) may have been emptied during the crisis that affected the patient before euthanasia.

PHPTH, present in 20 to 30% of patients with human MEN 2A, is usually mild and encompasses a number of conditions, from a solitary adenoma to hyperplasia in one or more parathyroid glands. Within the context of MEN 2A, most cases of PHPTH do not manifest clinical symptoms; however, hypercalciuria and renal lithiasis can be observed (Brandi *et al*., 2001). Hypercalcemia, hypophosphatemia and increase PTH, were decisive finding in diagnosis of PHPTH (Feldman, 2015). Solitary adenoma of parathyroid gland was present in our case, while hyperplasia in two parathyroid gland was reported by Peterson. The absence of lithiasis suggests the adenoma grew rapidly in the last stages of the animal's life (the last neoplasia to appear), and the clinical manifestations were more accentuated near the time the disease was diagnosed. However, in this case we believe it is hard to attribute the predominance of signs to one of the three types of neoplasia, since it is the three combined that defined the diverse manifestations observed in the animal. Histopathology confirmed PA, because of its morphological features.

By conclusion, definitive diagnosis of thyroid neoplasia required routinely use of IHC to avoid errors in classification, treatment and follow-up of MTC. When a PCC is found, it is of vital importance to rule out the presence of thyroid or parathyroid tumor; likewise, in the presence of MTC, it is advisable to rule out PCC.

Finally, we would like to highlight the importance of IHC; without it, the diagnosis of MTC and its phenotypic association with MEN 2A would not have been possible.

Conflict of interest

The authors declare that there is no conflict of interest

References

Behrend, E.N. 2015. Canine hyperadrenocorticism, in: Feldman, E.C., Nelson, R.W., Reusch C.E., Scott-Moncrieff, J.C.R. and Behrend, E.N. Canine & Feline Endocrinology, fourth edition, Elsevier, pp: 377-451.

Bennett, P.F. and Norman, E.J. 1998. Mitotane (o'p'-DDD) resistance in a dog with pituitary-dependent hyperadrenocorticism and phaeochromocytoma. Aust. Vet. J. 76(2), 101-103.

Brandi, M.L., Gagel, R.F., Angeli, A., Bilezikian, J.P., Beck-Peccoz, P., Bordi, C., Conte-Devolx, B., Falchetti, A., Gheri, R.G., Libroia, A., Lips, C.J., Lombardi, G., Mannelli, M., Pacini, F., Ponder, B.A., Raue, F., Skogseid, B., Tamburrano, G., Thakker, R.V., Thompson, N.W., Tomassetti, P., Tonelli, F., Wells, S.A.Jr. and Marx, S.J. 2001. Guidelines for diagnosis and therapy of MEN type 1 and type 2. J. Clin. Endocrinol Metab. 86(12), 5658-5671.

Buffa, R., Rindi, G., Sessa, F., Gini, A., Capella, C., Jahn, R., Navone, F., De Camilli, P. and Solcia, E. 1987. Synaptophysin immunoreactivity and small clear vesicles in neuroendocrine cells and related tumours. Mol. Cell Probes. 1(4), 367-381.

Cox, T.M., Fagan, E.A., Hillyard, C.J., Allison, D.J. and Chadwick, V.S. 1979. Role of calcitonin in diarrhoea associated with medullary carcinoma of the thyroid. Gut 20(7), 629-633.

Feldman, E.C. 2015. Hypercalcemia and primary hyperparathyroidism, in: Feldman, E.C., Nelson, R.W., Reusch C.E., Scott-Moncrieff, J.C.R. and Behrend, E.N. Canine & Feline Endocrinology, fourth edition, Elsevier, pp: 579-624.

Gilson, S.D., Withrow, S.J., Wheeler, S.L. and Twedt, D.C. 1994. Pheochromocytoma in 50 dogs. J. Vet. Intern. Med. 8(3), 228-232.

Gould, V.E., Wiedenmann, B., Lee, I., Schwechheimer, K., Dockhorn-Dworniczak, B., Radosevich, J.A., Moll, R. and Franke, W.W. 1987. Synaptophysin expression in neuroendocrine neoplasms as determined by immunocytochemistry. Am. J. Pathol. 126(2), 243-257.

