



XXIII congresso AIPVet
23-25 Aprile 2026
San Benedetto del Tronto

Superare i confini:
la multidisciplinarietà per il
patologo veterinario

Beyond boundaries:
a multidisciplinary approach
for the veterinary pathologist

Book of Abstract





XXIII congresso AIPVET 23-25 Aprile 2026

San Benedetto del Tronto

Superare i confini: la multidisciplinarietà per il patologo veterinario

Beyond Boundaries: Multidisciplinary for veterinary pathologists

Venue: Hotel Calabresi

Viale Marinai d'Italia 1, San Benedetto del Tronto (AP)

<https://www.hotelcalabresi.it/>

Giovedì 23 Aprile 2026

Ore 13:00-13:30 - Registrazione e light buffet

13:30-14:00 Saluti e avvio lavori

14:00-15:15 Moderatori: Giacomo Rossi e Marco Galeotti

Keynote speakers - Patologia degli organismi acquatici in un'ottica eco-ambientale

14:00-14:15 *Introduzione sulle attività locali e regionali in itticultura* - Alessandra Roncarati

14:15-14:30 *Il contributo della didattica UNICAM alla conoscenza delle specie aliene acquatiche* - Dr. Felici

14:30-15:00 *Sorveglianza sanitaria in molluschi e pesci: analisi delle patologie e delle mortalità nel Mezzogiorno d'Italia* - Gianluigi Paduano

15:15-16:00 Moderatori: Fabio Marino e Cinzia Centelleghes

Presentazioni Orali

- *The potential resilience in Mediterranean mussels (*Mytilus galloprovincialis*) to climate-induced ocean acidification* – Luciana Mandrioli

- *Soft tissue surgery in aquarium fish: diagnostic imaging, microscopic investigation, tailored surgical techniques and post surgical management* – Gianpiero Nieddu

- *Histopathological and molecular correlations of polystyrene nanoplastics-induced intestinal lesions in the European eel (*Anguilla Anguilla*)* - Evaristo Di Napoli



XXIII congresso AIPVET 23-25 Aprile 2026

San Benedetto del Tronto

16:00-16.30 Coffee Break & Sessione Poster (Poster da 1 a 14)

16:30-18:30 Moderatore: Luciana Mandrioli e Elena Maria Bozzetta

Presentazioni Orali

- *Pathological evaluation of wild fish for zoonotic bacterial and parasitic agents in freshwater ecosystems* – Martina Ossola
- *The emerging role of Mycobacterium chelonae as a shared pathogen in fish mycobacteriosis and human dermatitis* – Marta Polinas
- *An unsung diagnostic tool: what 5 years of fish cytology can teach us* - Gaia Beatrice Maria Bianchi
- *Pathological and environmental findings during a clam mortality episode* – Gabriella Di Francesco
- *Hematodinium perezii detection in blue crab (Callinectes sapidus) and its temporal and spatial prevalence in Northern Adriatic Lagoons* – Tobia Pretto
- *Systemic Scuticociliatosis by Philasterides dicentrarchi (Ciliophora, Scuticociliatida) in gilthead sea bream (Sparus aurata)* – Luana Cortinovia
- *Root Mean Square-based tracking of post-mortem muscle electrical changes in Oreochromis niloticus over 24 hours: implications for post-mortem interval estimation* – Lanteri Giovanni

19:00-21:00 Welcome party e Assemblea soci



Venerdì 24 Aprile 2026

8:30-9:30 Moderatori: Franco Mutinelli e Cristiano Cocumelli

Keynote speaker - *Specie aliene invasive: dalla normativa al contesto italiano* - Piero Genovesi

9:30-10:45 Moderatori: Orlando Paciello e Ranieri Verin

Presentazioni Orali

- *Histopathological effects of Monocystis sp. infection in Eisenia fetida* – Rebecca Leandri
- *Pathological effects of arsenic on the digestive gland of Lymnaea stagnalis* – Giorgia Rosato
- *Impact of biodegradable and conventional microplastics on Eisenia fetida S.: an ecotoxicological and ecopathological perspective* – Karen Power
- *Histological characterization of bone marrow adipose tissue in emaciated dogs: differentiating starvation from cachexia – a forensic perspective* – Cristiano Maino
- *Bodies recovered from water: evaluation of the post-mortem interval and diatom test in veterinary forensic pathology* – Vittoria Romano

10:45-11:15 **Coffee Break & Sessione Poster (Poster da 15 a 27)**

11:15-11:45 Moderatore: Giovanni Pellegrini

Plenary Talk – *Carcinogenesi virale in studi di tossicologia preclinica* - Cecilia Gola e Filippo Torrigiani

11:45-12:30 Moderatori: Camilla Recordati e Federico Armando

Presentazioni orali

- *An Open Source Pipeline for Automated Quality Control in Large Scale Digital Pathology* – Giovanni Pellegrini
- *A novel therapeutic strategy for neurofibromatosis type 1 associated tumors: histopathology and immunohistochemistry in a murine model* – Paolo Finotti
- *Probiotic Modulation of Hypoxia-Induced Changes in Duodenal Tight Junction Proteins and HIF-1 α in a Mouse Model* – Lucia Biagini

12:30-14:00 **Light lunch & Sessione Poster**



XXIII congresso AIPVET 23-25 Aprile 2026

San Benedetto del Tronto

12:30 -14.00 Round table lunch - Patologia sperimentale - *New Approach Methodologies (NAMs): the new frontier for modeling diseases and assessing adverse drug effects while reducing animal use* - Federico Armando e Nadine Stokar-Regenscheit

14:00-14:30 Moderatore: Paola Roccabianca

Keynote speaker: *Didattica innovativa in patologia veterinaria: quale vantaggio?* – Laura Cavicchioli

14:30-15:30 Moderatori: Paola Roccabianca e Laura Cavicchioli

Keynote speaker: *Using case-based learning and open-book assessment to develop the diagnostic reasoning skills of veterinary undergraduates: the Dublin experience* - Joseph Cassidy

15:30-16:00 Moderatori: Massimo Castagnaro e Giuliano Bettini

Presentazioni orali

- *A grain of sand in the swine lung pathology world* - Giovanni Pietro Burrari
- *SPIRO-AI: application of artificial intelligence to the pathological investigation of ovine respiratory diseases* – Ludovica Mura

16:00-16:30 Coffee Break & Sessione Poster (Poster da 28 a 41)

16:30-17:00 Moderatori: Raffaella De Maria e Saverio Paltrinieri

Presentazioni orali

- *MiRNAs in canine mammary tumour angiogenesis and modulated angiogenesis-related signalling pathways* - Jessica Maria Abbate
- *Potential targets for precision therapy in canine primary splenic sarcomas: a preliminary study* – Alessandra Verdi

17:00-18:15 Moderatore: Raffaella De Maria

Keynote speakers - *OMICS in Patologia Veterinaria* - Raffaele Calogero e Fabrizio Ceciliani

18:45-20:00 Attività scientifica condivisa



XXIII congresso AIPVET 23-25 Aprile 2026
San Benedetto del Tronto

Ore 20:00 Cena sociale



Sabato 25 Aprile 2026

8:30-10:30 Moderatore: Chiara Brachelente

Keynote speakers - *Lesioni infiammatorie e neoplastiche del cavo orale nel cane e nel gatto: casi selezionati con approccio diagnostico interattivo* - Silvia Ferro, Silvia Lucia Benali, Giuseppe Giglia

10:30-11:00 Coffee Break

11:00-12:30 Moderatori: Silvia Ferro, Silvia Lucia Benali e Giuseppe Giglia

Presentazioni orali

- *Feasibility of HER2 Status Assessment by FISH in Cytologic Samples of Canine Bladder and Urethral Carcinomas* – Maral Anjomanibenisi
- *Isoform-informed FGFR1/2 targeting in canine osteosarcoma: 3D models and long-read sequencing to map splicing diversity* – Francesca Ciaramellano
- *Anatomical-site and EcPV2-dependent variation in M2 macrophage polarization in equine squamous cell carcinoma* – Ginevra Martinoli
- *The good, the bad and the oncolytic: comparative analysis of BoHV-4 and BoHV-1 oncolytic potential in canine osteosarcoma and histiocytic sarcoma* – Melania Di Pentima
- *Beyond morphology: immunohistochemical profile of canine oral spindle cell tumors* – Ilaria Porcellato
- *Interplay between tumor immune microenvironment and FcaPV infection in feline nasal planum squamous cell carcinoma* – Livia De Paolis

12:30 Chiusura e Light lunch



XXIII congresso AIPVet
23-25 Aprile 2026
San Benedetto del Tronto

Oral Presentations



MicroRNA-driven regulation of angiogenesis and key signalling pathways in canine mammary tumours

Jessica Maria Abbate¹, Barbara Brunetti², Maral Anjomanibenis², Francesca Arfuso³, Alessia Giannetto¹, Domenico Giosa¹, Giovanni Lanteri¹

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina (ME)

²Department of Veterinary Medical Sciences, University of Bologna, Bologna (BO)

³Department of Veterinary Sciences, University of Messina, Messina (ME)

Angiogenesis is a hallmark of cancer enabling tumour growth and metastasis. MicroRNA (miRNAs) regulate angiogenesis post-transcriptionally, but signatures in canine mammary tumours (CMTs) remain poorly defined. We profiled 24 miRNAs with putative pro- and anti-angiogenic roles in CMTs ($n=22$) by RT-qPCR and compared their expression with normal mammary glands. MiRNA-modulated pathways were inferred through target-gene prediction (multiMiR), followed by Gene Ontology and KEGG pathway enrichment analysis (clusterProfiler). MiRNA expression was correlated with angiogenesis metrics - microvascular density (MVD) and endothelial area (EA) – quantified on Factor VIII-immunolabeled sections using QuPath. MVD and EA were higher in malignant than in benign CMTs, peaking in grade III carcinomas (MVD: 40.0 ± 24.4 vs 21.5 ± 7.1 microvessels/mm²; EA: $38,665.3 \pm 21,146.9$ vs. $24,232.6 \pm 4,266.1$ μm^2). Malignant CMTs showed a progressive shift towards a pro-angiogenic profile, with significant upregulation of pro-angiogenic miR-9, miR-20a, miR-98, miR-210 and miR-21 ($p < 0.05$). Conversely, putative anti-angiogenic miRNAs displayed a heterogenous, context-dependent expression pattern: miR-152-3p and miR-542-3p were downregulated in benign CMTs vs. normal gland, whereas miR-34a and miR-205 increased in malignant CMTs ($p < 0.05$), with miR-205 higher in malignant than in benign tumors (mean FC 1.16 vs. 0.57). In malignant CMTs, MVD and EA were positively correlated ($r = 0.8$, $p = 0.0003$); EA correlated with miR-98 ($r = 0.67$, $p = 0.006$) and tumour size with miR-210 ($r = 0.58$, $p = 0.03$). Target prediction identified 16,910 genes, and pro- and anti-angiogenic miRNAs shared 86.5% of predicted targets, indicating substantial overlap and potentially competing regulation within common gene networks; however, functional validation remains essential. KEGG enrichment identified 100 significant pathways ($\text{FDR} < 0.05$), including MAPK ($\text{FDR} = 1.45 \times 10^{-13}$; 286 genes), PI3K-Akt ($\text{FDR} = 1.91 \times 10^{-9}$; 332 genes), HIF-1 ($\text{FDR} = 3.07 \times 10^{-5}$; 104 genes), VEGF ($\text{FDR} = 1.72 \times 10^{-3}$; 57 genes), and breast cancer pathway ($\text{FDR} = 6.36 \times 10^{-8}$; 142 genes). MAPK1/MAPK3 were the most frequently targeted genes, followed by PIK3CA, AKT1/AKT2 and mTOR. Pathways converged on VEGF/VEGFR, HIF-1, PI3K/Akt/mTOR, and MAPK signalling, underscoring the translational relevance of CMTs and the potential utility of miRNA-based biomarkers for tumour stratification and targeted anti-angiogenic approaches.

Feasibility of HER2 Status Assessment by FISH in Cytologic Samples of Canine Bladder and Urethral Carcinomas

Maral Anjomanibenis¹, Ginevra Martinoli¹, Barbara Bacci¹, Maria Giulia Ferrari¹, Enrico Bottero², Enrico Di Oto³, Francesco Dondi¹, Giovanni Tallini⁴, Barbara Brunetti¹

¹Department of Veterinary Medical Sciences, University of Bologna, Italy

²MyLav Veterinary Diagnostic Laboratory, Milan, Italy

³OaCP IE, LTD, Cork, IE

⁴Department of Experimental, Diagnostic and Specialty Medicine, Bologna, Italy

Cytological examination is the method of choice for diagnosing neoplasms of the canine urinary bladder and urethra. The most frequently diagnosed neoplasms are carcinomas, particularly urothelial carcinomas. Currently, therapeutic options for these tumors remain limited. This study aimed to evaluate the feasibility of assessing HER2 status via fluorescence *in situ* hybridization (FISH) in cytological samples and to determine the frequency of HER2 gene amplification, with the prospect of applying HER2-targeted therapies currently available in human medicine. This retrospective study included 22 cytological samples collected for diagnostic purposes. Cytological samples consisted of brush imprints and squash smears. Samples were obtained from 22 dogs (15 mixed breed; mean age 11 years), with a prevalence of females (14 spayed, 3 intact), 4 intact males, and 1 neutered male. Lesions were located in the bladder (12/22), urethra (9/22), or both (1/22). Slides stained with May–Grünwald–Giemsa or Diff-Quik were decolorized with xylene washes, followed by passes in 100% ethanol. FISH analysis was performed using HER2 and CRIBA1 probes on the most cellular areas of the slides. CRIBA1 is used as a control gene to detect polysomy of chromosome 9. Among the 22 samples, 7 (31.8%) were non-evaluable and excluded from further analysis, due to low cellularity and/or poor quality after destaining. Of the 15 evaluable cases, 6 (40%) showed HER2 gene amplification, including 3 cases with cluster amplification (>10 copies), while 9 (60%) were non-amplified. This highlights the need for further studies with a larger case series to better assess non-evaluable cases. These results demonstrate the feasibility of assessing HER2 status by FISH on previously stained cytological samples, including archived material. Furthermore, the identification of HER2 gene amplification in a subset of cases provides a rationale for future studies investigating the potential application of HER2-targeted therapies in these carcinomas.

Probiotic Modulation of Hypoxia-Induced Changes in Duodenal Tight Junction Proteins and HIF-1 α in a Mouse Model

Lucia Biagini¹, Laura Bonfili¹, Francesco Maria Grasselli¹, Giacomo Rossi¹, Anna Maria Eleuteri¹

¹School of Biosciences and Veterinary Medicine, University of Camerino, Matelica, Italy

Chronic hypoxia is a pathophysiological condition associated with several diseases, including heart failure, chronic kidney disease, and neurodegenerative disorders. This study evaluates the potential of probiotic supplementation to modulate hypoxia-induced intestinal barrier dysfunction, focusing on the duodenum. C57BL/6 mice were divided into four groups: normoxia control (NC, n = 10), maintained under standard atmospheric oxygen (~21% O₂); normoxia probiotic (NP, n = 10), maintained under normoxia and receiving probiotics (SLAB51®); hypoxia control (HC, n = 10), exposed to hypoxic conditions (12% O₂); and hypoxia probiotic (HP, n = 11), exposed to hypoxia while receiving probiotics. Treatments lasted two months. Formalin-fixed, paraffin-embedded tissues were processed according to standard immunohistochemical protocols. Tight junction protein expression (Zonula Occludens-1, Claudin, Occludin) was evaluated using a semi-quantitative immunoreactive score (0 to 12), combining the extent of biomarker expression and staining intensity. Hypoxia Inducible Factor (HIF)-1 α and E-cadherin were quantified by counting immunoreactive cells in five microscopic fields at $\times 40$ magnification. ZO-1 expression substantially decreases under hypoxic condition (HC) and, to a lesser extent, following probiotic supplementation (HP) ($p < 0.05$). Occludin expression significantly increased after probiotic supplementation (NP) when compared to the other groups ($p < 0.001$). Claudin increased in both hypoxic groups (HC, HP) compared to normoxia (NC, NP) ($p < 0.001$). E-cadherin expression was elevated in HC compared to HP, and significantly increased in all experimental groups in contrast to the control normoxia (NC) ($p < 0.001$). HIF-1 α was predominantly expressed by lamina propria infiltrating macrophages and the highest expression, observed under hypoxic conditions (HC) ($p < 0.001$), was partially reduced by probiotic supplementation (HP). Overall, hypoxia alters TJP expression and induces HIF-1 α , which in turn is involved in the regulation of TJP. Probiotic administration appears to modulate these responses, partially restoring intestinal barrier conditions closer to those observed under normoxic conditions.

An unsung diagnostic tool: what 5 years of fish cytology can teach us

Bianchi G.B.M.¹, Ghisleni G.^{1,2}, Nieddu G.³, Luciana Mandrioli L.⁴, Giudice C.¹

¹ Dipartimento di Medicina Veterinaria, Università di Milano, Lodi (LO)

² Consulente Veterinario per la Sicurezza Alimentare dei Prodotti Ittici, Mercato Ittico, Milano (MI)

³ Acquario e civica stazione idrobiologica di Milano (MI)

⁴ Dipartimento di Scienze Mediche Veterinarie Alma Mater Studiorum Università di Bologna, Ozzano dell'Emilia, Bologna (BO)

Cytology is a rapid and minimally invasive diagnostic technique that has long been widely used in mammalian and avian veterinary medicine. In contrast, its application in fish remains comparatively limited, despite its considerable diagnostic potential. The aim of this retrospective study is to give an overview of the most common cytological cases that a pathologist approaching this world is likely to deal with, underlining the advantages of this diagnostic tool.

Fish cytologic cases were selected from the Veterinary Anatomic Pathology archive, University of Milan, during the years 2020-2025. Smears were air-dried and stained with May-Grünwald Giemsa. All cytological findings were confirmed by histology. For each case the following data were collected: fish origin and species, sampled organ, smear technique, cytological and histological diagnosis.

24 cases were selected and etiologically classified in 9 neoplasms, 7 parasitosis, 2 bacterial agents and a virosis, while in 6 cases the etiologic agent wasn't found. Skin and muscle were the most sampled organs with 11 and 7 cases, respectively, followed by gills (2 cases), single cases in testis, oral cavity and thyroid, and a multisystemic disease. 12 smears were obtained by scraping, 6 by fine needle aspirations (FNA) and 6 by imprint. 15 fish had ornamental (pet trade) origin while 9 cases were food-production species from the commercial aquaculture chain.

This study reflects the various records that a brand new ichthyopathology service can face and confirm cytology to be a reliable and practical diagnostic tool in teleost fish, particularly effective for detecting skin tumours, among which Peripheral nerve sheath tumours prevail, and protozoan parasites, inducing cysts or granulomatous reactions, often rich in melanomacrophages. An active role of the pathologist by prompting fish cytology to clients and colleagues, paired with simple clear instructions, has proved to be crucial to promote its use as a routinary element.

A grain of sand in the swine lung pathology world

Giovanni Pietro Burrai^{1,3}, Ludovica Mura¹, Tiziana Cubeddu¹, Marina Antonella Sanna¹, Silvia Dei Giudici², Carla Cacciotto^{1,3}, Michele Pazzola¹, Alberto Alberti^{1,3}, Elisabetta Antuofermo^{1,3}, Marta Polinas¹

¹ Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Sassari

² Istituto Zooprofilattico Sperimentale della Sardegna, Sassari

³ Mediterranean Center for Disease Control, Università degli Studi di Sassari, Sassari

As a grain of sand represents an infinitesimal part of a splendid beach, a single virus can provide only a partial answer to a complex problem. From this holistic perspective, we investigated Porcine Respiratory Disease Complex (PRDC), a syndrome in which viruses and bacteria interact with environmental, management, and genetic factors, leading to severe lung lesions. For histological and biomolecular analyses, six samples were collected from each of thirty lungs from 10 piglets (PG), 10 post-weaning pigs (PW), and 10 sows (SW) showing clinical signs of PRDC.

