A One pathology, Multicentre Portuguese approach to thyroid tumours of dogs and cats

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Introduction

Thyroid tumours are the most common endocrine tumours of dogs and cats. Feline thyroid tumours are essentially functional adenomas curable with thyroidectomy, whereas most canine thyroid tumours are non-functional carcinomas either unresectable or recurrent within two years in up to half of the cases [1,2]. The oncobiology of these tumours remains unclear, but investigating this aspect could contribute to the development of targeted therapies.

Diagnosis, prognosis, and management of human thyroid carcinoma (TC) rely on mutation screening of *BRAF* and *RAS* genes, and *TERT* promoter. *BRAF*, *NRAS*, *HRAS*, and *KRAS* encode proteins that are key effectors of MAPK signalling pathway, an important kinase pathway, conserved in mammals [3] (**Figure 1**). Within the frame of a 'One Health' perspective, we aimed to characterise a series of thyroid tumours of dogs and cats, focusing on correlating histopathological findings with molecular alterations.

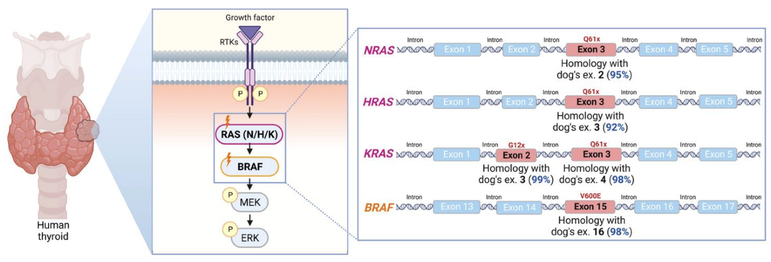


Figure 1. The molecular pattern behind human TC – mutations in codon 600 of *BRAF* and codons 12, 13, and 61 of the *RAS* genes are common events of human TC that result in constitutive activation of MAPK signaling pathway. Dogs and cats have conserved exons within these genes (**> 92% homology**); the human-canine homology of the key hotspots is represented. Created with BioRender.com (2023).

Materials and Methods

Sixty-seven canine and sixteen feline thyroid tumours from seven diagnostic centres were re-evaluated using HE stained slides by a team of veterinary and human pathologists following the most recent WHO criteria [4,5]. The diagnosis of mixed compact-follicular histotype was attributed whenever the follicular and compact components ranged between 40-60%, although criteria simply refers "approximately equal amounts". Mitotic count (MC) was assessed in all cases by two observers in a consistent area of 2.37 mm^2.

The activation status of MAPK and mTOR signalling pathways was assessed by evaluating the immune profile of the phosphorylated downstream effectors (pERK for MAPK, pS6 for mTORC1, and pAKT for mTORC2). This evaluation was carried out by three observers who attributed a score to intensity [faint (1), moderate (2), intense (3)] and extension [5-25% (1), 26-50% (2), 51-75% (3), 76-100% (4)]. A product of both was then obtained (a staining score from 0-12).

