international concordance study for mammary tumours in dog and cat, potentially fuelling an international discussion on critical issues.

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MALIGNANCY ANALYSIS IN 26,224 HISTOPATHOLOGIC DIAGNOSES OF CANINE TUMOURS: RESULTS FROM A MULTICENTRE STUDY IN CENTRAL ITALY (2008–2023)

N. Fonti[1], F. Parisi[1], A. Carnio[2], C. Cocumelli[2], E. Sophie Dhein[3], C. Eleni[2], V. Galietta[2], F. Guscetti[3], A. Lachi[4], A. Poli[1], P. Scaramozzino[2], F. Millanta[1]. [1] *University of Pisa, Pisa, Italy;* [2] *Istituto Zooprofilattico Sperimentale (IZS) delle Regioni Lazio e Toscana, Rome, Italy;* [3] *University of Zurich, Zurich, Switzerland;* [4] *Institute of Clinical Physiology - National Research Council of Italy (IFC-CNR), Pisa, Italy*

Background: Animal Cancer Registries (ACRs) significantly enhance our understanding of oncology, providing insights into epidemiological and clinical trends, and fostering comparative research. This study aimed to provide data on a large set of canine tumours, analysing their distribution and malignancy profile by risk factors.

Materials & Methods: Vet-ICD-O-canine-1 was used to code 26,224 histologically diagnosed tumours from two pathology-based ACRs in central Italy between 2008 and 2023. The Cochrane-Armitage test and logistic regression analysis were performed to investigate the influence of different variables on the binary outcome (malignant versus benign tumours). Odds ratios (ORs) for specific histotypes were reported.

Results: Benign and malignant tumours were 10,902 (41.6%) and 15,316 (58.4%) out of the total, respectively. The risk of malignancy was shown to increase by 8% with each year of age. Females had a higher risk of developing a malignant tumour (OR = 2.16, CI 95% 2.04-2.29). Neutering status did not affect overall malignancy but contributed to histotype-specific risks. The ORs of developing malignant tumours and specific histotypes were largely influenced by breed. The Dogo Argentino had the highest malignancy risk (OR = 2.18, CI 95% 1.49-3.25), with an increased risk for squamous cell tumours (OR = 5.15; CI 95% 3.35-7.71), while the West Highland White Terrier had the lowest (OR = 0.54, CI 95% 0.38-0.76), and an increased risk for histiocytic tumours (OR = 3.03, CI 95% 1.35-6.08).

Conclusion: This study confirmed previous findings, revealed novel potential risk factors and breed predispositions and highlights the value of multicentre collaboration in identifying high-risk oncology patients.

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PRELIMINARY STUDY ON TUMOUR-ASSOCIATED MACROPHAGES IN FELINE ALIMENTARY LYMPHOMAS

F. Parisi[1], N. Fonti[1], A. Poli[1], F. Millanta[1]. [1] *University of Pisa, Pisa, Italy*

Background: Interactions between tumour cells and tumour-associated macrophages (TAMs) appear to have significant effects on treatment response and prognosis in oncological patients, as testified by previous studies both in human and veterinary medicine. This study aimed to characterize TAMs in feline alimentary lymphomas.

Materials & Methods: Thirty-four feline alimentary lymphomas were retrieved from the Animal Cancer Registry of the Department of Veterinary Sciences, University of Pisa. Histopathology was carried out to classify lymphomas as mucosal or transmural. Immunohistochemistry was performed to ascertain the immunophenotype (anti-CD3 and CD20 antibodies) and to highlight total (anti-Iba1), M2-polarized (anti-CD204) and recently recruited (anti-MAC387) macrophages. Immunostained cells were counted in 2.37 mm^2 and M1-polarized macrophages calculated as the difference between total and CD204-positive macrophages. Mann–Whitney U test, Kruskall–Wallis test and Dunn's post-hoc analysis were used to assess differences among tumour groups.