Samples were screened for viral nucleic acids and enriched by nested Pan-Rep PCR (*Circoviridae*), RCA (*Anelloviridae*) and SISPA (linear DNA viruses) and then analysed by MinION Nanopore sequencing. Histologically, inflammation was mainly interstitial in all groups, with piglets having higher interstitial inflammation than PW ($p = 0.023$) and higher alveolar inflammation than PW ($p = 0.033$) and SW ($p = 0.00069$). MinION sequencing revealed age-related differences in viral distribution. PCV1 was detected in all age classes, PCV2 predominated in PW and SW, and PCV3 in piglets. *Anelloviridae* were found in all groups, whereas *Parvoviridae* and *Orthoherpesviridae* were less represented. Nested PCR confirmed widespread *Circoviridae* positivity. After normalization, the virome remained age-related, with PCV3 predominating in piglets and PCV2 in post-weaning pigs. In accordance with the “*Grain of Sand Theory*” our findings suggest that no single virus can explain the complexity of PRDC. Like a beach shaped by countless grains of sand, PRDC emerges from the interplay of many factors. As the saying goes, “*If you are not at the table, you are on the menu.*” Only by earning our place at that table, through a truly holistic approach, will we be able to recognize the leading dancers, distinguish them from the chorus, and give meaning to the intricate choreography of disease.

Isoform-informed *FGFR1/2* targeting in canine osteosarcoma: 3D models and long-read sequencing to map splicing diversity

Francesca Ciaramellano ^{1*}, Marcella Massimini ^{1*}, Luana Fiorella Mincarelli ², Mariarita Romanucci ¹, Marco Di Domenico ², Valentina Rinaldi ¹, Umberto Maggiolini ¹, Raffaella De Maria ³, Cesare Cammà ², Carlos E. Alvarez ⁴, Leonardo Della Salda ¹

¹ Department of Veterinary Medicine, University of Teramo, Teramo, Italy.

² Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, 64100 Teramo, Italy.

³ University of Turin, Grugliasco, Turin, Italy.

⁴ The Ohio State University Colleges of Medicine and Veterinary Medicine.

* These authors contributed equally

Canine osteosarcoma (OSA) is an aggressive, metastatic bone cancer and a valuable comparative model for human OSA. Fibroblast growth factor receptors *FGFR1/2* are actionable targets, yet alternative splicing of the IgIII domain (IIIb/IIIc) can alter ligand specificity and may contribute to primary response or resistance to *FGFRs* inhibition. The goal of this work is to build an isoform-resolved experimental framework to define how 3D microenvironmental cues modulate *FGFR1/2* splicing *in vitro*, enabling drug testing in the most informative conditions, and to discover previously unrecognized *FGFRs* splicing isoforms by long-read sequencing in both cell models and OSA tissues, supporting future isoform-selective strategies including isoform-specific inhibitors and immunotherapy.

Three canine OSA cell lines with distinct biological behavior were cultured in 2D and in two 3D systems (type I collagen embedding and spheroids), sampled at different time points and analyzed by isoform-specific RT-qPCR for *FGFR1/2* and their IIIb/IIIc variants. Full-length *FGFR1* variable-region amplicons were sequenced using Oxford Nanopore and processed with a transcript-isoform workflow. In parallel, a small, preliminary set of OSA tissues and healthy bone controls was assessed by RT-qPCR to obtain initial translational signals.

Across all cell lines, IIIc was the most abundant isoform at baseline, while total *FGFR1/2* expression levels differed markedly between lines ($p < 0.001$), suggesting intrinsic variability that could inform sensitivity to *FGFR* inhibitors. Upon 3D culture, D17 showed increased *FGFR1* IIIc ($p < 0.001$), whereas Wall displayed broader reprogramming with evidence of *FGFR1/2* IIIb switching ($p < 0.001$), a feature expected to be informative for stratifying drug response. Long-read sequencing confirmed substantial *FGFR1* transcript diversity *in vitro*, supporting the feasibility of isoform discovery. Preliminary tissue data also indicated higher *FGFR1/2* expression in OSA versus healthy bone, with enrichment of IIIc, warranting validation in larger cohorts.

Next steps include long-read sequencing of OSA tissues to link novel isoforms with clinical follow-up, and systematic *in vitro* testing of *FGFR1/2* inhibitors in 3D conditions to determine whether specific splicing variants enhance efficacy or drive primary resistance.

Systemic scuticociliatosis by *Philasterides dicentrarchi* (*Ciliophora*, *Scuticociliatida*) in gilthead sea bream (*Sparus aurata*)

Luana Cortinovis¹, Valentina Paolini¹, Andrea Basso¹, Tobia Pretto¹

¹ Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro (PD) Italy

Scuticociliatosis is a severe parasitic disease affecting marine fish and often associated with high mortality outbreaks in aquaculture. It is caused by opportunistic scuticociliates that normally live as free-living scavengers but may become histophagous endoparasites under stressful conditions. In January 2025, three gilthead sea bream (*Sparus aurata*) carcasses (38–50 cm S.L.) were submitted to the IZSVe laboratory following a creeping mortality event in a concrete tank containing approximately 2,000 broodstock fish. Water temperature during the outbreak ranged from 14 to 17 °C. Necropsy revealed prolapsed rectum, haemorrhagic ascites, petechial haemorrhages affecting testicles, visceral fat and swim bladder, catarrhal enteritis with intestinal dilatation, and severe encephalic congestion. Bacteriological examinations performed on spleen, kidney, brain and testes were negative or showed only scarce nonspecific polymicrobial growth. Histological analysis highlighted a massive presence of ciliates within seminiferous tubules and testicular stroma, as well as in the pancreas and visceral fat. Parasites were also observed in the intestinal and gastric lumen and within the brain, suggesting systemic scuticociliatosis. For pathogen identification, total genomic DNA was extracted from affected tissues. A fragment of the small-subunit rRNA gene was amplified and sequenced, producing sequences identical to both *Philasterides dicentrarchi* and *Miamiensis avidus*. To discriminate between the two closely related species, the α -tubulin gene was analysed, allowing definitive identification of *Philasterides dicentrarchi*. Since no effective treatments are currently available for scuticociliatosis, the entire fish batch was culled to prevent further spread of the infection. To our knowledge, this represents the first report of systemic scuticociliatosis caused by *Philasterides dicentrarchi* in gilthead sea bream, highlighting the importance of preventive management practices in aquaculture systems.

Interplay between tumor immune microenvironment and FcaPV infection in feline nasal planum squamous cell carcinoma

Livia De Paolis^{1,2}, Melania Di Pentima³, Filippo Dell'Anno^{1,2}, Federico Armando³, Ilaria Porcellato², Chiara Brachelente², Luca Mechelli², Benedetta Passeri³, Giovanni Pietro Burrai⁴, Lucia Rita Gibelli⁵, Floriana Fruscione¹, Malgorzata Ciurkiewicz⁶, Wolfgang Baumgärtner⁶, Elvio Lepri², Elisabetta Razzuoli¹

¹Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, National Reference Center of Veterinary and comparative Oncology (CEROVEC), Genova

²Department of Veterinary Medicine, University of Perugia, Perugia

³Department of Veterinary Medicine, University of Parma, Parma

⁴Department of Veterinary Medicine, University of Sassari, Sassari

⁵Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Milano

⁶University of veterinary medicine of Hannover, Hannover

Cutaneous squamous cell carcinoma (cSCC) is the most common malignant skin tumor in cats, accounting for 15–48% of feline cutaneous neoplasms and frequently affecting non-pigmented areas such as the nasal planum. While chronic UV exposure is a well-established etiological factor, increasing evidence supports the involvement of feline papillomavirus (FcaPV) in a subset of cases. In this context, aiming to provide useful insights into the biology of feline nasal planum cSCC, the tumor immune microenvironment (TIME) and FcaPV status of these tumors were investigated.

Forty-three archival FFPE samples were retrospectively selected and qPCR was performed to assess the presence of FcaPV DNA. FcaPV-2, -3, and -4 DNA was detected in 19/43, 11/43 and 4/43 cases, respectively. Six cases showed co-infections.

The expression of immune-related markers was investigated by IHC using anti-CD3, -CD20, -IBA1, -CD204, and -FOXP3 antibodies. Overall, the inflammatory infiltrate was predominantly macrophagic, with abundant IBA1⁺ cells, whereas CD3⁺ and CD20⁺ lymphocytes were less represented. Comparison between intra- and extratumoral compartments showed that CD3⁺, IBA1⁺, and CD204⁺ cells were more abundant within the intratumoral tissue, whereas CD20⁺ cells were more numerous in the extratumoral compartment.

Significant positive correlations were observed between CD3⁺ cells and CD20⁺ ($p = 0.029$), IBA1⁺ ($p = 0.004$), and CD204⁺ cells ($p = 0.020$). CD20⁺ cells were also positively correlated with IBA1⁺ ($p = 0.022$) and CD204⁺ cells ($p = 0.018$). Moreover, a positive correlation was detected between IBA1⁺ and CD204⁺ cells ($p = 0.008$), suggesting a possible M2 polarization of tumor-associated macrophages (TAMs). The consistent immune infiltration observed in these tumors may reflect both their locally invasive growth pattern and the anatomical localization of the nasal planum, an area chronically exposed to microtrauma and ulceration. Overall, these findings indicate a macrophage-rich TIME in feline nasal planum cSCC, potentially characterized by M2-polarized TAMs.

Pathological and environmental findings during a clam mortality episode

Gabriella Di Francesco¹, Federica Di Giacinto¹, Chiara Profico¹, Romina Lipa¹, Diana Ruscio², Sara Di Donato², Angelo Cameli¹

¹Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale" IZS Teramo- Italy

²Volontarie del Servizio Civile Universale presso IZS Teramo-Italy

In December 2025, a localised mortality event was recorded in natural bank of *Chamelea gallina* in the Compartimento Marittimo of Pescara, between the mouths of Vibrata and Salinello Rivers, almost 0.3 Nautical Miles (NM) far from the coast of Teramo (Abruzzi Region, Italy). Three samplings were carried out and 2541 clams were analysed. Inspection of specimens revealed the presence of dead clams (17%) with marked emaciation of the soft tissues. Histopathological examination revealed multifocal tissue alterations characterised by gill epithelial degeneration and mucocyte hyperplasia of the surface epithelia and digestive gland degeneration consistent with physiological stress responses rather than infectious disease. Occasional inflammatory reactions and lesions involving the pallial cavity were observed, suggesting exposure to non-specific environmental stressors. Mortality data were compared with those collected in the same areas during the monitoring programme of clam resource *C. gallina*. In 2025, routine monitoring activities were complemented by an exceptional translocation action associated with the construction of breakwaters. This initiative combined sustainable fishery management measures with the relocation of clams from areas intended for construction works. A total of six translocation campaigns were carried out between March and October 2025, during which 31.9 tonnes of clams were moved from sites designated for breakwater construction. Clams of commercial size (≥ 22 mm) were released into authorised fishing grounds (12.3 tonnes), while the juveniles (10.7 tonnes) were restocked in areas located more than 1 NM offshore. In December 2025, the clam mortality was significantly higher compared to the previous ones collected during translocation activities ($p < 0.001$). Overall, histological findings supported the hypothesis that mortality was attributable to adverse environmental conditions rather than transmissible pathogens. Despite this event, biomass values remained above the reference points defined in Commission Delegated Regulation (EU) 2020/3. Continued monitoring is recommended to support adaptive management of bivalve fisheries and ensure long-term sustainability.

Histopathological and molecular correlations of polystyrene nanoplastics-induced intestinal lesions in the European eel (*Anguilla Anguilla*)

Evaristo Di Napoli¹, Costantino Parisi², Francesco Addeo¹, Antonio Rinaldi³, Rubina Paradiso³, Emanuele Esposito³, Giovanni Piccolo¹, Fabio di Nocera³, Giuseppe Iovane³, Giorgia Borriello³, Orlando Paciello³, Maria Carmela Ferrante¹

¹Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy

²International Institute for Cell and Molecular Biology, Warsaw, Poland

³Experimental Zooprophyllactic Institute of Southern Italy (IZSM), Portici (NA), Italy

Microplastics (MPs; 1 µm–5 mm) and nanoplastics (NPs; <1 µm) are solid, insoluble, and persistent synthetic polymer particles, considered emerging pollutants due to their ubiquitous distribution in aquatic ecosystems, resistance to degradation, and ability to interact with biological matrices and co-adsorbed contaminants. This study evaluated the effects of subchronic oral exposure to polystyrene nanoplastics (PS-NPs) on the intestine of the European eel (*Anguilla anguilla*) using an integrated morphological and molecular approach. Eighty-five animals were randomly divided into three groups: control (standard diet), low-dose PS-NP, and high-dose PS-NP. Treatment lasted 28 days and was administered through supplemented feed. At the end of the exposure period, animals underwent necropsy and anterior and posterior intestine were sampled. Tissues were processed for histopathological analysis using hematoxylin and eosin staining. Lesions were assessed using a standardized semiquantitative scoring system, considering degree and extent of inflammatory infiltrate; integrity of the mucosal epithelium (erosions, desquamation, loss of brush border); architectural alterations (shortening or fusion of folds, mucosal disorganization); edema and/or congestion; and mucus and goblet cell changes (number/activity). Intestinal samples were analyzed by RT-qPCR to quantify the expression of genes involved in the inflammatory response (IL-1β, IL-6, TNF-α) and tight junction integrity (occludin, claudin-3, claudin-5), to evaluate intestinal barrier functionality. Statistical analysis (Shapiro–Wilk, one-way ANOVA, Bonferroni; $P < 0.05$) confirmed a significant dose-dependent histopathological effect in PS-NPs-treated groups. At low doses, mild chronic enteritis was observed, characterized by modest inflammatory infiltration and slight epithelial alterations. At high doses, lesions were moderate to severe, with more extensive inflammatory infiltrates, marked mucosal disorganization, and evident epithelial damage. Molecular analysis showed compartment-specific modulation, more pronounced in the anterior intestine, and not consistently correlated with lesion severity. This finding suggests a possible temporal dissociation between transcriptional response and morphological damage. Subchronic exposure to PS-NPs induced progressive, dose-dependent intestinal injury, confirming the value of an integrated morpho-molecular approach in teleost species.

The good, the bad and the oncolytic: comparative analysis of BoHV-4 and BoHV-1 oncolytic potential in canine osteosarcoma and histiocytic sarcoma

Melania Di Pentima¹, Sergio Minesso¹, Valentina Franceschi¹, Gaetano Donofrio¹, Elisabetta Razzuoli², Wolfgang Baumgärtner³, Melania Andrani¹, Giuseppina Basini¹, Simona Bussolati¹, Rosanna Vescovini⁴, Silvia Fante¹, Marcella De Maglie¹, Benedetta Passeri^{1,*}, Federico Armando^{1,*}

¹Dipartimento di Medicina Veterinaria, Università degli Studi di Parma, Parma, Italia

²Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle D'Aosta

³Department of Pathology, University of Veterinary Medicine Hannover, Bünteweg, Germany

⁴Dipartimento di Medicina e Chirurgia, Università degli studi di Parma, Parma, Italia

*these authors contributed equally

Canine osteosarcoma (cOSA) and histiocytic sarcoma (cHS) are aggressive tumors characterized by poor prognosis and resistance to conventional therapies. Oncolytic virotherapy represent an alternative strategy. Bovine herpesviruses-4 (BoHV-4) and-1 (BoHV-1) are promising candidates due to their inability to cause disease in dogs and their oncolytic potential in human tumors. In our previous study, we demonstrated the primary oncolytic effects of BoHV-4 in cOSA, cHS, and primary cutaneous fibroblasts (FDOG) cell lines. Based on these findings, the present study aimed to extend the same experimental approach to BoHV-1 and to comparatively evaluate both viruses. FDOG, primary and continuous cOSA cell lines (OSA2 and D17), and a continuous cHS cell line (DH82) were infected at different multiplicity of infection (MOI, 0.01–1) and monitored from 12 to 96 hours post-infection (hpi). One MOI and 48–96 hpi were selected for further investigations to allow comparison with BoHV-4. Infected cells were detected by immunofluorescence and flow cytometry using anti-glycoprotein D (gD+) antibody. Cell death was quantified using 7AAD staining. As opposed to BoHV-4, BoHV-1 showed limited cytotoxicity in tumor cell lines compared to FDOG. Recovery assays demonstrated that all cell lines supported productive infection by at least one virus. Secondary oncolytic effects like dysregulation of tumor cell motility and angiogenesis were also investigated. Scratch assay revealed a transient effect on tumor cell motility caused by both viruses. A canine endothelial cell proliferation assay showed also a transient effect only on OSA2 and DH82 cell lines. In conclusion, due to the lack of selectivity and cytotoxicity of BoHV-1 for cancer cells, it will not be included in further studies. On the other hand, due to the higher selectivity for cancer cells and the ability to exert both direct and indirect oncolytic effects, BoHV-4 will deserve future investigations.

A novel therapeutic strategy for neurofibromatosis type 1 associated tumors: histopathology and immunohistochemistry in a murine model

Paolo Finotti¹, Valentina Zappulli¹, Ranieri Verin², Andrea Rasola³, Francesco Ciscato³, Ionica Masgras³
Maria Egle Castorina³, Margherita Toffanin³, Samuele Negro³

¹Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro (PD)

²Department of Veterinary Science, University of Pisa, Pisa (PI)

³Department of Biomedical Sciences, University of Padova, Padova (PD)

Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder caused by loss-of-function mutations in the NF1 tumor suppressor gene predisposing to the development of several neoplasms, including malignant nerve sheath tumors (NSTs) that are frequently resistant to conventional therapies.

Genetically engineered mouse models and innovative molecular approaches have paved the way for new therapeutic strategies.

In the present study, we investigated the *in vivo* effects of a novel therapy based on Hexokinase-2-displacing peptides (HK2pep), in a murine model of NF1-associated NST.

NF1^{flox/flox}; p53^{flox/flox}; PLP-Cre⁺ mice received tamoxifen chow to induce specific ablation of NF1 and p53 genes in Schwann cells. Following sciatic nerve crush injury, all mice developed a neoplasm at the site of nerve damage approximately 30 days after surgery. Tumor development was monitored *in vivo* through ultrasonography and three-dimensional reconstruction. Histopathology and immunohistochemistry confirmed the diagnosis of a malignant NST.

The therapeutic efficacy of HK2pep, was evaluated in this model in 10 tumor-bearing and 10 untreated mice. The peptide was administered intraperitoneally twice a day starting 30 days after crush. Tumor progression was monitored by ultrasound imaging, and animals were sacrificed at 10 and 15 days after treatment for histopathology and immunohistochemistry.

Treated mice showed a significant reduction ($p<0,01$) in tumor mass compared with controls at ultrasound and a mild reduction in tumor cell density at histology. Immunohistochemistry demonstrated a trend towards an increase in CD4⁺ lymphocytes at 15 day post treatment ($p=0,188$) and, at 10 days post treatment, a mild reduction in mitoses and Ki-67⁺ cells. A significant increase in infiltrated M1 macrophages and decrease in M2 macrophages was observed in the treated mice ($p<0,02$). No differences were observed in CD8⁺ lymphocytes, total macrophages and mast cells. Further studies are needed to explore these results, however mainly based on the tumor mass reduction, HK2pep seem promising a new approach for NF1-associated NST.