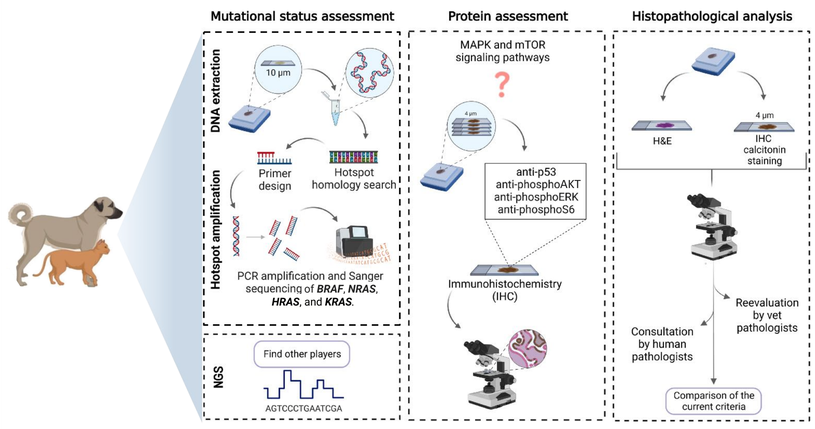


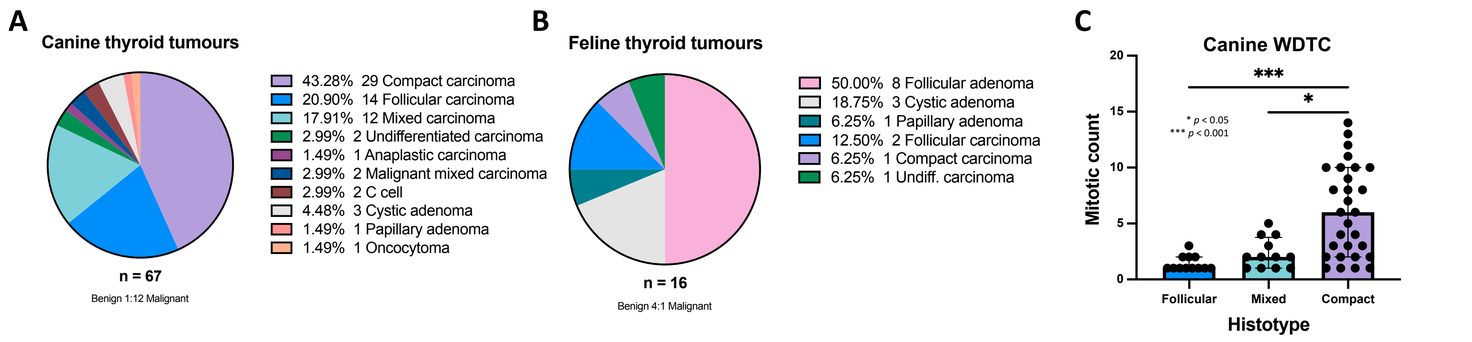
Figure 2. Representation of the project's multistep pipeline; p53 IHC and NGS are on-going tasks. Created with BioRender.com (2023).

Results and Discussion

Histopathological evaluation

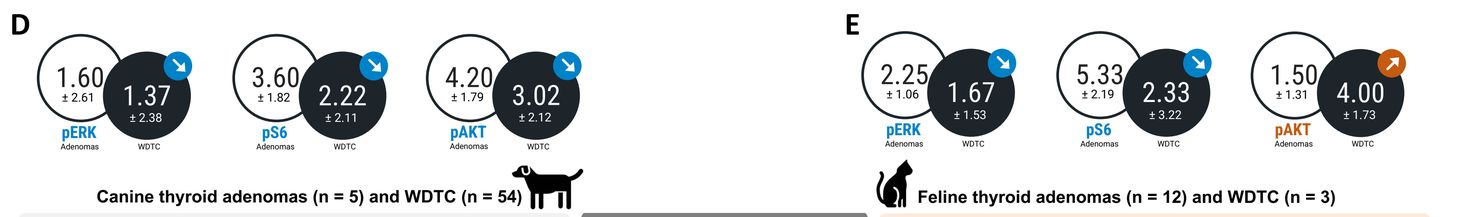
In dogs, 62 (93%) were carcinomas and 7 (7%) were adenomas, in line with previous reports; most canine carcinomas arose in pure-breeds (57%) and were of compact-cellular (47%), follicular (23%), and mixed compact-follicular (termed "mixed") (19%) type (**A**). In cats, 12 (75%) were adenomas, below other reports (up to 95%); most feline adenomas were follicular (67%) and all feline tumours occurred in European shorthairs (**B**).