Results: Thirty-three lymphomas were classified as 22 transmural lymphomas (seven B cell, 12 T cell, three non-B non-T neoplasms) and the remaining 11 as T-cell mural lymphomas. Iba1-, CD204- and MAC387-positive macrophages were higher in transmural lymphomas compared with mucosal ones ($P < 0.01$, $P < 0.01$ and $P < 0.05$, respectively). CD204-positive cells were higher in T-cell lymphomas ($P < 0.01$), while differences in M1-polarized macrophages were not significant.

Conclusion: Our results are comparable to previous studies on canine lymphoma and highlight the need to further clarify the role of TAMs and the tumour microenvironment in feline alimentary lymphomas.

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INTRA-TUMOURAL HETEROGENEITY OF ANISOKARYOSIS IN CANINE CUTANEOUS MAST CELL TUMOURS

C.A. Bertram[1], A. Haghofer[2], H. Janout[2], E. Parlak[1], F. Wilm[3], M. Aubreville[4], S.M. Winkler[2], M. Kiupel[5], A. Bartel[6]. [1] *University of Veterinary Medicine, Vienna, Austria;* [2] *University of Applied Sciences Upper Austria, Hagenberg, Austria;* [3] *Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany;* [4] *Technische Hochschule Ingolstadt, Ingolstadt, Germany;* [5] *Michigan State University, Lansing, United States;* [6] *Freie Universität Berlin, Berlin, Germany*

Background: Anisokaryosis of tumour cells is an important histological malignancy criterion. For canine cutaneous mast cell tumours (ccMCTs) anisokaryosis, referred to as 'karyomegaly', is routinely evaluated as part of the grading system. This study aimed to evaluate the distribution of anisokaryosis throughout the tumour section (intratumoural heterogeneity) to investigate whether 1) region selection influences anisokaryosis evaluation and 2) heterogeneity of anisokaryosis is prognostically relevant.

Materials & Methods: Anisokaryosis was evaluated in whole slide images (WSIs) of 96 ccMCTs with known tumour-specific death (TSD). A previously established deep learning-based algorithm was used to measure the area of all tumour nuclei within the manually or algorithmically segmented tumour area of each WSI. The standard deviation (SD) of nuclear size was calculated for small tumour regions each containing 112 cells on average.

Results: For a single, manually selected tumour region, the SD of nuclear area had an area under the curve (AUC) of 0.943 for TSD. Intratumoural distribution analysis of SD of nuclear area revealed higher values and a larger spread of values for cases with TSD. Cases with TSD had on average 44.4% hotspots (locations above the threshold of $\geq 11.5\ \mu m^2$), while the other cases had on average 5.8% hotspots. The percentage of hotspots was indicative for TSD (AUC = 0.948).

Conclusion: This study demonstrated variable intratumoural distribution of anisokaryosis in some cases indicating that region selection can influence prognostication. Further studies should therefore evaluate the influence on inter-rater reproducibility. Intratumoural heterogeneity of anisokaryosis seems to carry good prognostic information.

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EXPLORATION OF DISEASE-RELATED SEQUENCES IN AA AMYLOIDOSIS IN MIXED-BREED CATS

N. Kobayashi[1], M. Kaneda[1], S. Iwaide[1], Y. Itoh[1], M. Hisada[1], N. Sedghi Masoud[1], K. Owaku[1], K. Nomra[2], M. Tsuneyasu[3], T. Akamine[3], T. Murakami[1]. [1] *Tokyo University of Agriculture and Technology, Tokyo, Japan;* [2] *MLT Co., Ltd., Osaka, Japan;* [3] *Watanabe Animal Hospital, Anicom Specialty Medical Institute Inc., Oita, Japan*

Background: AA amyloidosis is characterized by functional impairment due to abnormal deposition of misfolded serum amyloid A (SAA) protein in various organs. While some pure-breed cats are known to inherit AA amyloidosis, there has been a growing recognition of its occurrence in mixed-breeds. This analysis aims to identify disease-associated genes by comparing SAA genes in cats with and without AA amyloidosis.

Materials & Methods: SAA sequences from five AA amyloidosis-affected and five amyloidosis-free mixed-breed cats were sequenced. 3D structural differences in each sequence were predicted using AlphaFold2 software. Hepatic and renal amyloid deposits were subjected to LC-MS/MS to identify SAA sequences that form amyloid in tissues.