Root Mean Square-based tracking of post-mortem muscle electrical changes in *Oreochromis niloticus* over 24 hours: implications for post-mortem interval estimation

Giovanni Lanteri¹, Giancarlo Consolo², Simone Palazzolo³, Maria Giovanna Piro¹, Luca Cicero⁴,
Giuseppe Curcio¹, Francesca Arfuso⁵, Jessica Maria Abbate¹

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina (ME)

²Department of Mathematical, Computer, Physical and Earth Sciences, University of Messina, Messina (ME)

³Veterinary Practitioner, Brescia (BS)

⁴Experimental Zooprophyllactic Institute of Sicily "A. Mirri" (IZS), Palermo (PA)

⁵Department of Veterinary Sciences, University of Messina, Messina (ME)

Accurate estimation of the post-mortem interval (PMI) remains difficult in forensic casework, prompting growing interest in improved technologies that are often tested in experimental animal models. Here, we used an oscilloscope-signal generator system to quantify post-mortem changes in skeletal electrical conductivity by measuring the previously validated root mean square (RMS) voltage. RMS was recorded every 15 minutes for 24 hours in 21 Nile tilapias (*Oreochromis niloticus*) maintained at controlled ambient temperatures (15°C, Group 1; 20°C, Group 2; 25°C, Group 3). Pre-euthanasia blood samples were analysed for haematological variables, total protein, and electrolytes. At the end of the experiment, all fish underwent gross necropsy and histopathological examination. Serum electrolytes, total protein, and protein fractions did not differ among groups. Over the 24-hour monitoring period, the output signals showed a high degree of concordance, revealing a consistent pattern: RMS voltage increased initially, reached a peak, and then gradually declined toward approximately baseline values, consistent with a progressive reduction in electrical impedance associated with post-mortem autolysis. Ambient temperature significantly affected the timing of the RMS peak (T_{max}), occurring at approximately 15 h in fish maintained at 15°C and at approximately 5 h in fish maintained at higher temperatures (20°C and 25°C) ($p < 0.05$). Within groups, T_{max} correlated positively with chloride and potassium at 15°C; with sodium, chloride and calcium at 20°C; and with sodium and chloride at 25°C ($p < 0.05$). T_{max} occurred earlier at 20-25°C than in our previously published data on healthy control fish, potentially due to subacute-to-chronic inflammatory lesions recorded here - mainly involving the spleen, liver and cranial kidney - and often containing intralesional bacilli. The consistent early post-mortem RMS pattern across individuals supports RMS as a candidate marker for early PMI categorization. RMS dynamics may relate to *rigor mortis* progression and loss of membrane selectivity, processes modulated by temperature and inflammation. Overall, RMS measurement represents a rapid, non-destructive, low-cost method for assessing early post-mortem muscle dielectric changes relevant to PMI estimation.

Histopathological effects of *Monocystis* sp. infection in *Eisenia fetida*

Rebecca Leandri¹, Giorgia Rosato¹, Gennaro Volpe¹, Gionata De Vico¹, Karen Power¹

¹Department of Biology, University of Naples Federico II (NA)

The earthworm *Eisenia fetida* is widely used in laboratory ecopathological and ecotoxicological studies, due to its rapid reproductive rate and easy-maintenance. Ecotoxicological end points consist in the assessment of biomass and reproduction, measured respectively through body weight and cocoon production. During routine maintenance of a population of *Eisenia fetida* under laboratory conditions, the absence of cocoon was observed, suggesting a potential impairment of reproductive functions. To investigate the cause, routine histological analysis were performed on one hundred individuals. Schmorl staining was conducted to detect oxidizable intracellular pigments. Microscopic examination revealed the presence of large gametocysts containing pear-shaped sporocysts within the seminal vesicles of 63% of collected individuals. Molecular analysis confirmed the presence of *Monocystis* sp. (Apicomplexa: Gregarinomorpha: Arthrogregarida). Histomorphometric analysis highlighted the replacement of the $36.59 \pm 15.58\%$ of vesicles tissue with *Monocystis* sp. gametocysts and associated immune response using ImageJ. The activation of immune responses against *Monocystis* sp. within seminal vesicles in *Eisenia fetida* reported in this study is in contrast with previous evidences of immunological evasion reported in *Lumbricus terrestris*. In particular, inflammatory nodules, hemocytes presence and formation of characteristic brown bodies surrounding *Monocystis* sp. gametocysts within the seminal vesicles were observed. Our findings indicate that *Monocystis* sp. in *E. fetida* can induce substitution of reproductive tissue and activate energy-consuming immune responses, biasing the results of ecotoxicological endpoints in breeding systems.

Histological characterization of bone marrow adipose tissue in emaciated dogs: differentiating starvation from cachexia – a forensic perspective

Cristiano Maino¹, Veronica L'Erario¹, Silvia Sabattini¹, Lorenzo Ressel², Giuliano Bettini¹, Emanuele Ricci²

¹ Department of Veterinary Medical Sciences, Alma Mater Studiorum – University of Bologna, Ozzano dell'Emilia (BO), Italy.

² Department of Veterinary Anatomy, Physiology and Pathology, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool (UK).

Emaciation is a severe physical condition characterized by extensive loss of adipose tissue and significant muscle wasting, that may result from either starvation or cachexia. In starvation, involuntary caloric deficiency leads to a gradual decrease in basal metabolic rate, with sequential depletion of fat and lean body mass. In such instances, extreme weight loss may be considered neglect and thus has forensic importance. Conversely, cachexia is linked to primary pathological conditions that cause metabolic changes, leading to weight loss regardless of food intake.

This study aimed to histologically characterize femoral bone marrow adipose tissue in emaciated dogs to identify potential differences between starvation and neoplastic cachexia. Twenty-six dogs submitted for necropsy were retrospectively included: 10 that died from starvation, 8 with neoplastic cachexia, and 8 normal-weight controls that died from unrelated causes. Femoral bone marrow was evaluated macroscopically and histologically using haematoxylin-eosin and Alcian-PAS staining. Gelatinous transformation of bone marrow (GMT) was graded from 0 (normal) to 3 (severe). Adipocyte diameter and the percentage of Alcian-positive GMT were further quantified using digital image analysis.

Starved dogs exhibited moderate (30%) to severe (70%) GMT. In contrast, mild GMT was observed in only 3 (38%) cachectic dogs, while the remaining cachectic and all control dogs showed normal histological features. Adipocyte diameter was significantly smaller in starved dogs (22.7 μm ; range 13.9–33.8 μm) compared with cachectic (62.1 μm ; range 44.6–77.0 μm) and control dogs (56.9 μm ; range 49.7–73.7 μm ; $P < 0.001$). The percentage of GMT was significantly higher in starved dogs (43.0%) than in cachectic (15.3%) and control animals (6.4%; $P < 0.001$).

These preliminary findings suggest a strong association between GMT and starvation-related emaciation in dogs. Histological evaluation combined with digital image analysis may provide objective pathological criteria to support forensic investigations in suspected cases of neglect.

The potential resilience in Mediterranean mussels (*Mytilus galloprovincialis*) to climate-induced ocean acidification

Luciana Mandrioli¹, Antonina De Marco¹, Davide Nordio¹, Maral Anjomanibenis¹,
Maria Chiara Baiano¹, Nicolas Greggio², Alessio Bonaldo¹

¹Dipartimento di Scienze Mediche Veterinarie, Alma Mater Studiorum Università di Bologna, Bologna (BO)

²Department of Biological, Geological, and Environmental Sciences, Alma Mater Studiorum Università di Bologna, Bologna (BO)

Rising atmospheric CO₂ levels are driving seawater acidification, with projections suggesting a decrease in ocean surface pH by 0.3–0.5 units by 2100. This could impact the calcified shell of important commercial species such as the mediterranean mussel *Mytilus galloprovincialis*.

This multidisciplinary study addressed if and how predicted acidification scenarios may influence physiological responses. Commercial juveniles (shell length= 3.5 ± 0.5 mm) *M. galloprovincialis* were exposed to four experimental decreasing pH treatments (n= 450) over a 90-day exposure period. To evaluate the effect of the exposure we used different parameters: byssus production, animal tendency to aggregate (clumping), elemental fingerprint of the shells using ICP-OES. Moreover, routine histology was conducted on 48 whole samples and separately on mantle coats. Epithelial tubules of digestive gland were semiquantitatively analyzed and the percentage of atrophic tubules was scored. Mantle sections were evaluated according to their composition, the vesicular-connective tissue cells and adipogranular cells (ADG) and Alizarin Red S staining was also applied to detect presence and distribution of calcium.

After three months, byssus production did not show any differences between pH treatments, whereas as the pH decreases, the clump formation quantity decreased. No differences in calcium distribution were noted at the beginning and among the groups at the end of the trial. Also, a reduction in trace elements was observed, indicating that mussels preserve calcification but modify the fine-scale structure of their shells. The thickness of the digestive gland tubules was similar among the groups, suggesting that the animals were eating. In the mantle tissue, a trend toward reduction of the ADG with lowering pH was observed.

The integration of several indicators enhanced the understanding of the mussel' adaptability and resilience. Its plastic physiological responses might suggest a notable resilience to future global change scenarios. These types of studies are encouraged supporting sustainable aquaculture mitigation strategies.

Anatomical-site and EcPV2-dependent variation in M2 macrophage polarization in equine squamous cell carcinoma

Martinoli Ginevra¹, Avallone Giancarlo¹, Brunetti Barbara¹, Dario De Biase², Ressel Lorenzo³, Grillini Alessia⁴, Franceschini Tania⁴, Fiorentino Michelangelo^{4,5}, Sarli Giuseppe¹, Bacci Barbara¹

¹ Department of Veterinary Medical Sciences, University of Bologna, Italy

² Department of Pharmacy and Biotechnology (FaBiT), University of Bologna, Bologna, Italy

³ Department of Veterinary Anatomy Physiology and Pathology, Institute of Infection, Veterinary and Ecological Sciences, Faculty of Health & Life Sciences, University of Liverpool, Liverpool, CH64 7TE, UK

⁴ Pathology Unit, Dipartimento Interaziendale di Anatomia Patologica di Bologna (DIAP), Maggiore Hospital, Bologna, Italy

⁵ Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Italy

Macrophages play a central role in shaping the tumor microenvironment, influencing tumor progression. However, macrophage infiltration in equine squamous cell carcinomas (SCCs) remains poorly characterized.

This study aimed to quantify total macrophages and pro-tumorigenic M2 subtype in equine SCCs from anatomical locations and to investigate their association with papillomavirus infection and p53 and Ki67.

Thirty-six equine SCC samples were analyzed, including 17 ocular, 16 genital (11 penile and 5 vulvar), and 3 cutaneous tumors. Immunohistochemistry was performed using IBA1 (total macrophages) and CD204 (M2 macrophages). Positive cells were digitally quantified across the entire tumor area using QuPath and expressed as cells/mm². The M2 macrophage proportion was calculated as the CD204+/IBA1+ ratio. Results were correlated with *Equus caballus papillomavirus* type 2 (EcPV2) infection detected by *in situ* hybridization, p53 abnormalities (immunohistochemical overexpression and TP53 mutations identified by next-generation sequencing), and Ki67 proliferation index.

EcPV2 positivity was detected in 13/36 cases, TP53 mutations in 13/28 cases, and p53 overexpression in 19/36 cases. Median macrophage densities were 693.9 IBA1+ cells/mm² and 228.68 CD204+ cells/mm², with a mean CD204/IBA1 ratio of 0.49. Genital SCCs showed a significantly higher CD204/IBA1 ratio compared with ocular and cutaneous tumors ($p = 0.002$). EcPV2-positive tumors had higher total macrophage density (853 vs 484 cells/mm²; $p = 0.019$) and a higher M2 proportion (0.79 vs 0.40; $p = 0.022$). No significant associations were found between macrophage infiltrate (density or M2 proportion) and p53 IHC overexpression or TP53 mutations. However, CD204+ macrophage density positively correlated with Ki67 proliferation index ($r = 0.37$; $p = 0.024$).

Despite study limitations due to lack of correlation with clinical and prognostic data, these findings suggest that the macrophage infiltrate in equine SCC varies according to anatomical site and EcPV2 status, with proportions of pro-tumorigenic M2 macrophages in genital locations and in EcPV2-associated tumors.

SPIRO-AI: application of artificial intelligence to the pathological investigation of ovine respiratory diseases

Ludovica Mura¹, Mohammad Furqan Arshad¹, Tesfalem Mehari Berhe², Giovanni Lai¹, Antonio Varcasia¹, Maria Luisa Pinna Parpaglia¹, Elisabetta Antuofermo¹, Marta Polinas¹, Marina Antonella Sanna¹, Maria Giovanna Cancedda³, Ciriaco Ligios³, Davide Adami², Delaram Ghadir², Setayesh Ghadir², Stefano Giordano², Giovanni Pietro Burrai¹

¹ Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Sassari

² Consorzio Nazionale Interuniversitario per le Telecomunicazioni, Dipartimento di Ingegneria dell'Informazione, Università di Pisa,

³ Istituto Zooprofilattico Sperimentale della Sardegna, Sassari

With an estimated population of over three million sheep, the ovine sector represents a cornerstone of Sardinia's food security and agri-food economy. However, respiratory diseases continue to pose a major constraint, making post-mortem inspection essential for investigating pulmonary pathology and informing flock management. Since such diagnoses are largely retrospective, their value for timely on-farm intervention remains limited. In this context, we present SPIRO, an AI-based diagnostic tool for the real-time assessment of ovine lung disease.

A dataset of 993 JPEG lung images was macroscopically classified into four categories: healthy, inflammatory, neoplastic, and undetermined. To reduce class imbalance, augmentation techniques including rotation, translation, and flipping were applied. A two-stage AI pipeline was developed, combining YOLOv11 for lung region localization with EfficientNetB0 for lesion classification. At gross examination, 180 lungs were classified as healthy, 654 as inflammatory, 51 as neoplastic, and 108 as undetermined, whereas histology – used as the gold standard – identified 229 healthy lungs, 728 inflammatory lungs, and 36 neoplastic lungs. With the application of the two-stage pipeline, the neoplastic class achieved the best performance, with 99% of cases correctly classified, supporting the model's reliability in identifying tumor lesions. The healthy and undetermined classes achieved correct classification rates of 85% and 82%, respectively. The inflammatory class, as expected considering the different macroscopic patterns related to different etiologies, showed slightly lower accuracy (80%). Overall, the model achieved a weighted average precision of 0.89, recall of 0.90, and F1-score of 0.89. The trained EfficientNetB0 model based on 993 images was integrated into a web-based application (link: <https://lung-classification.streamlit.app/>) developed using the Streamlit framework, enabling users to upload lung images in commonly used formats (e.g., JPG, PNG) for automated analysis.

This study supports the integration of AI-based tools into veterinary surveillance systems to enhance disease monitoring, epidemiological data collection and rapid flock-level intervention.

soft tissue surgery in captive fish: diagnostic imaging, microscopic investigation, tailored surgical techniques and post surgical management

Gianpiero Nieddu¹, Carlo Paoletti², Mathilde Charrasse², Gaia Beatrice Maria Bianchi³, Gabriele Ghisleni^{3,4}, Tim Barbé⁵, Luciana Mandrioli⁶

¹ *Acquario e Civica Stazione Idrobiologica di Milano (MI)*, ² *Veterinary Hospital AniCura Advetia, Paris, France*, ³ *Dipartimento di Medicina Veterinaria e Scienze Animali-DIVAS, Università di Milano, Lodi (LO)*, ⁴ *Consulente Veterinario per la Sicurezza Alimentare dei Prodotti Ittici, Mercato Ittico, Milano (MI)*, ⁵ *FishCareVet, Lennik, Belgium*, ⁶ *Dipartimento di Scienze Mediche Veterinarie Alma Mater Studiorum Università di Bologna, Ozzano dell'Emilia, Bologna (BO)*

Soft tissue surgery is becoming increasingly relevant, both in “pet fish” area and in public aquaria, where the fish are housed for ornamental and teaching purposes. Nevertheless, systematic data on clinical outcomes and welfare and diagnostic protocols among the professionals of the sector are still poorly standardized. This report, based on authors’ clinical experience, focused on five selected cases further investigated through microscopic investigation: a laser-assisted wen removal in an oranda affected by impaired vision and abnormal swimming, a classic eyeball enucleation in a goldfish affected by an exophytic mass investigated with pre-operative skull radiography, a multiple cutaneous soft tissue masses treated with laser-therapy in goldfish, abdominal distension associated with celomic mass in koi carp, and finally a long term management a Mediterranean moray eel affected by multiple exophytic oral masses, investigated by radiography and computed tomography. The field of soft tissue surgery and the associated disciplines appears to have reach a turning point. The improved awareness by the “pet-fish” owners that the clinical-pathological procedures run in terrestrial species, with the appropriate adjustments can be extended to the aquatics, has provided a significant contribution. Moreover, by professional advances in anesthesia, microsurgical instrumentation, and postoperative care have made complex procedures increasingly feasible, but substantial gaps in knowledge, standardization, and training is still scarce. In addition, lacking of consensus of some frequent conditions affecting i.e. the goldfish, as the multiple cutaneous nerve sheath tumors, is a paradigmatic example that requires sharing information and cases between fish pathologists and fish health experts.

Pathological evaluation of wild fish for zoonotic bacterial and parasitic agents in freshwater ecosystems

Martina Ossola¹, Michele Macrelli¹, Giovanni Sala¹, Damiano Accurso¹, Marco Farioli², Cristian Salogni¹

¹Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", Brescia (BS)

²Dipartimento Veterinario e Sicurezza degli Alimenti di Origine Animale, Agenzia di Tutela della Salute dell'Insubria, Varese (VA)

Wild fish populations represent valuable site-specific sentinels of aquatic ecosystem health. The evaluation of pathogen circulation requires correlating pathogen detection with the identification of pathological lesions. In the context of climate change and the expanding distribution of zoonotic pathogens, accurate pathological diagnosis is crucial for safeguarding public health and preserving ecosystem integrity. To investigate these aspects, five commercially and ecologically relevant freshwater fish species from the heavily anthropized Lake Varese (Northern Italy) were examined to assess the occurrence of zoonotic infectious agents and associated pathological findings. During 2024, a total of 59 fish specimens underwent anatomopathological examination, bacteriological cultures on internal organs, and parasitological investigations for the detection of encysted metacercariae or plerocercoids in the muscular tissue by transillumination, followed by molecular identification. Bacterial growth was detected in 49.2% of specimens; 93.1% of isolates were *Aeromonas* spp. (*A. sobria*, *A. caviae*, *A. hydrophila*) and 6.9% *Pseudomonas* spp.. No macroscopic lesions typically associated with *Aeromonas* infection were observed post-mortem, suggesting visceral dissemination from skin, gills, or gut, potentially triggered by fishing-related stressors or by post-mortem spread. Therefore, lesion identification is essential, as the detection of certain bacterial species alone does not necessarily indicate disease. *Mycobacterium hiberniae*, a non-tuberculous mycobacterium, was isolated from wels catfish. The nematode *Eustrongylides excisus* was detected in 16.9% of fish, with localization varying by species: either free in the coelomic cavity or encysted in the muscular tissue. Since macroscopic lesions caused by different zoonotic parasites may appear similar, knowledge of the epidemiology and life cycle of each pathogen is essential for accurate identification. These results emphasize that systematic pathological examination, together with knowledge of the epidemiology and ecology of the etiological agents, is essential for the detection and interpretation of disease and for monitoring fish health, highlighting the need for continuous, site-specific surveillance in freshwater ecosystems.

An Open-Source Pipeline for Automated Quality Control in Large-Scale Digital Pathology

Lisa Vicentini¹, Giovanni Pellegrini¹, Lucia Minoli¹, Sara Munaro¹, Luca Bertola¹, Marco Berzacola¹, Valeria Bertani^{1*}, Federico Rocchi^{1*}

¹Evotec Aptuit (Verona) Srl, Campus Levi-Montalcini, Verona (VR)

*VB and FR contributed equally as senior authors.

Digital pathology has become a foundational component of modern diagnostic practice, enabling laboratories to transition from traditional microscopy to scalable, data-driven workflows. As institutions increasingly digitize large collections of slides, they face operational challenges related to file naming consistency, metadata extraction, and post-acquisition quality control (QC). When performed manually, these tasks are time-intensive, prone to human error, and difficult to standardize at scale.

To address these issues, we developed a fully open-source pipeline that automates slide preparation and quality assessment through a combination of publicly available tools and machine learning algorithms. Our workflow integrates GrandQC, an open-source machine learning framework for whole-slide image quality evaluation, with optical character recognition (OCR) to automatically validate slide labels and ensure consistency between physical and digital identifiers. The QC module performs systematic detection of common acquisition defects including blur, pen marks, tissue folds, debris, and out-of-focus regions providing a comprehensive and reproducible assessment of slide integrity. This automated approach significantly reduces manual workload while improving standardization and reliability across high-throughput scanning environments.