Hedinger, C., Williams, E.D. and Sobin, L.H. 1989. The WHO histological classification of thyroid tumors: A commentary on the second edition. Cancer 63(5), 908-911.

Lee, J.J., Larsson, C., Lui, W.O., Höög, A. and Von Euler, H. 2006. A dog pedigree with familial medullary thyroid cancer. Int. J. Oncol. 29(5), 1173-1182.

Moline, J. and Eng, C. 2011. Multiple endocrine neoplasia type 2: An overview. Genet. Med. 13(9), 755-764.

Parenti, G., Zampetti, B., Rapizzi, E., Ercolino, T., Giachè, V. and Mannelli, M. 2012. Updated and new perspectives on diagnosis, prognosis and therapy of malignant pheochromocytoma/paraganglioma. J. Oncol. Article ID 872713. doi:10.1155/2012/872713.

Patnaik, A.K., Lieberman, P.H., Erlandson, R.A., Acevedo, W.M. and Liu, S.K. 1978. Canine medullary carcinoma of the thyroid. Vet. Pathol. 15(5), 590-599.

Patnaik, A.K. and Lieberman, P.H. 1991. Gross, histologic, cytochemical, and immunocytochemical study of medullary thyroid carcinoma in sixteen dogs. Vet Pathol, 28(3), 223-233.

Peterson, M.E., Randolph, J.F., Zaki, F.A. and Heath, H. 3rd.1982. Multiple endocrine neoplasia in a dog. J.

Am. Vet. Med. Assoc. 180(12), 1476-1478.
Reusch, C.E. 2015. Pheochromocytoma and multiple endocrine neoplasia, in: Feldman, E.C., Nelson, R.W., Reusch, C.E., Scott-Moncrieff, J.C.R. and Behrend, E.N. Canine & Feline Endocrinology, fourth edition, Elsevier, pp: 521-551.
Rigopoulou, D., Gómez Lobo, I., Guadalix Iglesias, S. and Calatayud Gutiérrez, M. Carcinoma de tiroides. 2008. Clasificación. Manifestaciones Clínicas. Diagnóstico. Actitudes terapéuticas. TSHrh y tiroglobulina sérica en el manejo del carcinoma diferenciado tiroideo. Medicine 10(14), 904-913.
Sako, T., Kitamura, N., Kagawa, Y., Hirayama, K., Morita, M., Kurosawa, T., Yoshino, T. and Taniyama, H. 2001. Immunohistochemical Evaluation of a Malignant Pheochromocytoma in a Wolfdog. Vet. Pathol. 38(4), 447-450.
Subramaniam, R. 2011. Pheochromocytoma-Current concepts in diagnosis and management. Trends Anaesth. Crit. Care 1(2), 104-110.
Thuróczy, J., Van Sluijs, F.J., Kooistra, H.S., Voorhout, G., Mol, J.A., van der Linde-Sipman, J.S. and Rijnberk, A. 1998. Multiple endocrine neoplasias in a dog: Corticotrophic tumour, bilateral adrenocortical tumours, and pheochromocytoma. Vet Q. 20(2), 56-61.
Toledo, S.P.A., Lourenço, D.M.Jr., Santos, M.A., Tavares, M.R., Toledo, R.A. and Correia-Deur, J.E. 2009. Hypercalcitoninemia is not pathognomonic of medullary thyroid carcinoma. Clinics (Sao Paulo) 64(7), 699-706.
Von Dehn, B.J., Nelson, R.W., Feldman, E.C. and Griffey, S.M. 1995. Pheochromocytoma and hyperadrenocorticism in dogs: Six cases (1982-1992). J. Am. Vet. Med. Assoc. 207(3), 322-324.
Wright, K.N., Breitschwerdt, E.B., Feldman, J.M., Berry, C.R., Meuten, D.J. and Spodnick, G.J. 1995. Diagnostic and therapeutic considerations in a hypercalcemic dog with multiple endocrine neoplasia. J. Am. Anim. Hosp. Assoc. 31(2), 156-162.