MC significantly varied among canine well-differentiated thyroid carcinomas (WDTC): higher in compact carcinomas > mixed > follicular (**C**). This had never been reported and could be useful to improve the stratification of the histotypes. Other variables (e.g., sex, age) and poor prognosis features (e.g., tumour size, multifocality, vascular invasion, tumour margins) did not differ among histotypes.



Immunohistochemistry assays

Due to the available number of cases for evaluating the pERK, pS6, and pAKT immune profiles, only canine WDTC and feline thyroid adenomas were used for comparison with clinical-pathological features. Among intensity, extension, and staining score, solely the intensity retrieved significant results. The mean staining score values (± SD) for the three antibodies were compared between thyroid adenomas and WDTC in dogs (**D**) and cats (**E**); only the pAKT mean staining score values in cats increased with malignity (p = 0.025).



Increased pAKT intensity was significantly associated with smaller tumours (< 4 cm) (p = 0.004)	**Tumour size**	Significantly increased pERK intensity was associated with smaller tumours (r = -0.564, p = 0.04)
Increased pS6 intensity was associated with unifocal tumours (p = 0.062)	**Multifocality**	Increased pAKT intensity was significantly associated with multifocal tumours (p = 0.027)
Peripheral pS6 intensification was associated with the presence of vascular invasion (p = 0.018)	**Vascular invasion**	Not applicable
Increased pS6 intensity was associated with clean margins (R0)	**Tumour margins**	Not applicable

Mutational status

From all canine thyroid tumours, we detected three different missense mutations, only within WDTCs (n = 54): **one on *HRAS* (n = 1/52, 1.9%)** and **two on *KRAS* (2/54, 3.7%)**; however, only one of the *KRAS* mutations [**c.179G>C(p.Gly12Arg)**] had also been described in human TC [6]. All the screened sequences were wild-type on the remaining homologous exons: exon 16 of *BRAF* and exon 2 of *NRAS*.

Concerning the feline species, **two mutations were detected on *KRAS***, in one adenoma **(n = 1/11, 9.1%)** and in one WDTC **(n = 1/2, 50.0%)**: a missense mutation that had never been reported in human nor feline thyroid tumours [**c.696CAA>CAT(p.Gln232His)**]. No other mutations were found.

Conclusions

Dogs and cats are completely different in terms of thyroid disease. We seek to unveil the full translational value of dogs and cats in this field. Contrarily to the literature, the non-mixed histotypes constitute more than half of the canine thyroid tumours of our series, which may in part be due to the subjectivity of the current criteria. We observed that the mitotic count has a discriminative power among canine WDTC, and we propose its use to fortify the stratification system.

Concerning the protein studies, since low staining score levels were verified in both adenomas and WDTCs, we conclude that MAPK and mTOR pathways are not preponderant in thyroid carcinogenesis of dogs. This is in line with the low mutation burden detected. In relation to feline thyroid tumours, definitive conclusions cannot be drawn due to the reduced number of cases; nevertheless, mean staining scores for pERK and pS6 were found to be higher in feline adenomas compared to canine WDTCs, implying a greater involvement in feline thyroid disease. Generally, except for pAKT in cats, all the proteins appear to be linked with favourable prognostic characteristics. The significance of pAKT in feline WDTC warrants further investigation. To sum up, **whereas dogs do not seem to be good models to study human thyroid cancer, it remains to be determined the full utility of studying feline thyroid tumours for that purpose**.

References: **1**-Lunn & Boston (2012), in Withrow and MacEwen's Small Animal Clinical Oncology, **2**-Campos *et al.* (2014), **3**-Cuenda A *et al.* (2018), **4**-Kiupel *et al.* (2008), in Histological classification of tumors of the endocrine system of domestic animals, **5**-Rosol & Meuten (2017), in Tumors of the Endocrine Glands **6**-Xing *et al*. (2013). **Acknowledgements:** the authors would like to acknowledge the support of the i3S Scientific Platforms Genomics and Histology and Electron Microscopy (HEMS), the Vet-OncoNet for their logistical support, and the diagnostic centres for providing all the cases.

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