A central strength of our pipeline is the exclusive use of libraries released under permissive open-source licenses, ensuring adaptability and unrestricted deployment in both academic and commercial settings. Leveraging OpenSlide, the system supports virtually all major whole-slide image formats, maximizing interoperability across scanner vendors and laboratory infrastructures.

Overall, our framework demonstrates how open-source tools and machine learning algorithms can streamline large-scale slide digitization, reduce QC bottlenecks, and enhance the consistency of downstream computational pathology workflows. By offering a transparent, flexible, and broadly compatible solution, this pipeline supports laboratories in improving throughput, minimizing errors, and strengthening the foundations for AI-assisted diagnostic analysis.

The emerging role of *Mycobacterium chelonae* as a shared pathogen in fish mycobacteriosis and human dermatitis

Marta Polinas¹, Tiziana Cubeddu¹, Luca Pilloni², Giovanni Pietro Burras^{1,3}, Marina Antonella Sanna¹, Claudio Murgia¹, Clara Gerosa², Rossano Ambu², Daniela Fanni², Elisabetta Antuofermo^{1,3}

¹Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Sassari (SS)

²Divisione di Patologia, Dipartimento di Scienze Mediche e Sanità Pubblica, Università degli Studi di Cagliari, Cagliari (CA)

³Mediterranean Center for Disease Control, Università degli Studi di Sassari, Sassari (SS)

Non-tuberculous mycobacteria (NTM) are ubiquitous environmental pathogens in aquatic ecosystems and play an often underestimated role in both aquatic animals and humans. *Mycobacterium marinum* is the main pathogen responsible for fish mycobacteriosis and has traditionally been considered the main agent of “fish tank granuloma” in humans. *Mycobacterium chelonae*, part of the *M. chelonae-abscessus* complex, causes fish mycobacteriosis and may play an underrecognized role in granulomatous infections at the human–animal interface.

This retrospective comparative study investigated formalin-fixed, paraffin-embedded (FFPE) granulomatous lesions from 26 marine fish (Mugilidae, *Sparus aurata*, and *Dicentrarchus labrax*) and 12 human skin biopsies, previously negative for acid-fast bacilli by Ziehl–Neelsen staining. All samples underwent histopathological examination and PCR amplification with sequencing of the *hsp65* gene to identify the causative *Mycobacterium* species.

In fish, lesions consisted of multifocal chronic granulomas with a central eosinophilic necrotic core surrounded by epithelioid macrophages and peripheral spindle cells, mainly affecting the liver, while human skin biopsies showed nodular to diffuse granulomatous dermatitis, mainly involving the upper limbs. *Mycobacterium chelonae* was detected in 20/34 fish samples (58.8%) and 6/12 human biopsies (50%), with 99–100% nucleotide identity to reference strains. Phylogenetic analysis demonstrated clustering of fish- and human-derived sequences, suggesting shared environmental reservoirs.

Overall, the Ziehl–Neelsen negativity in both species highlights the diagnostic limitations of conventional histochemistry and underscores the necessity of molecular tools for an accurate detection of paucibacillary mycobacterial infections. Our results identify *M. chelonae* as a major etiological agent of piscine mycobacteriosis in Mediterranean aquaculture species and provide molecular evidence supporting its role in human granulomatous dermatitis clinically consistent with tank granuloma.

Beyond morphology: immunohistochemical profile of canine oral spindle cell tumors

Ilaria Porcellato¹, Martina Visaggio¹, Martina Bordoni¹, Chiara Brachelente¹, Monica Sforna¹

¹Dipartimento di Medicina Veterinaria, Università degli Studi di Perugia (PG)

Canine oral malignant spindle cell tumors represent a diagnostic challenge due to morphological overlap and lack of specific histological features or immunohistochemical markers. The main differential diagnoses typically include spindle cell amelanotic melanoma, spindle cell carcinoma, soft tissue sarcomas (such as fibrosarcoma, peripheral nerve sheath/perivascular tumor), hemangiosarcoma and fibroblastic osteosarcoma. Immunohistochemistry may play a pivotal role in improving diagnostic precision. The aim of this study was to investigate the immunohistochemical panel expression in canine oral spindle cell tumors to assess its usefulness in classifying these neoplasms. Fifty-two cases of canine oral spindle cell tumors were retrospectively selected, including 27 melanomas (diagnosed on pigment presence and/or previous immunohistochemical positivity for melanocytic markers), and 25 undifferentiated oral spindle cell tumors. Immunohistochemistry for Melan-A, PNL2, Sox-10, Olig-2, α -SMA, calponin, NGFR, and α -synuclein was performed. When the differential diagnosis included hemangiosarcoma, osteosarcoma or carcinoma, specific markers were additionally tested (i.e. CD31, RUNX2, pancytokeratin). Melanomas were characterized by Melan-A⁺/PNL2⁺, SOX-10⁺, calponin⁻, α -SMA⁻, Olig-2⁻, alphaSyn⁺, NGFR⁺. Among the 25 undifferentiated spindle cell neoplasms, six different groups, in terms of marker expression, were identified: 5 were reclassified as melanomas according to the above marker expression, 10 were defined as undifferentiated neuroectodermal tumors (UNT) (Sox-10⁺, α -synuclein⁺ and NGFR⁺), 4 as undifferentiated spindle cell tumors (USPT) (negative to all tested markers), 3 as osteosarcomas (negative to all tested markers, but RUNX2⁺), 2 as myoid sarcomas (α -SMA⁺/calponin⁺) and one as cytokeratin-positive anaplastic carcinoma. Results identify a peculiar phenotypical entity of the oral cavity of dogs, which shows expression of neuroectodermal markers such as Sox-10, α -synuclein and NGFR. These group of tumors may be undifferentiated spindle cell melanomas, but this hypothesis has to be proven by further biomolecular characterization; the definition of their cellular origin, together with the description of their biological behavior, is currently under investigation.

Impact of biodegradable and conventional microplastics on *Eisenia fetida* S.: an ecotoxicological and ecopathological perspective

Karen Power¹, Rebecca Leandri¹, Giorgia Rosato¹, Lorenzo Riccio², Monica Zizolfi¹, Giulia Maisto¹, Gionata De Vico¹

¹ Department of Biology, University of Naples Federico II, Naples, Italy

² Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy

Microplastics (MPs) represent an emerging issue endangering all ecosystems including soils, where the impact of both conventional and biobased ones remains controversial. Previous studies have investigated the ecotoxicological effects of conventional and biodegradable microplastics, by analysing endpoints like mortality, growth, and reproduction rate in soil invertebrates. Earthworms are part of the macrofauna of soil and are vital "ecosystem engineers" that enhance soil health by improving structure, aeration, and fertility. By living in direct contact with soil they can be exposed to a great variety of contaminants, among microplastics which can impair their health and their ecological role.

The study aimed to assess the effects of two concentrations (1% and 2%) of biodegradable and conventional high-density polyethylene microplastics on the earthworm *Eisenia fetida* (Savigny, 1826) using a combined ecotoxicological and ecopathological approach. Earthworms were raised according to OECD procedures and mortality and biomass were assessed at 0,7,14, 28 days, while routine histopathological analysis were performed on samples at day 14 to evaluate sublethal effects. Moreover, collagen deposition and mucin excretion was evaluated by Picrosirius red and Pas-ab staining respectively.

Ecotoxicological results showed increased mortality and decreased biomass across all treated groups after 14 days of exposure, while a significant reduction in offspring was only observed in 1%-biodegradable microplastics group. Ecopathological analysis revealed inflammatory and/or degenerative phenomena in the epidermal and muscular layers in all treated groups after 14 days of exposure, suggesting the presence of sublethal effects which could impair the well-being of individuals. Overall, our results suggest that the ecopathological approach combined with the classical ecotoxicological one can help explain pathological events which are behind the ecotoxicological endpoints and underline the existence of fine tissue and cell damage even when no changes are observed during ecotoxicological studies.

***Hematodinium perezii* detection in blue crab (*Callinectes sapidus*) and its temporal and spatial prevalence in northern Adriatic lagoons**

Tobia Pretto¹, Nicola Lago¹, Valentina Paolini¹, Andrea Basso¹

¹Centro Specialistico di Ittiopatologia, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD)

The expansion of the blue crab, *Callinectes sapidus*, along the northern Adriatic coast of Italy is threatening local ecosystems and aquaculture, particularly the production of Manila clam (*Ruditapes philippinarum*). This invasive species can host the parasitic dinoflagellate *Hematodinium perezii*, but no records of this infection in blue crabs exist for the northern Adriatic Sea. To investigate the presence and prevalence of *H. perezii* in *C. sapidus* in this region, seven lagoons were surveyed in spring 2024. Thirty commercial-sized specimens for each sampling site were analysed using a combination of cytological and histological analyses alongside a specific real-time PCR assay. Haemolymph was collected for rapid cytological screening and total DNA extraction. Histology of multiple organs was performed and the heart was specifically targeted to score the severity of *H. perezii* infection. *Hematodinium perezii* was detected in four lagoons, and this finding was further confirmed by Sanger sequencing. In one lagoon, monthly monitoring from March 2024 to May 2025 revealed substantial temporal variation in *H. perezii* infection prevalence. Moreover, histological evaluation in ovigerous females revealed high prevalence of aseptate hyphae in the egg mass referable to oomycetes further characterised by end-point PCR and Sanger sequencing as *Lagenidium callinectes*. The rapid population expansion and high local densities of the blue crab likely influence the observed distribution and infection patterns of *H. perezii*.

bodies recovered from water: evaluation of the post-mortem interval and diatom test in veterinary forensic pathology

Vittoria Romano¹, Giuseppe Piegari¹, Valeria Russo¹, Agata Tibullo¹, Evaristo Di Napoli¹, Davide De Biase² and Orlando Paciello^{1,3}

¹ Department of Veterinary Medicine and Animal Productions, University of Naples, Naples (NA)

² Department of Pharmacy, University of Salerno, Fisciano (SA)

³ Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA)

In both human and veterinary forensic practice, determining the cause of death in bodies recovered from water remains a major challenge. Several ancillary tests have been proposed to support the diagnosis of drowning; among these, the diatom test received considerable attention. However, its main limitation is low specificity, mainly due to the risk of post-mortem contamination. In human forensic pathology, bone marrow is considered the tissue least affected by post-mortem diatom penetration, but only few studies investigated this aspect in animals. Based on these considerations, the aims of the present study were to: 1) evaluate the decomposition process in cat cadavers experimentally submerged in water; 2) examine histological alterations in different tissues in relation to increasing submersion times; and 3) assess post-mortem diatom penetration into bone matrix and bone marrow. To these aims, 12 cats were divided into two different groups according to their age: Group A (5-7 years old) and Group B (< 1 year). The animals were submerged in water under standardized conditions of diatom concentration (160-178 in 10 ml of sample) and environmental temperature. In both groups, complete necropsy, histological examination and diatom test were performed at 7, 14 and 21 days post-submersion. Cadavers in Group A showed progressive stages of decomposition, with the chromatic phase evident at 7 days post-immersion and the emphysematous phase at 21 days. Group B also showed the colliquative phase at 7 days and reached skeletonization at 21 days. In both groups, the histological changes were mainly characterized by loss of cellular detail and nuclear fragmentation, increasing over time. Postmortem diatom contamination not detected in any assessed cases, in both bone matrix and bone marrow. These findings provide a useful reference framework for the interpretation of drowning cases in Veterinary Forensic Pathology.

Pathological effects of arsenic on the digestive gland of *Lymnaea stagnalis*

Giorgia Rosato¹, Rebecca Leandri¹, Teresa Chianese¹, Martina Dentato¹, Gionata De Vico¹, Anna Capaldo¹, Karen Power¹

¹Department of Biology, University of Naples Federico II, Naples, Italy

Industrialisation and urbanisation are recognized as significant sources of heavy metals in the environment. Human activities, such as mining, agricultural and industrial processes, and wastewater treatment can release arsenic into the environment. Significant amounts of arsenic have been frequently detected in aquatic systems, posing a serious toxicological threat to aquatic biodiversity.

This study investigates the effects of arsenic exposure on the digestive gland of the freshwater *Lymnaea stagnalis*, a model organism widely used in ecotoxicological and ecopathological studies. Ten specimens of *L. stagnalis* were exposed to 1360 µg/L arsenic for 7 days and subsequently processed for routine histopathological analyses. Moreover, the expression of Heat Shock Protein 70 was assessed by immunohistochemistry and western blotting to evaluate cellular stress. The digestive gland of control individuals consisted of digestive tubules containing three different cell types: basophilic cells, digestive cells and excretory cells. Arsenic-exposed snails showed loss of normal glandular tissue structure, a reduction in the number of basophilic cells, increased vacuolisation of digestive cells and the presence of necrotic areas associated with inflammatory infiltration. HSP70 appeared to be more highly expressed in exposed samples compared to controls, and strong immunoreactivity was detected in basophilic cells.

Our results suggest that arsenic exposure alters the physiological structure of the digestive gland and can impair metabolic functions in *L. stagnalis*. The tested concentration may exceed the detoxification capacity of the gland, potentially impairing digestive and metabolic functions. Further investigations are required to clarify the molecular mechanisms underlying arsenic toxicity in *L. stagnalis*.

Potential targets for precision therapy in canine primary splenic sarcomas: a preliminary study

Alessandra Verdi¹, Paola Roccabianca¹, Valeria Buonfrate², Silvia Dell'Aere¹, Caterina Romanello¹, Damiano Stefanello¹, Elisa Maria Gariboldi¹, Chiara Giudice¹, Roberta Ferrari¹

¹ Department of Veterinary Medicine and Animal Sciences – DIVAS, University of Milan, Lodi (LO), Italy

² Department of Veterinary Medicine, University of Bari, 70010 Valenzano (BA), Italy

Canine non-angiomatous-non-lymphomatous sarcomas (NANLs) are rare but generally aggressive primary neoplasms arising from diverse splenic stromal cell lineages. Due to their rarity and historically inconsistent classification, data on their biological behavior are limited. Beyond splenectomy, conventional cytotoxic chemotherapy has shown little to no efficacy in improving outcomes. This preliminary study evaluated the expression of potential molecular therapeutic targets, including platelet-derived growth factor receptor β (PDGFR β), CD117 (KIT), fibroblast activation protein (FAP) and, oxytocin receptor (OXTR) on retrospective NANLs.

One normal spleen (to test normal stroma components) and 14 canine NANLs were collected. Formalin-fixed, paraffin-embedded sections were stained for vimentin, Iba-1, CD31, α -smooth muscle actin (α SMA), and nerve growth factor receptor (NGFR) for the definitive diagnosis and for PDGFR β , CD117, FAP, and OXTR with antibodies for human targets but validated in the canine species. Molecular target expression was semi-quantitatively scored for percentage of positive neoplastic cells (0–10%, 11–50%, 51–80%, 81–100%), staining intensity (low, moderate, high) and expression pattern (nuclear, cytoplasmic, membranous). A molecule was considered as a promising target when more than 80% of neoplastic cells were positive.

All NANLs expressed vimentin and were Iba1 and CD31 negative. Labelling for α SMA and NGFR varied. According with morphology and phenotype, the caseload included 11 undifferentiated stromal sarcomas, 2 leiomyosarcomas and 1 liposarcoma. Normal stromal components were negative for all the considered therapeutic targets. All NANLs were CD117-negative; PDGFR β was identified in 2/14 NANLs (26 to 100% of positive neoplastic cells), FAP in 5/14 (11-80%), and OXTR (21-100%) in 13/14 cases. Staining was usually of low to moderate in intensity, with a cytoplasmic and membranous pattern for PDGFR β and FAP, and cytoplasmic only for OXTR.

Despite the limited sample size, according with the preliminary results OXTR emerges as a promising candidate molecular target for NANLs, warranting further investigations.



XXIII congresso AIPVet
23-25 Aprile 2026
San Benedetto del Tronto

Poster Presentations



Intracerebral papillary ependymoma in a cow: gross, histological and immunohistochemical characterization

Stefano Bagatella^{1,2*}, Ronja Kauer^{1,3}, Margherita Polidori^{1,4}, Anna Oevermann¹

¹ Division of Neurological Sciences, DCR-VPH, Vetsuisse Faculty, University of Bern, Bern, Switzerland

²Department of Veterinary Sciences, University of Turin, 10095 Grugliasco, Italy (current affiliation)

³Tierarztpraxis Ostalb GmbH, 73431, Aalen, Germany (current affiliation)

⁴Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland

*Presenting author

Intracranial tumors in cattle represent an exceptional finding. Among these, bovine ependymomas have only been reported sporadically, mostly appearing in surveys on bovine spongiform encephalopathy (BSE) surveillance or on cattle tumors. Despite this, morphological descriptions are exceedingly rare, and no prior immunohistochemical characterization of bovine ependymomas has been performed. Here, we describe the gross, histologic, and immunophenotypic features of a papillary ependymoma in the brain of a 7.5-year-old cow presented as a BSE suspect case. Grossly, a large, intra-axial, well-demarcated mass occupied the left cerebral hemisphere, extending from the frontal to the occipital lobe and communicating with the lateral ventricle. Histologically, the neoplasm was composed of elongated to columnar cells arranged predominantly in perivascular pseudorosettes with a prominent papillary architecture. Immunohistochemically, >95% of neoplastic cells showed strong cytoplasmic GFAP immunoreactivity, with labeling accentuated in perivascular processes, and approximately 20% were immunoreactive for Vimentin. Neoplastic cells were negative for Pan-Cytokeratin, S100, Olig2, NeuN, Synaptophysin, and E-cadherin. The combined morphologic and immunophenotypic findings supported a diagnosis of papillary ependymoma and excluded potential morphological mimics such as astroblastoma (also positive for GFAP and Vimentin, but with histological features distinct from ependymomas, including broad/coarse perivascular processes, vascular hyalinization, rhabdoid features, and eosinophilic cytoplasmic inclusions), choroid plexus tumor (positive for Pan-Cytokeratin, Vimentin, E-cadherin, GFAP) and papillary glioneuronal tumor (positive for NeuN, Synaptophysin, GFAP, S100, Olig2).

This case highlights the diagnostic challenge posed by the morphological overlap of neuroectodermal tumors, the necessity of immunohistochemistry in reaching a definitive diagnosis, and the importance of immunohistochemical characterization of brain tumors in underrepresented species like cattle. Moreover, it underscores the need for characterization of uncommon tumors in animals through immunohistochemistry, both for diagnostic accuracy and to advance our understanding of comparative pathology across various species.

Preliminary Study on the Application of Immunofixation in Serum of Cats with Inflammatory Gammopathies

Virginia Bettoni¹, Angelica Stranieri¹, Linda Pavone¹, Saverio Paltrinieri¹

¹ Department of Veterinary medicine and Animal Science – DIVAS University of Milan, Lodi

The concentration and type of immunoglobulins in serum may vary as a result of infectious-inflammatory or neoplastic diseases. Serum protein electrophoresis is a useful diagnostic and monitoring tool; however, it provides only quantitative information and does not allow the identification of the individual proteins. Immunofixation, on the other hand, makes it possible to identify the antibody isotypes present in the electrophoretic peaks. To date, no validation studies of this method have been performed in cats, and it has been used only in gammopathies of neoplastic origin, not in inflammatory forms. The aims of this study were to establish the appropriate protocol for better visualization of positive bands on feline serum, and to preliminarily assess the immunoelectrophoretic pattern in cats with inflammatory polyclonal gammopathy. For the optimization of the protocol, the immunoelectrophoretic pattern was compared in undiluted cat serum and in serum diluted 1:2, 1:4, 1:8, and 1:16. The native serum and the different dilutions were labelled with anti-whole serum, anti-IgG, anti-IgA, anti-IgM, anti- λ -chain, and anti- κ -chain antibodies. The antibodies were used both undiluted and diluted 1:2. The bands were more clearly visible when using undiluted antibodies and when serum was diluted 1:8 for labelling with anti-whole serum and anti-IgG, and 1:2 for labelling with the other antibodies. These dilutions were then applied to serum samples collected from 15 cats, 13 of which showed polyclonal inflammatory gammopathies on serum agarose gel electrophoresis and 2 without gammopathy. In all subjects, strong polyclonal positivity for IgG and λ chains was observed, together with less intense polyclonal positivity for IgM and IgA. A single case showed a possible weak polyclonal positivity to κ -chains. The results of this exploratory study highlight a largely homogeneous immunoelectrophoretic pattern in the cases considered. Further studies involving a larger number of cases and more comprehensive clinical data are therefore needed.

Atypical presentation of a nictitating membrane gland tumor in a dog: a case report.

Ilaria Bronzini¹, Marco Rondena¹, Valeria Baldassarre¹, Francesco Godizzi¹

¹ Clinica Veterinaria San Marco, Veggiano, Padova

Lacrimal gland tumors in dogs and cats are rare but well-recognized neoplasms that may arise from the gland of the nictitating membrane (NLG) or the main lacrimal gland. A recent veterinary study applied the new human WHO classification to canine and feline lacrimal gland tumors in order to improve subtype classification for research and prognostic purposes. This case report describes and characterizes, from histological and immunohistochemical perspectives, an NLG tumor in a dog with an atypical presentation, morphologically overlapping with a lipid-rich epithelial tumor similar to adenocarcinomas described in the mammary gland. A 5-year-old Boxer dog was referred for an incisional biopsy of a lesion involving the third eyelid. Based on the biopsy sample, the initial morphological diagnosis was a malignant neoplasm compatible with liposarcoma, additional immunohistochemical investigations were recommended. The dog subsequently underwent enucleation, and the infiltrative neoplasm involving the NLG was ultimately diagnosed as a poorly differentiated carcinoma. The majority of neoplastic cells were characterized by markedly distended cytoplasm containing one or two well-demarcated achromatic vacuoles causing nuclear displacement and indentation, resembling lipid-rich mammary carcinoma. Immunohistochemistry was performed using pancytokeratin (CKAE1/AE3), cytokeratin 14 (CK14), cytokeratin 5/6 (CK5/6), cytokeratin 7 (CK7), vimentin, p63, and α -smooth muscle actin (α -SMA), together with special stains including PAS, PAS-D and Alcian blue. Tumor cells were positive for CKAE1/AE3, CK14, CK5/6 and p63, and negative for vimentin, CK7, α -SMA, PAS, PAS-D and Alcian blue. Morphological and immunohistochemical findings support an epithelial/myoepithelial origin of the tumor cells, while lipid accumulation was inferred from histological features characterized by large cytoplasmic vacuoles. To the best of our knowledge this is the first report of this type of presentation for this anatomical site, expanding the spectrum of tumors reported to arise from the NLG.

Bacterial co-infection in European sea bass intended for experimental research: implications for health monitoring in aquaculture and research facilities

Fabiano Capparucci¹, Emil Gjursevic², Jessica Maria Abbate¹, Claudio Gervasi¹, Giovanni Lanteri¹, Fabio Marino¹, Carmelo Iaria¹

¹ Institute for Comparative, Experimental, Forensic and Aquatic Pathology (ICEFAP) “Slavko Bambir”, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy;

² Department for Biology and Pathology of Fishes and Bees, Faculty of Veterinary Medicine, University of Zagreb, Croatia

This study reports a case of mass mortality in European sea bass (*Dicentrarchus labrax*) intended for experimental research and originating from a Sicilian mariculture facility, caused by a natural co-infection involving three bacterial taxa. During quarantine at the Institute for Comparative, Experimental, Forensic and Aquatic Pathology (ICEFAP) “Slavko Bambir” of the University of Messina, affected fish showed anorexia, behavioural changes, and severe ulcerative lesions with deep muscle necrosis and, up to complete tail loss in advanced cases. Cytology revealed the concurrent presence of filamentous bacteria, bipolar rod-shaped bacteria and occasional scattered comma-shaped bacteria. In total, 109 specimens were examined macroscopically and by cytology and histopathology; bacterial taxa were identified by 16S rRNA gene sequencing as *Tenacibaculum maritimum*, *Photobacterium damsela* subsp. *piscicida* and *Vibrio* spp. Histopathological analysis documented systemic disease, with coagulative and/or colliquative myonecrosis, granulomatous lesions in spleen and kidney containing abundant intralesional bacteria, and chronic inflammation of mesenteric adipose tissue, consistent with a local reaction to the oil-adjuvated vaccine inoculation site. On this basis, the affected fish were excluded from experimental procedures. These findings highlight the critical role of health monitoring in mariculture facilities supplying animals for experimental research, as bacterial co-infections can undermine study validity and promote dissemination of opportunistic pathogens. The case further suggests that environmental conditions, transport-related stress, and vaccination may modulate host susceptibility. Integrated prevention – combining appropriate vaccination programs, environmental optimization, and stringent quarantine – remains essential to ensure pathogen-free animals for experimental protocols.

Proliferative disorders of the thyroid gland in teleost fish. The paradox of iodine deficiency in marine species.

Fabiano Capparucci¹, Andrea Martino^{2,3}, Jessica Maria Abbate¹, Carmelo Iaria¹, Luciana Mandrioli⁴, Gaia Pesce⁵, Maral Anjomanibenisi⁴, Barbara Brunetti⁴, Fabio Marino¹

¹ Institute for Comparative, Experimental, Forensic and Aquatic Pathology (ICEFAP) “Slavko Bambir”, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy;

² Aquarium of Genoa, Tropical Department, Costa Edutainment S.p.A., Area Porto Antico, Ponte Spinola, Genoa, 16128, Italy

³ Aquaculture Research Group (IU-ECOQUA), University of Las Palmas de Gran Canaria, Carretera de Taliarte, s/n, Telde, Las Palmas, Canary Islands, 35200, Spain

⁴ Department of Veterinary Medical Sciences, University of Bologna, Italy

⁵ Aquarium of Genoa, Veterinary Department, Costa Edutainment S.p.A., Area Porto Antico, Ponte Spinola, Genoa, 16128, Italy

Although proliferative lesions of thyroid follicular cells, distinguished in hyperplasia (simple, nodular, ectopic), adenoma (follicular and papillary types), and carcinoma (follicular, and solid types), have been documented in fish, their description can be useful for health monitoring and managing breeding conditions. The present study contributes to understanding these disorders in aquarium fish, supports the characterisation of neoplasms, and highlights the role of environmental factors in their pathogenesis. Tissue samples from three fish, *Chelmon rostratus*, *Diplodus vulgaris*, and *Apolectichthys xanthurus*, housed at the Genoa Aquarium were submitted for diagnostic purposes. They were euthanized due to highly vascularized, broad-based gill masses that were not suitable for surgical excision. In *C. rostratus*, the mass was in the right oropharyngeal cavity, whereas in the other two fish, the masses involved the left side. Grossly, the lesions were irregular, lobulated, compact, and reddish. Histopathological examination revealed the presence of diffuse hyperplastic thyroid tissue in *C. rostratus* and *D. vulgaris*, characterized by varying-sized and distended colloid-containing follicles lined by hyperplastic/hypertrophic follicular epithelial cells. In *A. xanthurus*, neoformation was predominantly a solid cellular growth pattern, with loss of thyroid follicular structure. Histopathological findings were consistent with a diagnosis of nodular hyperplasia in both *C. rostratus* and *D. vulgaris* and solid carcinoma in *A. xanthurus*. All thyroid proliferations were positive for panCK, E-cadherin, and claudin-1, and variably for PCNA, whereas TTF-1 did not show immunoreactivity. The thyroid proliferative lesions were most likely linked to iodine deficiency, given the predisposing husbandry factors such as reduced water exchange in Recirculating Aquaculture Systems (RAS), inadequate inorganic salt supplementation, ozonation, and nitrate accumulation. These conditions likely reduced bioavailable iodine, promoting thyroid proliferative disorders in fish reared in RAS. The results led to explore husbandry strategies to prevent iodine depletion in RAS, including supplementation through water and diet.

Betanodavirus infection: comparative histopathological findings in naturally infected *Epinephelus marginatus* and experimental infected *Dicentrarchus labrax*

Jessica Maria Abbate¹, Fabiano Capparucci¹, Carmelo Iaria¹, Anna Toffan², Simone Palazzolo³, Fabio Marino¹

¹ Institute for Comparative, Experimental, Forensic and Aquatic Pathology (ICEFAP) “Slavko Bambir”, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy;

² Istituto Zooprofilattico delle Venezie, National Reference Centre for Fish Mollusk and Crustacean Diseases, Legnaro, Italy.

³ Freelancer

Histopathological evaluation is a cornerstone of Viral Nervous Necrosis (VNN) diagnosis because it reveals the disease's distinctive lesion profile, corroborates viral neurotropism, and supports differential diagnosis against other neurological conditions. Furthermore, it provides a practical diagnostic approach for laboratories lacking dedicated VNN molecular testing. This study aims to provide a comprehensive description of VNN-associated histopathological lesions in *Epinephelus marginatus*, affected during a Mass Mortality Event (MME) in the Mediterranean Sea, and in *Dicentrarchus labrax* experimentally infected with Betanodavirus (RGNNV genotype) via intramuscular inoculation. In *E. marginatus*, histological examination revealed well-recognised inflammatory and neurodegenerative changes, characterised by multifocal, severe, lymphocytic–plasmacytic meningoencephalitis, spongiosis and perivascular edema, and neuronal necrosis with associated gliosis and neuronophagia. Compared with grouper, experimentally infected *D. labrax* developed lesions of moderate severity. In addition to the classic neurodegenerative and inflammatory changes affecting the brain and retina, consistent with those reported in natural infections, the experimental intramuscular challenge enabled identification of additional, underreported lesions involving the spinal cord and dorsal root ganglia. In the spinal cord, vacuolar change affected both white and grey matter and was occasionally associated with neuronal necrosis. Within the dorsal root ganglia, most neurons displayed cytoplasmic swelling, central chromatolysis and marginal pyknotic nuclei, with occasional mild macrophage and lymphocyte infiltration. Overall, Betanodavirus-induced lesions are broadly comparable across fish species and infection settings (natural vs. experimental), although severity may vary. Beyond the classic target organs (brain and retina), viral tropism for other nervous system structures appears to depend on the route of host entry and is more readily characterized under experimental conditions. Integrating histopathology with virology improves diagnostic accuracy, clarifies pathogenetic mechanisms and species-specific susceptibility, and has important implications for health surveillance and mortality management in aquaculture and natural environments. Incorporating evidence from natural and experimental infections provides a more complete picture of lesion development.

Isolation of circulating extracellular vesicles in cats: preliminary insights into their role in cancer and obesity

Cilli E.¹, Rinaldi V.¹, Giusti I.³, Poppa G.³, Musi A.², Baffoni M.¹, Boari A.¹, Dolo V.³, Crisi PE.¹, Bongiovanni L.^{1,2}

¹Dipartimento di Medicina Veterinaria, Università di Teramo, Teramo (Italy)

²Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University (Netherlands)

³Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila (Italy)

In human medicine, the link between obesity and cancer onset and progression has already been suspected and, in some cases, demonstrated. Often, obese/overweight cats with cancer are examined in clinics. We hypothesize that cancer may show different outcomes in obese/overweight and not-obese cats, since obesity and related factors released by adipocytes can influence the biological behavior of cancer cells. Among these factors, circulating extracellular vesicles (EVs) and their cargo might play a role and represent useful new biomarkers.

Aim of the present study was to isolate and analyze circulating EVs in different feline patients, establishing a protocol for future analyses.

Blood samples were collected from four groups of feline patients i) obese/overweight without cancer (3); ii) obese/overweight with cancer (6); iii) not-obese without cancer (1); iv) not-obese with cancer (5). Samples were processed to isolate EVs, by size exclusion chromatography and ultracentrifugation. The presence of EVs was confirmed by Western blot analysis of EV markers and TEM; the number of particles was counted by NTA (nanoparticle tracking analysis).

With this study we developed a protocol to isolate EVs from feline plasma that can be applied in future investigations. Statistical analysis was performed using the Mann–Whitney test to assess differences between groups and linear regression models to investigate the relationships among the variables. Even though no differences were observed in the number of particles among patients' groups, variation of particles' size was recorded.

The limited number of cases included and the EV isolation methods applied, based only on size, could have influenced the data, and improvement to the test will be implemented in future investigations.

Although preliminary, these data lay the basis for the development of a new blood-based test to predict cancer prognosis in obese/overweight cats.

Preferred type of presentation: Oral presentation

Spatial regulation of HBV replication

Michele D'Antonio¹, Monica Giannotta¹, Chiara Perucchini^{1,2}, Donato Inverso^{1,3}

¹ Division of Immunology, Transplantation, and Infectious Diseases, Unit of Vascular Pathobiology, San Raffaele Scientific Institute, Milan (MI)

² Division of Immunology, Transplantation, and Infectious Diseases, Unit of Dynamics of Immune Responses, San Raffaele Scientific Institute, Milano (MI)

³ Vita-Salute San Raffaele University, Milan (MI)

As blood flows from the portal area (PA) to the centrilobular vein (CV) of the liver lobule, endothelial cells (ECs) and hepatocytes are exposed to gradients of oxygen, nutrients, and metabolites, generating spatial heterogeneity. The Wnt/ β -catenin signalling pathway is the primary driver of this zonation, and ECs of the CV are the main source of Wnt ligands. Hepatitis B virus (HBV) is a hepatotropic virus responsible for persistent infections, cirrhosis, and hepatocellular carcinoma in humans. Because HBV replication is higher around the CV, this study aims to investigate the molecular mechanisms underlying HBV zonation and to determine how these spatial differences contribute to chronic infection.

HBV replication-competent transgenic mice were intravenously injected with an AAV8 vector overexpressing R-spondin-1, an enhancer of the Wnt/ β -catenin pathway, or with a control vector (n = 5 per group; two doses tested). Total RNA was isolated from frozen liver tissue (Promega, Z611), treated with DNase (Invitrogen, AM1907) and reverse transcribed (Invitrogen, 11756050). Gene expression was analysed by qPCR using TaqMan assays for mouse R-spondin-1 (Mm00507077_m1), glutamine synthetase (Mm00725701_s1), and E-cadherin (Mm01247357_m1), two well-known zonation markers. Nucleic acids were extracted from serum (Qiagen, 57704), and viremia was quantified by comparison with a standard curve generated from HBV plasmid DNA using a Core-specific TaqMan probe. Immunofluorescence and immunohistochemical analyses were performed using the following primary antibodies: rabbit anti-glutamine synthetase (Abcam, ab49873), goat anti-E-Cadherin (R&D, AF748), and rabbit anti-HBcAg (Abcam, ab115992).

Preliminary results indicate that hepatocyte-specific overexpression of R-spondin-1 disrupts liver zonation by expanding pericentral, GS-positive hepatocytes at the cost of periportal, E-cadherin-positive hepatocytes, at both mRNA and protein levels. This change is accompanied by an enlarged area of HBcAg-positive hepatocytes and elevated viremia levels. A better understanding of the molecular machineries differentiating pericentral and periportal hepatocytes could open new therapeutic strategies for patients with chronic HBV infection.

First report of *Cyathostoma* spp. associated airsacculitis in a rose-breasted cockatoo (*Eolophus roseicapilla*)

Chianese B.¹, Perrucci S.², Biagini L.³, De Bellis D.³, Galosi. L.³, Rossi G.³

¹Diaz Veterinary Clinic, Naples (NA)

²Department of Veterinary Sciences, University of Pisa, Pisa (PI)

³School of Bioscience and Veterinary Medicine, University of Camerino, Camerino (MC)

Avian syngamid nematodes are parasites of the respiratory tract. *Cyathostoma* spp. (family *Syngamidae*) have been well documented as occurring in birds of different orders. A young female rose-breasted cockatoo (*Eolophus roseicapilla*) was presented with an acute onset of depression and dyspnoea. Following stabilisation and radiographic studies, the bird died overnight and was referred for post-mortem examination. Endoscopy revealed diffuse pulmonary and air sac opacization with significant thickening of the right thoracic air sacs, which contained several adult nematodes. The left coelomic cavity was obstructed by caseous material, disrupting the normal regional anatomy. Necropsy demonstrated diffuse multiorgan lesions, most notably severe fibrinous airsacculitis with yellowish caseous exudate occupying most of the thoracic and caudal sac cavities, interspersed with nematodes. Histopathology showed moderate diffuse active-chronic lymphocytic hepatitis and moderate glomerulonephritis with congestion and abundant calcium urate tubular precipitates. The air sac lamina and the pericardium were markedly thickened by inflammation containing amorphous necrotic and eosinophilic exudate, including trapped strongyle-type eggs and fragmented portions of adult worms. A focal parabronchial infiltration, composed of lymphocytes, heterophiles, and eosinophils from hyperplastic BAL, was also observed. Morphological features, especially the collar structure surrounding the oral cavity, enabled the identification of the parasite as a nematode species belonging to the genus *Cyathostoma*. Although the presence of *Cyathostoma* spp. nematodes is often asymptomatic, severe forms of colonization may lead to secondary bacterial infections and death. To our knowledge, this is the first report of *Cyathostoma* spp. involvement in captive-bred parrots.

My big, fat, polyploid giant cancer cells: preliminary in vitro characterization of drug-induced polyploid giant cancer cells in canine osteosarcoma cell lines

Marcella De Maglie¹, Melania Di Pentima¹, Rosanna Vescovini², Melania Andrani¹, Silvia Fante¹, Benedetta Passeri¹, Carla Perego³, Monica Galbusera³, Damiano Stefanello⁴, Paola Roccabianca⁴, Camilla Recordati^{4,*}, Federico Armando^{1,*}

¹Dipartimento di Medicina Veterinaria, Università degli Studi di Parma, Parma, Italia

²Dipartimento di Medicina e Chirurgia, Università degli Studi di Parma, Parma, Italia

³Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milano, Italia

⁴Dipartimento di Medicina Veterinaria, Università degli Studi di Milano, Lodi, Italia

*these authors contributed equally

Canine appendicular osteosarcoma (c-OSA) is the most common form of primary bone cancer in dogs, characterized by high malignancy, local invasiveness, and limited responsiveness to conventional treatments. The primary cause of c-OSA treatment failure is chemoresistance. In several tumor types, chemoresistance has been associated with the presence of polyploid giant cancer cells (PGCCs), a small and specialized neoplastic subpopulation developing within the tumor. Although their critical role in genomic instability, tumorigenesis, metastasis, and therapy resistance is increasingly recognized, their origin and development remain poorly understood. This study aims to induce PGCCs in c-OSA tumor cell lines following chemotherapeutic treatment to further characterize PGCCs. Continuous (D17) and primary (OSA2) c-OSA cell lines as well as primary canine cutaneous fibroblast cell line were treated with increasing concentrations of carboplatin and doxorubicin and comparatively evaluated against not treated controls. After 24- and 72-hours post incubations, cell viability was assessed by MTT assay to evaluate the cytotoxic effects of the treatments. To detect PGCCs we performed flow cytometry assessing ploidy by using propidium iodide nuclear staining to measure DNA content. The results demonstrated a dose dependent decrease in overall cell viability compared to untreated controls and allowed the identification of sublethal dose capable of inducing PGCCs. Flow cytometry revealed an increased percentage of PGCCs following treatment. Combining the drug concentration and exposure time able to induce the lowest cell toxicity and the highest number of PGCCs we chose the optimal treatment to obtain PGCCs in vitro. Additional analyses, including PGCCs LC-3 expression, further confirmed their identity. These preliminary results suggest that this in vitro model may represent a useful tool for studying PGCCs induction and discover their potential as a target for novel therapeutic strategies in c-OSA.

Set up and application of an immunohistochemical method to evaluate health biomarkers in *Ostrea edulis*

Francesca Errani¹, Donatella Volpatti¹, Andrea Venerando¹, Alessia Vetri², Nicolas Scapin², Giuseppe Arcangeli², Marco Galeotti¹

¹Dipartimento di Scienze Agroalimentari, Ambientali e Animali, Università degli Studi di Udine, Udine, Italia

²Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italia

In recent years, research on bivalve mollusks has gained popularity for their importance in terms of biodiversity and economic value.

Immunohistochemistry (IHC) could be proposed as an ancillary method to investigate bivalve mollusks biology and pathology. However, the need for validated antibodies, optimized for aquatic animals and particularly for mollusks, remains a challenge.

The aim of this study was to assess the reactivity of commercially available antibodies targeting several health biomarkers, including Heat shock protein 70 (HSP70), Caspase-3 (Casp-3), Cyclooxygenase 2 (Cox-2), Cytochrome P450 1A (CYP1A) and inducible Nitric Oxide Synthase (iNOS) to allow IHC applications on flat oyster (*Ostrea edulis*).

For the implementation of the research, Western immunoblotting (WB) and IHC were employed to assess selected antibodies' specificity. Fresh-frozen tissues of flat oysters were used for WB investigations. While for the IHC, an indirect peroxidase-based assay was applied to Davidson-fixed flat oyster samples.

Although an immune labeling was detected for all the tested markers, WB revealed a reliable cross-reactivity only for three out of five antibodies, particularly HSP70, iNOS, and Caspase-3. These results underscore the importance of proper antibodies' validation and IHC optimization for mollusk research in order to obtain accurate results and avoid misinterpretations.

The selected and validated antibodies were also applied to sixty flat oysters' samples collected within the Interreg MARINET project, which aims to evaluate the health condition of flat oysters deriving from integrated multi-trophic aquaculture (IMTA) sites located in Italy and Croatia. Immunopositivity was detected in some of the oysters' tissues (gills, mantle, digestive gland and digestive system). A microscopical semi-quantitative evaluation of antigens (markers) expression level in the positive samples will be performed in order to obtain valuable information for monitoring the integrity of wild/farmed flat oyster populations, improving rearing techniques and ensuring the animal's well-being.

***Apodemus* mice as reservoirs of pathogenic *Leptospira* in Europe: evidence from published studies**

Daniele Fabbri^{1,2}, Luca Dorigo³, Laura Grassi⁴, Paola Beraldo^{1,2}

¹Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via Sondrio 2/A, Udine (UD), Italy.

²NBFC, National Biodiversity Future Center, Piazza Marina 61, Palermo (PM), Italy.

³Museo Friulano di Storia Naturale, Via C.G. Sabbadini, Udine (UD), Italy.

⁴Istituto Zooprofilattico Sperimentale delle Venezie, Via della Roggia, 100, Campoformido (UD), Italy

Leptospirosis is a major zoonotic disease caused by Gram-negative spirochetes of the genus *Leptospira*. While most human infections remain asymptomatic, severe forms such as Weil's disease and severe pulmonary haemorrhagic syndrome may occur, with mortality reaching 15%. Globally, leptospirosis is estimated to cause approximately 60,000 deaths annually. Wild rodents play a central role in the epidemiology of the pathogen, as *Leptospira* colonises the renal parenchyma of reservoir hosts and is chronically shed in urine, contaminating soil and water and facilitating transmission to susceptible hosts.

This study synthesises published evidence on the occurrence of pathogenic *Leptospira* in European *Apodemus* populations. Several species, including *Apodemus flavicollis*, *A. sylvaticus*, and *A. agrarius*, have tested positive for pathogenic leptospires such as *Leptospira interrogans*, *L. kirschneri*, and *L. borgpetersenii*. Transmission occurs through direct or indirect contact with environments contaminated by infected urine, highlighting the importance of environmental persistence in the pathogen's life cycle.

Ecological characteristics of *Apodemus* populations may contribute to the maintenance of leptospires in natural ecosystems. Rodent behaviour and habitat use can sustain environmental contamination and create infection hotspots, increasing the likelihood of exposure for humans and other hosts, particularly in peri-urban landscapes. The available evidence therefore supports the role of *Apodemus* species as contributors to the environmental circulation of pathogenic *Leptospira* in Europe, with implications for zoonotic risk at the wildlife-human interface.

Oh my gosh, I thought it was a leech! First case of *Cathaemasia hians* infection in a black stork found in Italy

Silvia Fante¹, Tiziano Iemmi², Marta Fozzer³, Melania Di Pentima¹, Marcella De Maglie¹, Marco Genchi³, Alice Vismarra³, Laura Helen Kramer³, Benedetta Passeri¹, Federico Armando¹

¹Dipartimento di Medicina Veterinaria, Università degli Studi di Parma, Parma, Italy

² Wildlife Rescue Center Rifugio Matildico - San Polo d'Enza. Reggio Emilia, Italy

³ Dipartimento di Medicina Veterinaria, Università degli Studi di Parma, Parma, Italy

The Black Stork (*Ciconia nigra*) is a large migratory bird which breeds in summer in Eurasia and winters in Eurasia and Africa. Black storks inhabit mature wetlands where they feed mostly on fish, amphibians and invertebrates. This report presents the case of *C. nigra* coming from Poland and found in Emilia-Romagna (Italy). Gross post-mortem examination revealed multiple still-viable parasites on the oral and oesophageal mucosa. Diphtheroid lesions were present. In addition, multiple mural granulomas were present in the small intestine. Stereomicroscopy of the parasites confirmed the presence of adults containing operculated eggs and miracidia, allowing us to identify them as trematodes. Routine histology of the intestinal lesions revealed young and adult stages of the parasite within the intestinal granulomas. In addition, *Candida spp* was identified in the diphtheroid oral lesions by PAS histochemistry. Histological and stereomicroscopy morphological features such as position and extension of the vitellaria suggested *Cathaemasia hians* among others. To further support this diagnosis, there are a few cases of black storks infected by *Cathaemasia hians* are reported in east Europe. This trematode is transmitted eating amphibians and gives typical oral, oesophageal and intestinal lesions. Infection by this parasite debilitates animals and affects the rate of survival of the species, affecting migration and the demography of the flock. Given the morphology of the parasite, the origin of this stork and the typical lesions presented this could be the first case of *Cathaemasia hians* infection in a black stork reported in Italy.

Bad things come in threes: a case of portal vein hypoplasia, infection by *Capillaria hepatica* and *Pneumocystis spp* in a dog.

Silvia Fante¹, Marta Fozzer², Sara Maggi³, Melania Di Pentima¹, Marcella De Maglie¹, Marco Genchi², Alice Vismarra², Laura Helen Kramer², Andrea Corsini³, Benedetta Passeri¹, Federico Armando¹

¹Dipartimento di Medicina Veterinaria, Università degli Studi di Parma, Parma, Italia, Unità di Patologia Veterinaria

² Dipartimento di Medicina Veterinaria, Università degli Studi di Parma, Parma, Italia, Unità di Parassitologia

³ Dipartimento di Medicina Veterinaria, Università degli Studi di Parma, Parma, Italia, Ospedale Veterinario Universitario Didattico, Medicina Interna

A two-year-old, intact male, mixed breed dog was referred to the University Veterinary Teaching Hospital of Parma for severe chronic gastrointestinal signs and intermittent hyperthermia. The most relevant clinicopathological findings were consistent with marked chronic inflammation, hypoproteinemia and marked elevation of hepatobiliary parameters. Faecal microscopic examination and Baermann test were negative. Diagnostic imaging revealed an interstitial-alveolar pulmonary pattern with chronic pulmonary thrombosis, hepatopathy, reduced portal vein diameter, multiple acquired portosystemic shunts, abdominal effusion and gastroenterocolopathy. Liver biopsy histopathology showed portal venous hypoperfusion consistent with primary portal vein hypoplasia and ductular reaction. Immunohistochemistry for Ki67 was negative on the bile ducts supporting a diagnosis of ductal plate malformation. One month after initial presentation, the patient developed a marked increase in abdominal effusion, multiple spontaneous haemorrhages, and died. At gross post-mortem examination the dog presented severe ascites, liver atrophy and fibrosis, and multiple acquired extrahepatic portosystemic shunts. Routine histology of the liver revealed portal vein hypoplasia and the presence of abundant nematode eggs in the biliary ducts. Morphology and size of the eggs are compatible with those of *Capillaria hepatica*. In the lung, histology revealed a bronchointerstitial pneumonia with moderate, patchy pneumocytes type 2 hyperplasia and alveolar histiocytosis. Alveolar spaces and histiocytes were filled with numerous *Pneumocystis* trophozoites. The nematode *C. hepatica* is frequently found in rodents but is rare in dogs, which can be infected by ingestion of larvated eggs found in the environment. This represents an interesting case of a combination of three different co-morbidities in a single dog.

Lingual hemangioendothelioma associated with marked elastosis in a dog

Niccolò Fonti¹, Hena Annavini², Daniel Micozzi², Danilo Mancini³, Marta Giacomazzo¹, Giovanni Martino¹, Valentina Zappulli¹, Silvia Ferro¹

¹Dipartimento di Biomedicina Comparata ed Alimentazione, Università di Padova, Legnaro (PD)

²Sibillavet Clinica Veterinaria, Sarnano (MC)

³Lab. Analisi cliniche Dott. Fioroni s.r.l, San Benedetto del Tronto (AP)

A 16-year-old neutered male Setter crossbred dog presented with a small, firm, reddish mass measuring 1 cm in diameter on the lateral margin of the tongue at the level of the mid-third. The dog lived outdoors in central Italy and had been adopted from a shelter as an adult. Thoracic radiographs and cytologic evaluation of regional lymph nodes performed for staging were unremarkable. The mass was surgically excised and submitted for histopathologic examination. Microscopically, a partially elevated, well-defined, non-encapsulated, infiltrative, moderately cellular vascular lesion extending into the lamina propria was observed. It was composed of variably sized blood-filled channels lined by spindle to plump endothelial cells supported by a delicate fibrovascular stroma; thus, a diagnosis of hemangioendothelioma was made. The cells exhibited mild anisocytosis and anisokaryosis, with < 1 mitosis per 2.37 mm^2 . Immunohistochemistry showed cytoplasmic immunolabeling for von Willebrand factor. Both within and outside the tumor, diffuse marked elastosis characterized by abundant elastic fibers highlighted by Weigert–Van Gieson staining involved the interstitium and vascular walls. To the authors' knowledge, elastosis of the lingual mucosa has not previously been reported in dogs, nor has lingual hemangioendothelioma. In human and veterinary pathology, cutaneous solar elastosis represents a hallmark of chronic actinic damage and has been associated with the development of several epithelial and mesenchymal neoplasms, including vascular tumors. Considering the dog's outdoor lifestyle and the exposed location of the lesion, a possible actinic pathogenesis of the elastosis can be hypothesized. Alternatively, oral fibroelastotic lesions unrelated to UV exposure and possibly associated with chronic trauma have been described in humans under the term "focal oral elastosis". This case highlights an unusual association between lingual hemangioendothelioma and marked subepithelial elastosis, further suggesting that elastosis may reflect an underlying etiopathogenetic process potentially contributing to neoplastic development.

Extracellular vesicles in aquatic species: a scoping review of molecular markers employed in EV characterization

Silvia Gerosa¹, Serena Ferraresso¹, Valentina Moccia¹, Cinzia Centelleghé¹, Massimo Milan¹,
Valentina Zappulli¹

¹Dipartimento di Biomedicina Comparata e Alimentazione (BCA), Università degli Studi di Padova, Legnaro (PD)

Extracellular vesicles (EVs) are lipid bilayered nanoparticles involved in intercellular communication both in humans and animals, holding potential applications in biomarker discovery, diagnostics and therapeutics. In Veterinary Medicine, EV research is a rapidly evolving field; however, EV role in aquatic species remains poorly investigated due to insufficient standardization in EV isolation and characterization protocols and to the lack of specific EV-associated molecular markers.

This scoping review aims to map existing evidence and methodological trends to support the identification for a robust panel of EV molecular markers in aquatic species of economic and ecological relevance.

The literature search was conducted in PubMed and Web of Science databases. Additionally, manual searches were performed to maximize relevant studies retrieval. Studies were included when EV isolation followed current EV study international guidelines (MISEV23) and when the characterization protocol encompassed the detection of EV markers.

Of the 92 initially identified peer reviewed studies, 49 met all inclusion criteria and were subjected to data extraction.

Results highlight that EV markers for aquatic species have been predominantly characterized by Western Blot using commercial antibodies, with few studies employing in-house produced antibodies. In particular, transmembrane proteins (e.g., tetraspanins) and cytosolic proteins (e.g., Flotillins) constitute the most investigated EV marker categories, with supporting immunoreactivity reported across several taxa. Nevertheless, key methodological gaps have been evidenced. First, EV markers to assess sample purity and detect co-isolated nanoparticles are not routinely included. Second, targeted proteins often show different molecular weights compared to the expected values. Finally, a lack of transparency is evident, as several studies do not report product details (e.g., antibodies catalogue numbers).

These findings highlight the need for a robust multimarker strategy aligned with international guidelines. Furthermore, greater transparency in reporting antibody references is essential for reproducibility, and comprehensive assessment of antibody cross-reactivity within the target species is required.

A Unique Canine Cutaneous Tumor with Squamous and Melanocytic components

Marta Giacomazzo¹, Erica Melchioti², Claudia Zanardello², Marta Vascellari², Giovanni Martino¹, Niccolò Fonti¹, Silvia Ferro¹, Valentina Zappulli¹

¹Department of Comparative Biomedicine and Food Science (BCA), Veterinary Medicine, University of Padova, Legnaro (PD)

²Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro (PD)

Squamomelanocytic tumor is a neoplasm characterized by an intimate admixture of neoplastic squamous epithelial cells and melanocytes. It is occasionally described in human medicine, but extremely rare in veterinary pathology, with only two reports involving the canine oral cavity. The aim of this study was to describe the clinicopathologic and immunophenotypic features of a primary cutaneous tumor with squamous and melanocytic components in a dog. A 10-year-old neutered female German Shepherd dog was presented for evaluation of a mass growing near the ventral attachment of the left pinna. Fine-needle aspiration cytology suggested melanoma and the mass was surgically excised. Staging, including cervical lymph node ultrasound and thoracic radiographs, revealed no evidence of metastasis. Histologically, the lesion was a well-demarcated, non-encapsulated, infiltrative biphasic neoplasm composed of heterogeneous, moderately atypical cellular populations embedded in a mild fibrous to hyalinized stroma. A minor epithelial component was arranged in cords and trabeculae and showed multifocal squamous differentiation with intracytoplasmic keratohyalin granules. This component was intimately admixed with a prevalent population of abundant spindle-to-pleomorphic cells arranged predominantly in whorls. Multifocally, angulated to spindle-shaped cells containing intracytoplasmic melanin pigment were observed singly or in small clusters. Diffusely, marked anisocytosis and anisokaryosis, together with a high mitotic index, were observed. Immunohistochemically, approximately 80% of neoplastic cells expressed vimentin, approximately 50% showed pan-cytokeratin immunoreactivity, and 20–30% were positive for Melan-A, whereas von Willebrand factor was negative. Integration of the histomorphological and immunophenotypic findings supported the diagnosis of a cutaneous malignant squamomelanocytic tumor. Differential diagnoses included malignant melanoacanthoma and combined spindle cell melanoma with sarcomatoid squamous cell carcinoma, both described in humans. To the authors' knowledge, this entity has not previously been reported in canine haired skin. This case expands the morphologic spectrum of canine cutaneous neoplasms and underscores the importance of histopathologic evaluation supported by immunohistochemistry.

What's New in the Fox? Discovery of a Novel Papillomavirus with a Putative Association with Genital Lesions in *Vulpes vulpes*

Valeria Buonfrate¹, Francesco Pellegrini¹, Marco Cordisco¹, Giulia Carone¹, Rosa Leone¹, Mauro Di Blasi², Gianvito Lanave¹, Vito Martella¹, Nicola Zizzo¹, Adriana Lo Giudice¹

¹Dipartimento of Veterinary Medicine, University of Bari – ALDO MORO, Valenzano (BA)

²Veterinary Practice, via Dietrosanto, 11, Rodio (SA)

Papillomaviruses (PVs) are host-specific pathogens recognized as well-established causes of cutaneous and mucosal lesions and neoplasms across a wide range of species. To date, 112 distinct non-human PV types have been genomically characterized, with many cases reported in various wildlife species. However, information on PVs in red fox (*Vulpes vulpes*) remains scarce. This report describes the findings from a red fox submitted for necropsy. Macroscopic examination revealed lesions consistent with genital warts, particularly affecting the vaginal mucosa. Tissue samples were collected for histology and molecular analysis. The formalin-fixed sample was first processed for histologic examination, which confirmed the non-neoplastic papillomatous nature of the lesion. The fresh tissue sample was then subjected to DNA extraction. The extracted DNA was processed using an independent enrichment protocol for circular DNA by rolling circle amplification (RCA). The resulting product was treated with T7 endonucleases and then sequenced on Oxford Nanopore MinION R10.4.1 flow cells. Sequencing revealed the presence of a viral genome (6590x reads), assembled in a complete circular sequence with Depth of Coverage of 318x. Sequence analysis showed a maximum nucleotide identity of 73.71% with *Canis familiaris* papillomavirus 28 (BK066742). These low levels of homology confirm the discovery of a highly divergent and previously undescribed *Vulpes* papillomavirus. The detection of the viral genome with the lesion suggests a possible association between the virus and the observed vaginal papilloma. This study highlights the utility of RCA–T7–Oxford Nanopore sequencing protocols for the unbiased detection of circular DNA viruses in veterinary diagnostic pathology.

Since the present findings rely solely on the identification of viral DNA, which does not confirm active viral replication, ongoing investigations, including immunohistochemistry and transcriptomic analyses, are being conducted to further evaluate the association between the virus and the lesion and to assess evidence of viral activity within affected tissues. This study reports a novel papillomavirus, demonstrating the efficacy of RCA–ONT–T7 protocols for unbiased pathogen discovery in veterinary pathology diagnostics.

Cellular death mechanisms in Pacific oysters *Crassostrea (Magallana) gigas* during pre-fattening stage in the Adriatic Sea

Livio Galosi¹, Laura Bonfili¹, Anna Maria Eleuteri¹, Alessandra Roncarati¹, Gian Enrico Magi¹

¹Scuola di Bioscienze e Medicina Veterinaria, Università di Camerino, Matelica (MC)

In last decades, demand for bivalves has increased substantially worldwide. In the Adriatic Sea, oysters farming is a profitable reality but batches are afflicted by mortality phenomena, usually in or after the summer season. A batch of Pacific oysters *Crassostrea (Magallana) gigas* was cultivated in a long-line plant in the medium Adriatic Sea, located 6 marine miles in front of the city of Civitanova Marche. Samples were collected from the moment of the diploid spat arrival from France to 10 months of farming, as five time points: December, March, July, August and September. During this period a significant growth rate was observed, but an increase of mortality in summer months. Molecular analysis did not reveal presence of specific pathogens in all the time points. In this study histological, immunohistochemical and biochemical analyses were performed to evaluate the pathological findings and to explore the pathway of cellular death thorough evaluation of specific marker as: cleaved caspase-3, LC3, cathepsin B and L and by evaluation of proteasome activity. Histologically the main pathological findings observed was atrophy of digestive gland. The degree of atrophy increased significantly during summer. IHC analyses reveled a significantly increase of LC3 and caspase-3 expression in digestive gland during summer. Biochemical analyses revealed an impairment of the ubiquitin-proteasome system (UPS) whereas lysosomal enzymes cathepsin L and cathepsin B activities significantly increased in summer as caspase-3 activity. Both the expression and activity of caspase 3 and the expression and activity of autophagic pathway increased significantly in the summer, during increased mortality months, suggesting that both apoptosis and autophagy were involved in oysters cell death.

The mollusc *Octopus vulgaris* (Linnaeus, 1758): a treasure trove of information

Luciana Mandrioli¹, Antonio Casalini¹, Barbara Brunetti¹, Sara Ciulli¹, Enrico Volpe¹,
Noemi Romagnoli¹, Carlotta Lambertini¹, Alessia Diana¹, Oliviero Mordenti¹

¹Dipartimento di Scienze Mediche Veterinarie, Alma Mater Studiorum Università di Bologna, Ozzano dell'Emilia, Bologna (BO)

The common octopus is one of the most interesting species in popular memory, due to its several intriguing features. It has a worldwide geographic distribution and is subject to active fisheries, including several FAO Major Fishing Areas, such as the Mediterranean Sea. It is a model species for biomedical and behavioural studies, and is also housed in aquarium exhibits. Despite these premises, the scientific literature documenting spontaneous diseases elicited by biological agents and abiotic factors is scattered and poorly reviewed. Favouring of reproductive trials of octopuses, which are characterized by a very peculiar biological cycle, provides several key benefits by expanding knowledge of behavioural repertoires under controlled conditions, optimizing husbandry and environmental parameters to improve welfare and reproductive output, refining and standardizing paralarval rearing and weaning, strengthening health management, and reducing reliance on wild-caught broodstock. The present report describes a multidisciplinary experience including 15 couples of adults. The animals were maintained at the aquaculture facility of Cesenatico for reproductive purposes. The water parameters of the housed tanks were periodically checked and were always within normal ranges. Different animals underwent different protocols. Microbiologic investigations looked for bacteria and viruses reported to be pathogenic to octopuses. One animal underwent clinical procedures to adapt the ultrasound and immersion anaesthesia protocols. After successful reproduction, all the animals spontaneously died in post-spawned senescence. The gross findings were Coccidiosis and whitish mineral concretions of the renal appendages in two animals, respectively. Histologically, nephridial lumina were filled with Dicyemida symbionts, which can be considered biological indicators of the whole animal's health status, as well as of the white body and the digestive gland. Managed care of these fascinating animals is crucial for monitoring physiological changes associated with senescence and for reducing the risk of disease.

Pathological findings from two stranded ocean sunfish (*Mola mola*, Linnaeus 1758)

Luciana Mandrioli¹, Sara Ciulli¹, Annalisa Zaccaroni¹, Enrico Volpe¹, Perla Tedesco¹, Andrea Gustinelli¹, Marina Silvi¹, Ángel Portillo-Moreno², David Fernández-Casado², Marcos Pérez López², Tobia Pretto³, Marilena Boscarato³, Anna Toffan³, Ludovica Di Renzo⁴, Federica Di Giacinto⁴, Chiara Profico⁴, Gabriella Di Francesco⁴

¹Department of Veterinary Medical Sciences, Alma Mater Studiorum University of Bologna (BO)

²Toxicology Unit. Veterinary School, Universidad de Extremadura, Cáceres, Spain

³Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD)

⁴Istituto Zooprofilattico Sperimentale Abruzzo e Molise Teramo (TE)

The ocean sunfish is a rare, pelagic, cosmopolitan species of negligible commercial interest and subject to unintended bycatch. Little is known about the species, with rare stranding cases and increasing non-scientific reports of its presence in the Mediterranean Sea.

Two adult ocean sunfish stranded along the northern and central Adriatic coast underwent comprehensive diagnostic investigation. Gross examination of the individual from 2023, weighed about 100 kg and exhibited crustacean copepods attached on the skin and parasites affecting various organs such as trematodes attached to the gills, cysts within the gastric mucosa and larval cestodes under the capsule of the liver. Histological examination disclosed the presence of plerocercoid larvae within the renal and hepatopancreatic tissue. Concerning the microbiological analyses (biochemical tests and MALDI-TOF), *Vibrio harveyi* was isolated and its presence in multiple tissues confirmed by PCR. About the fish stranded in 2024, it weighted about 1 ton displaying a severe helminthic infection affecting the gastrointestinal tract and a pathologic tissue around the left eye. The parasites attached to the gastric wall were histologically described as cestodes. In the hepatopancreas, sparse thin-walled parasitic cysts and multifocal peribiliary duct fibrosis were detected. The spleen showed melano-macrophagic centers hyperplasia and hypertrophic endothelia of the ellipsoids. The periocular tissue was characterized by multiple cystic-like structures deriving from the epidermis and multifocal granulomatous dermatitis; bacteriological investigation and genetic characterization of isolated bacteria identified *Staphylococcus equorum* associated to the periocular pathologic tissue. In both cases, brain tissue and eye tested negative for *Betanodavirus* by rRT-PCR. Histology revealed the female sex of both ocean sunfish. The potential role in stranding exerted by the biological agents, in conjunction with the toxicological findings (levels of essential metals and pyrethroids in the fish stranded in 2024) is discussed.

Congo Red–negative hepatic amyloidosis associated with liver rupture in a young cat

Giovanni Martino¹, Silvia Ferro¹, Federico Porporato^{2,3}, Anne-Cathrine Sarah Vogt^{4,5},
Marta Giacomazzo¹, Niccolò Fonti¹, Valentina Zappulli¹

¹Department of Comparative Biomedicine and Food Science, University of Padua, Legnaro, Italy

²Anicura Istituto Veterinario di Novara, Novara, Italy

³Clinica vet24, Pinerolo, Italy

⁴Department of Rheumatology and Immunology (RI), University Hospital, Bern, Switzerland

⁵Department for BioMedical Research (DBMR), Faculty of Medicine, University of Bern, Bern, Switzerland

This report describes an unusual case of Congo red-negative, Thioflavin S-positive hepatic amyloidosis in a juvenile cat, associated with hepatic fissuration and severe subcapsular hemorrhage. A 5-month-old male domestic shorthair cat was presented with spontaneous hemoabdomen suspected to result from hepatic rupture. Hematological evaluation showed marked abnormalities including severe anemia (low erythrocytes, hematocrit, and hemoglobin), leukocytosis, thrombocytopenia, and biochemical alterations with increased glucose, SDMA, BUN, phosphate, and hepatic enzymes (ALT, AST, ALP), together with decreased creatinine. Computed tomography revealed multifocal hypoattenuating subcapsular hepatic collections associated with irregularities of the hepatic profile, interpreted primarily as subcapsular hematomas possibly related to hepatic fissuration. Rare cavitory-like hepatic lesions and mild peritoneal effusion were also detected. At laparoscopy the liver revealed extensive areas of subcapsular hemorrhage. Multiple biopsies histologically showed severe multifocal subcapsular hemorrhages with fragmentation and necrosis of hepatic parenchyma and fibrin deposition. Throughout the whole parenchyma, a mild to moderate hyaline extracellular material was multifocally to diffusely observed predominantly along often atrophic hepatocellular cords with preserved sinusoidal lumina, suggesting deposition within the space of Disse. Occasional similar intracytoplasmic deposits were also observed in cells compatible with hepatic stellate cells and/or macrophages. Congo Red staining, repeated twice with positive controls, was negative. However, immunofluorescence staining with Thioflavin S demonstrated positive labeling of the hyaline material, consistent with amyloid aggregates. These findings support a diagnosis of hepatic amyloidosis associated with hepatic fissuration and hemorrhage. The Congo Red–negative but Thioflavin S–positive pattern highlights the importance of complementary techniques for amyloid detection. Given the very young age of the patient, a hereditary or familial form of amyloidosis cannot be excluded.

Fatal garlic (*Allium sativum*) toxicosis in a dog: gross and histopathological findings in a rare case of systemic hemolytic injury

Valentina Maza², Virginia Tagliati², Elena Biasibetti², Simona Zoppi¹, Alessia Di Blasio¹, Elena Bozzetta² and Marzia Pezzolato²

¹SS Patologia Animale Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino (TO);

²SS Istopatologia Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino (TO);

Garlic (*Allium sativum*) contains organosulfur compounds capable of inducing oxidative damage to canine erythrocytes, resulting in hemolytic anemia and methemoglobinemia. Although toxicosis caused by *Allium* species is relatively common in veterinary practice, fatal cases with detailed histopathological characterization are rarely described. This report describes a fatal case of garlic toxicosis in a 3-year-old female mixed-breed dog.

At necropsy, gastric contents consisted of food material and raw garlic cloves weighing approximately 16 g. The main gross findings included marked pulmonary hyperemia and hemorrhagic thoracic effusion.

Histopathological examination revealed multifocal myocardial hemorrhages, diffuse pulmonary capillary congestion, hepatic sinusoidal dilation associated with hemorrhagic areas, splenic hemorrhage, and dilation of renal glomerular capillaries. The overall pattern of lesions was consistent with systemic vascular injury and hypoxic damage secondary to oxidative erythrocyte injury and impaired oxygen transport.

The amount of garlic recovered from the stomach was lower than the toxic doses generally reported for dogs in the literature. This finding may suggest individual susceptibility to *Allium* toxicosis or incomplete recovery of the ingested material during necropsy.

This case highlights the diagnostic value of histopathological investigation in suspected fatal *Allium* toxicosis and contributes to expanding the limited literature describing fatal outcomes associated with garlic ingestion in dogs.

***In vitro* modelling of epithelial-fibroblast crosstalk in lung disease via 3D air-liquid interface (ALI) co-culture**

Marianna Sasso ¹, Lucia Minoli ¹, Marine Shatirishvili ¹, Federico Faggioni ¹,
Anna Sava ¹, Nicola Pianca

¹Evotec Aptuit (Verona) Srl, Campus Levi-Montalcini, Verona (VR)

The air–liquid interface (ALI) culture of primary human bronchial epithelial cells (HBECs) represents a valuable *in vitro* model that recapitulates key structural and functional features of the airway epithelium, including apical–basal polarity, epithelial barrier formation, mucociliary differentiation, and mucus secretion. However, conventional ALI monocultures lack the multicellular complexity of the lung microenvironment. In particular, epithelial–fibroblast interactions have recently been implicated as critical drivers in the pathogenesis of idiopathic pulmonary fibrosis (IPF). In this study, we developed and characterized a multicellular ALI co-culture model of HBECs and normal human lung fibroblasts (NHLF) to investigate epithelial–fibroblast crosstalk under physiological and pro-fibrotic conditions.

HBECs were seeded on Transwell inserts and cultured under submerged conditions until confluence, followed by air-lift to induce differentiation. At day 26, NHLF were seeded either on the underside of the Transwell membrane or at the bottom of the culture well. After 7 days of co-culture, cells were exposed to transforming growth factor- β 1 (TGF- β 1) for 7 days to model a pro-fibrotic environment. Cultures were characterized by histopathology, immunofluorescence for various epithelial components (ZO-1, β -acetylated tubulin, MUC5AC, and E-cadherin), and qPCR analysis of inflammatory cytokines (IL-8, IL1 β , TNF α , IL-6).

Both monoculture and co-culture systems formed a pseudostratified epithelium containing ciliated and goblet cells and expressing epithelial differentiation markers. Upon TGF- β 1 exposure, HBEC monocultures displayed marked morphological alterations, including epithelial thickening, irregular surfaces, and formation of cyst-like structures. In contrast, co-cultures maintained normal epithelial architecture. Consistently, TGF- β 1 induced approximately three-fold upregulation of IL1 β , TNF α , and IL-6 mRNA in monocultures, whereas no significant changes were observed in co-cultures.

Overall, fibroblasts exerted a protective effect against TGF- β 1-induced epithelial remodeling. This multicellular ALI model provides a physiologically relevant platform for investigating epithelial–fibroblast interactions in respiratory diseases such as IPF and may represent a valuable tool for future therapeutic discovery.

Neu5Gc Expression in Rabbit Tissues: An Exploratory Study Using Immunohistochemistry and HPLC

Maria Chiara Muollo¹, Fabio Gualazzi¹, Lucia Biagini¹, Danilo De Bellis¹, Laura Menchetti¹, Gabriele Brecchia², Sebastiana Failla³, Michela Contò³, Giacomo Rossi¹

¹Department of Veterinary Medicine, University of Camerino, Matelica (MC)

²Department of Veterinary Medicine, University of Perugia, Perugia (PG)

³ Council for Agricultural Research and Agricultural Economics Analysis, Research Centre for Animal Production and Aquaculture, Rome (RM)

Rabbits are a CMAH (cytidine monophosphate-N-acetylneuraminic acid hydroxylase) positive species, physiologically expressing the sialic acid N-glycolylneuraminic acid (Neu5Gc) synthesized from N-Acetylneuraminic acid (Neu5Ac) by the enzyme CMAH. Humans and several Western dog breeds carry a mutation in the CMAH gene, resulting in the absence of endogenous Neu5Gc. In these species, dietary intake of Neu5Gc from CMAH^{+/+} mammals can lead to its incorporation into endogenous glycoconjugates, inducing the production of circulating anti-Neu5Gc antibodies (xenosialitis). The aim of this study was to characterize the immunohistochemical distribution of Neu5Gc in rabbit gastrointestinal tract and skeletal muscle, supported by biochemical quantification. Twenty-one meat rabbits were slaughtered at 120 days of age. Samples of duodenum, ileum, cecum, colon, sacculus rotundus and longissimus dorsi muscle were collected. Neu5Gc concentrations were quantified by HPLC while, formalin-fixed, paraffin-embedded tissues were processed for immunohistochemistry using a chicken polyclonal anti-Neu5Gc antibody (BioLegend, 146903). HPLC confirmed the presence of the sialic acid in all tissues analyzed. Neu5Gc concentrations varied significantly among tissues ($p=0.001$), with the lowest median values observed in longissimus dorsi (7.27 mg/100 g) and the highest in colon (96.05 mg/100 g). In intestinal segments, immunoreactivity was predominantly observed in the epithelial compartment: a weak cytoplasmic labeling of enterocytes and a marked mucin-associated staining within goblet cells and luminal mucus were observed. Occasional discrete Neu5Gc-positive cells were detected in lymphoid areas of the sacculus. Skeletal myofibers resulted negative for Neu5Gc expression in IHC. The results suggest a tissue-specific distribution of Neu5Gc in rabbits, with predominant localization in the intestinal epithelium. The absence of immune-reaction in skeletal muscle using IHC may be due to limited antigen expression or tissue processing procedure. This information may be relevant for evaluating dietary exposure to Neu5Gc and may provide a comparative reference for interpreting the potential pathological significance of Neu5Gc in species lacking endogenous synthesis.

Oral metastatic melanoma in a captive gray wolf (*Canis lupus*): clinical management and outcome following electrochemotherapy

Margherita Orlandi¹, Rossana Cordon², Paolo Cavicchio², Andrea Zacchi², Leonardo Pasquini²,
Swan Specchi³, Ylenia Cuzzupè³, Pascaline Pey³, Riccardo Finotello³

¹ Laboratorio di Analisi Veterinarie MyLav, Rho (MI), Italy

² Giardino Zoologico di Pistoia srl, Pistoia (PT), Italy

³ Ospedale Veterinario "I Portoni Rossi", Zola Predosa (BO), Italy

Melanoma is a relatively frequent and highly aggressive oral neoplasm in domestic dogs, but is rarely reported in nondomestic canids. This report describes the clinical presentation, treatment, and outcome of a histologically confirmed oral melanoma in an 11-year-old, intact male, captive gray wolf (*Canis lupus*). The wolf was remotely darted to allow further investigations due to progressive dysphagia and sialorrhea. Oral examination under general anaesthesia revealed a 10-cm, cauliflower-like mass at the base of the tongue. Histopathology confirmed an incompletely excised, partially pigmented melanoma with a spindle–epithelioid histotype. Immunohistochemical staining for Ki-67 revealed an index >100, suggestive of aggressive biological behaviour, based on prognostic criteria established in the canine species. The tumour recurred locally two months later. Staging, including three-view thoracic radiographs, cytologic evaluation of regional lymph nodes, and abdominal ultrasonography, revealed no evidence of metastasis at the time of recurrence. Following protocols extrapolated from canine oral melanoma treatment, electrochemotherapy with intravenous bleomycin was administered two and three months after the initial diagnosis. The multimodal treatment yielded a partial tumour response and disease stabilization for 12 months; appetite and food intake normalized, with complete resolution of clinical signs. One year after diagnosis, local recurrence with regional and pulmonary metastases was documented. Palliative surgical debulking followed by electrochemotherapy was performed; however, the clinical condition declined, with the onset of apathy and anorexia four months later. The patient died during general anaesthesia and post-mortem examination confirmed widespread pulmonary and nodal metastases. To the authors' knowledge, reports of oral melanoma in gray wolves are rare. This case highlights the biological behaviour of oral malignant melanoma in a nondomestic canid, comparable to the aggressive clinical course observed in domestic dogs. Furthermore, it underscores the challenges of therapeutic decision-making in zoo species and the limited evidence guiding oncologic treatment in captive wildlife.

A rare histiocyte-rich myogenic cardiac sarcoma in a cat: resolving the differential diagnosis with stepwise immunohistochemistry

Francesca Parisi¹, Francesca Millanta¹, Laura Bigalli², Lorenzo Farace¹, Ranieri Verin¹

¹Dipartimento di Scienze Veterinarie, Università di Pisa, Pisa (PI)

² Ambulatorio Bigalli Pardini, San Giuliano Terme (PI)

Primary cardiac tumors are rare in cats and may represent a diagnostic challenge because of their low frequency and heterogeneous morphology. An 8-year-old spayed female DSH cat was presented with progressive clinical deterioration and thoracic effusion at clinical evaluation. Due to the worsening clinical condition, euthanasia was elected. At necropsy, up to 80% of the internal pericardial surface was effaced by multifocal, firm, whitish bulging irregularly round nodules. Similar lesions were also present on the endocardium and the underlying myocardium.

Histopathology was performed on FFPE tissues stained with hematoxylin and eosin. An immunohistochemical panel including cytokeratin (CK), vimentin, desmin, smooth muscle actin (SMA), Iba-1, CD163, CD204, MAC387, and iNOS was applied.

Histologically, the pericardium and epicardium were extensively effaced by a densely cellular infiltrative, poorly demarcated neoplastic proliferation extending into the superficial myocardium. The neoplasm was arranged in streams and solid areas of large spindloid to pleomorphic cells with well-defined cell borders, abundant eosinophilic cytoplasm, vesicular chromatin and prominent nucleoli. Frequent highly atypical multinucleated cells were observed.

By immunohistochemistry, neoplastic cells were cytokeratin-negative and vimentin-positive, ruling out mesothelioma and carcinoma. Iba-1 immunostaining showed non-specific background, limiting its diagnostic utility. CD163 immunolabeling highlighted numerous tumor associated macrophages (TAMs), whereas atypical neoplastic cells were negative, ruling out histiocytic sarcoma. Moreover, scattered CD204-positive, MAC387-positive and rare iNOS-positive cells further supported the presence of TAMs. Additional immunohistochemistry revealed desmin positivity in atypical neoplastic cells, while SMA was negative, supporting a myogenic origin.

Based on these findings, although α -actin was not performed, the lesion was considered consistent with a histiocyte-rich myogenic sarcoma compatible with rhabdomyosarcoma. This case highlights the importance of a stepwise immunohistochemical approach both in the diagnosis of poorly differentiated cardiac tumors in cats and in the study of TAMs, allowing differentiation between M1 and M2 macrophages.

Where are you coming from? From bioptical hypothesis to necropsy confirmation: a rare case of intra-abdominal mesothelioma in a horse

Benedetta Passeri¹, Silvia Fante¹, Rodolfo Gialletti², Silvia Napoli², Marco Ruggeri², Valentina Lazzari², Marcella De Maglie¹, Federico Armando¹, Melania Di Pentima¹

¹Dipartimento di Scienze Medico Veterinarie, U.O. Anatomia Patologica, Università degli Studi di Parma, Parma, Italia

²Dipartimento di Scienze Medico Veterinarie, U.O. Ippatria, Università degli Studi di Parma, Parma, Italia

A 11 year old female horse was presented with recurrent fever for approximately 20 days, depression, and abdominal distension. On clinical examination showed BCS 3/9, HR 76, RR 28, T 38.6 °C, mild anemia, hypoproteinemia, marked increase in serum amyloid and marked abdominal distension. Ultrasonographic examination revealed severe anechoic abdominal effusion, with numerous isoechoic masses with variably dimensions along the abdominal surface of the diaphragm, adhered to the splenic surface and the visceral peritoneum, as well as a 28 cm diameter cavitory mass with anechoic content in the right paralumbar fossa; the right kidney was not visualized.

A biopsy of the cavitory mass showed a pattern of branching papillae with fibrovascular cores lined by epithelioid cells, suggesting differential diagnoses like mesothelioma or a metastatic carcinoma perhaps of renal, pancreatic or ovarian origin.

Necropsy revealed abundant light-red ascitic effusion and nodular coalescing, whitish and firm masses adherent to the parietal and visceral peritoneum and omentum. The masses were often necrotic in their core. In the thoracic cavity, there was a little effusion and the lung showed a whitish mass in the caudal left lobe. Final histopathological diagnosis was of epithelioid mesothelioma, with tubular-papillary structures with multiple layers of neoplastic cells supported by scant fibrous stromal. Metastasis were found in local lymph nodes and left caudal lung. Immunohistochemistry showed tumor cells with cytoplasmic staining for cytokeratin, whereas vimentin was negative. Mesothelioma is a very rarely reported cancer in middle-aged horses with no obvious breed predisposition. In horses, the causes of mesothelioma are much less clearly defined than in humans: in most cases it is considered idiopathic/sporadic. Nevertheless, possible environmental factors may be taken into consideration.

Unexpected Predation: Anatomo-Pathological Description of Golden Jackal (*Canis aureus moreoticus*) Attacks on Donkeys

Pesaro Stefano ¹, Lorenzini Rita ², Benedetti Paolo ³, Baggio Alice ¹, Tomè Paolo ¹, Galeotti Marco ¹

¹ Centro di ricerca e Coordinamento per il Recupero della Fauna Selvatica; Dipartimento di Scienze Agro-alimentari, Ambientali e Animali; Università degli Studi di Udine

² Istituto Zooprofilattico Sperimentale del Lazio e della Toscana *M. Aleandri*, Grosseto

³ Ispettorato Forestale di Trieste e Gorizia, Regione Autonoma Friuli Venezia Giulia

The presence and distribution of meso- and large carnivores in Europe, particularly within the Italian territory, have markedly expanded over the last decades. The increase in carnivore populations has led to a rise in conflicts with human activities. To promote greater acceptance of these predators, authorities have introduced financial compensation schemes for damages caused by predation animals. However, access to such measures requires a professional and integrated approach. The golden jackal (*Canis aureus moreoticus*) is an opportunistic species, predominantly scavenging but also capable of active predation. However, knowledge of its predatory strategies, particularly toward larger prey, remains limited. The present study describes the lesions observed in cases of predation on donkeys attributed to the golden jackal in Friuli Venezia Giulia between 2016 and 2025. All eight individuals were females; four were found dead, while four were alive at the time of examination. Regarding body condition score, five animals were in good condition, two showed a moderate condition, and one was in poor condition. The main lesions observed were characterized by extensive and irregular lacerations involving the cutis and underlying musculature, predominantly localized in the perineal region. In particular, severe tissue disruption was evident at the level of the anus and external genitalia, with partial loss of soft tissues. The wound margins appeared uneven and frayed, with areas of hemorrhage and necrotic tissue. In some cases, lesions extended to the proximal tail region. The attribution of the predatory event was confirmed in two cases: in one instance through biomolecular investigations and in another through camera trapping. These findings highlight the importance of adopting an integrated diagnostic approach, which is essential not only for accurate ethological interpretation of predatory behavior but also, and above all, for supporting the competent authorities involved in the management of carnivores in Italy.

Histopathology in food control: the pathologist's role in ensuring food safety and authenticity

Marzia Pezzolato, Elena Biasibetti, Francesco Pennisi, Giovanna Esposito, Riccardo Provera, Alberico Franzin, Giaccio Nunzia, Elena Bozzetta

SS Istopatologia Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino (TO);

Histopathology is a fundamental tool in veterinary diagnostics, based on the recognition and interpretation of tissue alterations associated with biological, pathological, or degenerative processes. This approach, grounded in morphological pattern analysis and the critical evaluation of microstructural changes, can also be applied beyond the clinical field, including food safety and authenticity assessment. Within official controls on foods of animal origin, histological examination can detect structural alterations related to technological treatments or undeclared storage conditions.

A comprehensive validation study was performed on fish products. In Experiment 1, 142 swordfish samples were analyzed (71 fresh, 71 experimentally frozen at -20°C for ≥ 24 h). Experiment 2 included 113 samples from 36 fish species (86 frozen, 27 fresh). Experiment 3 comprised 60 marinated anchovy samples (33 frozen, 27 fresh). Experiment 4 included 118 smoked salmon samples (46 fresh, 36 frozen at -18°C , 36 stored at -3°C post-packaging). Experiment 5 analyzed 56 samples (28 fresh, 28 subjected to blast chilling at -35°C for 15 h), while Experiment 6 included 56 samples (28 fresh, 28 frozen at -40°C for 2 h). Experiment 7 evaluated 20 processed sashimi products (10 fresh, 10 frozen).

All samples were processed using standard histological protocols. A ring trial involving 10 Italian IZZSS laboratories was conducted to assess interlaboratory reproducibility.

Microscopic examination identified characteristic features of freezing damage, including sharply demarcated intracytoplasmic spaces consistent with ice crystal formation. Diagnostic performance, calculated across all experiments, showed a sensitivity of 94.2%, specificity of 97.7%, and a Cohen's kappa of 0.88, indicating strong agreement. Interlaboratory results confirmed good reproducibility.

The recent formalization of this method within a technical UNI standard highlights the growing acknowledgment of morphological analysis as a reliable tool for food authenticity assessment. In this context, histopathology assumes a strategic role, supporting official controls and contributing to the integration of biological knowledge, diagnostic methodologies, and regulatory frameworks for the prevention and detection of food fraud.

Prevalence of *Vibrio* spp. and OsHV-1 in farmed Pacific oyster (*Magallana gigas*) in Sardinia in the absence of mortality outbreaks

M. Polinas¹, G. Asara^{1,2}, D. Mandas³, F. Salati¹, C. Murgia¹, R. Zobba¹, G.P. Burrai¹, A. Alberti¹, A. Ruiu², E. Antuofermo¹

¹Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Sassari (SS)

²Istituto Zooprofilattico Sperimentale della Sardegna, Sezione di Oristano, Oristano (OR)

³Istituto Zooprofilattico Sperimentale della Sardegna, Sezione di Cagliari, Cagliari (CA)

The Pacific oyster (*Magallana gigas*) is the most farmed oyster species worldwide and is frequently affected by mass mortality events, often associated with *Vibrio* spp. and Ostreid herpesvirus-1 (OsHV-1) infections. In a previous study, we described a mortality episode associated with the presence of *Vibrio aestuarianus* on Sardinian oyster farms (Mandas *et al.*, 2020). Conversely, the present study aimed to conduct a prevalence survey of *Vibrio* spp. and OsHV-1 in Pacific oyster farms in Sardinia in the absence of mortality events.

Two hundred and ninety oysters were sampled from 9 farms in Sardinia. Samples underwent histopathology to evaluate and score hemocyte infiltration and PCR for the search of *Vibrio* spp. and OsHV-1. Histologically, 156/290 (54,8%) gill tissues and 131/290 (45,2%) mantle tissues exhibited nodular to multifocal inflammatory infiltrates with various degrees of hemocyte infiltration. Molecular analysis detected *Vibrio aestuarianus* in 5/290 (1,7%) of the gills and in 7/290 (2,4%) of the mantle tissue. *Vibrio splendidus* was found in 10/290 (3,4%) of the gills and in 12/290 (4,1%) of the mantle. OsHV-1 was detected in 31/290 (10,7%) of the gills and in 30/290 (10,3%) of the mantle.

Statistical analysis revealed a significant association between hemocyte infiltration and the presence of *V. aestuarianus* in both gill and mantle tissues only in the Tortoli lagoon ($p < 0.05$). No statistical association was observed with *V. splendidus* or OsHV-1 for any other parameter.

The results demonstrate that pathogens commonly implicated in oyster mortality events, such as *Vibrio* spp. and OsHV-1, can be detected in farmed Pacific oysters in Sardinia even in the absence of mortality outbreaks, circulating at subclinical levels within oyster populations. Establishing this baseline epidemiological scenario is important for health monitoring programs, as environmental or husbandry-related changes could shift the host–pathogen balance, potentially increasing pathogen virulence and leading to future mortality events.

Myoid markers are not normally co-expressed in canine oral spindle cell melanomas

Ilaria Porcellato¹, Ana-Alexandra Dobrin², Martina Visaggio¹, Martina Bordoni¹, Manuella Militaru², Chiara Brachelente¹, Monica Sforna¹

¹Dipartimento di Medicina Veterinaria, Università degli Studi di Perugia

² Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine of Bucharest

Canine oral melanomas are one of the most common and aggressive tumors in dogs. These tumors may pose puzzling diagnostic challenges particularly when neoplastic melanocytes are amelanotic and acquire a spindloid morphology.

In a larger study investigating the immunohistochemical profile of these tumors, in some cases we observed up to 20% of suspected neoplastic cells expressing myoid markers such as alpha-SMA and calponin. Nevertheless, the differentiation between neoplastic spindle cells and intraneoplastic reactive fibroblasts (or cancer associated fibroblasts, CAF) considering purely morphological criteria can be challenging, as the latter might express myoid markers and show some degree of atypia and occasional mitoses.

This investigation is intended to test if myoid and melanocytic markers are co-expressed by neoplastic cells in melanocytic tumors.

For this purpose, we selected 6 cases of Melan-A⁺ melanomas where alpha-SMA and calponin were multifocally expressed within the tumor (percentages ranging between 10-20% evaluated on immunohistochemistry) and submitted them to immunofluorescence to investigate potential co-localization with the nuclear melanocytic marker Sox-10.

Results showed that alpha-SMA and calponin were usually co-expressed within the tumor; however, calponin and Sox-10 never co-localized in any of the selected cases. In our experience, the number of intratumoral fibroblasts/myofibroblasts expressing alpha-SMA and calponin in canine oral spindle cell melanomas can be very high. These cells were mostly arranged in bundles, but occasionally showed a perivascular arrangement; sometimes scattered single positive cells were also detected within the tumor. The highest number of myoid cells was usually observed near areas of ulceration and at the margins of the tumor.

Our investigation shows that reactive fibroblasts/myofibroblasts can be numerous in canine oral melanocytic tumors and that the expression of myoid markers, such as calponin and alpha-SMA, appears to be restricted to a reactive, non-neoplastic, intratumoral spindle-cell population.

Preliminary investigation of polyploid giant cancer cells in spontaneous canine osteosarcoma

Caterina Romanello¹, Alessandra Verdi¹, Damiano Stefanello¹, Federico Armando², Paola Roccabianca^{1*}, Camilla Recordati^{1*}

¹Dipartimento di Medicina Veterinaria, Università degli Studi di Milano, Lodi, Italia

²Dipartimento di Medicina Veterinaria, Università degli Studi di Parma, Parma, Italia

*These authors contributed equally

Canine osteosarcoma (cOSA) represents 85% of all canine skeletal tumours and is generally characterised by high metastatic potential and poor prognosis. Polyploid giant cancer cells (PGCC) are a tumour cell subpopulation characterised by enlarged or multiple nuclei and increased DNA content. These cells can be induced by cellular stress, chemotherapy and have been associated with drug resistance, recurrence and tumour heterogeneity in diverse human malignancies. Though osteoclast-like cells or giant cells are frequently observed in osteosarcomas (in both humans and dogs) their morphology suggests they may represent a form of PGCCs. However, the role of PGCCs in canine tumors in general, and in cOSA in particular, remains largely unexplored. The aim of this study was to perform a preliminary investigation of PGCCs in cOSA (all sites). In this study, H&E-stained slides of 14 canine OSA (including a case sampled after antitumour adjuvant carboplatin treatment) were retrospectively selected and digitized. All tumours were classified according to anatomical site of development, histological subtype, grade, and mitotic count. In addition, the number of PGCCs (identified as multinucleated giant cells without association with osteoid or bone trabeculae) were counted in 2 mm². PGCCs were observed in 11/14 (79%), and among these, 6/11 (55%) had > 15 PGCCs per 2 mm². A high number of PGCCs was also observed in the case that received adjuvant carboplatin treatment (28 PGCCs in 2 mm²). No correlations between examined parameters were found, although high numbers of PGCCs were prevalent in low to intermediate grade cOSA. These preliminary findings highlight the presence of PGCCs in cOSA. Further studies including larger case numbers are warranted to determine PGCCs origin, investigate their prevalence, and clarify their biological and prognostic significance in cOSA.

Role of murine strain and chemotherapy on polyploid giant cancer cells induction in a preclinical model of high-grade serous ovarian cancer

Caterina Romanello^{1,2}, Laura Sala^{1,2}, Luca Bertola^{1,2}, Alessandra Capuano³, Samanta Muzzin³, Ilenia Pellarin³, Valentina Rossi^{3,4}, Francesca Basso-Valentina³, Gustavo Baldassarre³, Eugenio Scanziani^{1,2}, Camilla Recordati^{1,2}

¹Department of Veterinary Medicine and Animal Sciences (DIVAS), University of Milan, Lodi, Italy

²Mouse & Animal Pathology Laboratory (MAPLab), Unimi Foundation, Milan, Italy

³Centro di Riferimento Oncologico di Aviano (CRO), IRCCS, Division of Molecular Oncology, Aviano, Italy

⁴Department of Life Sciences, University of Trieste, Trieste, Italy

Ovarian cancer is one of the most prevalent malignancies in women, with high-grade serous ovarian cancer (HGSOC) representing its most aggressive and lethal subtype. Standard treatment consists of platinum-based chemotherapy; however, it has been associated with the induction of polyploid giant cancer cells (PGCCs). PGCCs are markedly enlarged neoplastic cells (at least 3 times larger than adjacent tumoral cells), characterised by a single giant pleomorphic nucleus or multiple nuclei, and tetraploid or higher DNA content. PGCCs are frequently observed in high-grade cancers, are implicated in drug resistance and tumour recurrence. Although the biological significance of PGCCs is not yet fully understood, they may represent a promising prognostic and therapeutic marker. The aim of this study is to retrospectively identify which murine strain and chemotherapy were associated with the induction of PGCCs in a murine HGSOC model. Seven studies were evaluated, involving a total of 145 mice intraperitoneally injected with murine ID8 p53^{-/-}/PTEN^{-/-} cell line and treated with different drugs (including Cisplatin, Carboplatin, a PARP inhibitor, a CDK12/13 inhibitor, and a Histone Methyltransferase Inhibitor). Tumoral burden was quantified in each case by counting intra-abdominal masses. PGCCs were identified using predefined morphological criteria and semi-quantitatively assessed. Among untreated control animals, the highest baseline number of PGCCs was observed in immune-compromised (nude and NSG) mice compared to immune-competent (C57BL/6) mice. The highest PGCCs scores were detected in mice treated with Cisplatin, Carboplatin, and the PARP inhibitor, while the CDK12/13 inhibitor, and the Histone Methyltransferase Inhibitor had no effect. These findings suggest that PGCCs induction is treatment-dependent and that the host immune response may contribute to PGCCs development, although the underlying mechanisms remain unclear. These observations highlight the importance of considering both murine strain and treatment strategy when evaluating PGCCs formation in experimental ovarian cancer models.

Immunohistochemical characterization of drug-induced Polyploid Giant Cancer Cells in a preclinical model of High Grade Serous Ovarian Cancer

Laura Sala^{1,2}, Luca Bertola^{1,2}, Caterina Romanello^{1,2}, Paola Roccabianca¹, Pietro Riccaboni¹, Alessandra Capuano³, Samanta Muzzin³, Ilenia Pellarin³, Francesca Basso-Valentina³, Gustavo Baldassarre³, Cristina Lenardi⁴, Monica Galbusera⁵, Carla Perego⁵, Eugenio Scanziani^{1,2}, Camilla Recor-dati^{1,2}

¹Department of Veterinary Medicine and Animal Sciences (DIVAS), University of Milan, Lodi, Italy

²Mouse & Animal Pathology Laboratory (MAPLab), Unimi Foundation, Milan, Italy

³Centro di Riferimento Oncologico di Aviano (CRO), IRCCS, Division of Molecular Oncology, Aviano, Italy

⁴Department of Physics, University of Milan, Milan, Italy

⁵Department of Pharmacological and Biomolecular Sciences (DISFEB), University of Milan, Milan, Italy

High-Grade Serous Ovarian Cancer (HGSOC) is the most prevalent subtype of epithelial ovarian cancer in humans and is characterized by high recurrence rates, metastatic spread, and poor prognosis. Chemotherapy remains the standard treatment; however, accumulating evidence suggests that it may induce the formation of Polyploid Giant Cancer Cells (PGCCs), a subpopulation implicated in tumor progression, therapeutic resistance, and relapse. Despite their potential clinical relevance, PGCCs remain poorly characterized and lack specific biomarkers. This study aimed to characterize PGCCs and overall tumoral response to chemotherapy using a panel of immunohistochemical (IHC) markers selected from the literature and to quantify their expression through Digital Image Analysis (DIA) in a pre-clinical HGSOC model. FFPE intra-abdominal tumors samples from 46 wildtype C57BL/6 female mice were analysed. Mice were intraperitoneally injected with 1×10^6 ID8 p53^{-/-}/PTEN^{-/-} HGSOC cells and treated with either vehicle (n = 23) or carboplatin (n = 23). Immunostained slides were digitized and analysed using QuPath (v0.5.1). Statistical analysis was performed using the Mann–Whitney U test. All tested antibodies stained PGCCs, although none demonstrated specificity. DIA revealed significant increases in γ H2AX (DNA damage), N-cadherin (EMT), CD44 (stemness), p21 (cell cycle), and LC3B (autophagy) expression in carboplatin-treated samples compared to controls. No significant differences were observed for Vimentin (EMT) and Cyclin D1 (cell cycle). These findings correlate with an increased presence of PGCCs in treated tumors and suggest that carboplatin-induced tumor responses associated with DNA damage, stemness, and autophagy, with a lesser contribution of EMT and cell cycle regulation. This integrated IHC-DIA workflow provided a comprehensive strategy for the investigation of carboplatin-related effects, even though none of the evaluated markers proved specific for PGCCs identification, highlighting the need for further studies to identify selective PGCCs biomarkers.

Funding

This research was funded by “Unione europea-NextGenerationEU, Centro Nazionale ICSC, CN00000013, Decreto MUR n. 1031 - 17/06/2022 (CUP: G43C24001920001)”.

Quantification of drug-induced Polyploid Giant Cancer Cells in a pre-clinical model of High Grade Serous Ovarian Cancer

Laura Sala^{1,2}, Luca Bertola^{1,2}, Caterina Romanello^{1,2}, Paola Roccabianca¹, Pietro Riccaboni¹, Alessandra Capuano³, Samanta Muzzin³, Ilenia Pellarin³, Francesca Basso-Valentina³, Gustavo Baldassarre³, Michelangelo Tronti⁴, Nicola Toschi⁴, Cristina Lenardi⁵, Monica Galbusera⁶, Carla Perego⁶, Eugenio Scanziani^{1,2}, Camilla Recordati^{1,2}

¹Department of Veterinary Medicine and Animal Sciences (DIVAS), University of Milan, Lodi, Italy

²Mouse & Animal Pathology Laboratory (MAPLab), Unimi Foundation, Milan, Italy

³Centro di Riferimento Oncologico di Aviano (CRO), IRCCS, Division of Molecular Oncology, Aviano, Italy

⁴Department of Biomedicine and Prevention, University of Rome-Tor Vergata, Rome, Italy

⁵Department of Physics, University of Milan, Milan, Italy

⁶Department of Pharmacological and Biomolecular Sciences (DISFEB), University of Milan, Milan, Italy

High-Grade Serous Ovarian Cancer (HGSOC) is the most prevalent and aggressive epithelial ovarian cancer subtype in humans, representing a heterogeneous disease with high recurrence, metastatic rate, and mortality due to late-stage diagnosis and drug resistance. First-line treatment is primarily based on chemotherapy, however, accumulating evidence indicates that this therapeutic approach may induce Polyploid Giant Cancer Cells (PGCCs). Since PGCCs numbers correlate with tumor grade, chemoresistance, progression, and prognosis, understanding and targeting PGCCs may improve therapeutic strategies and prognosis of HGSOC. This study aimed to develop and validate an integrated workflow combining conventional histopathology, Digital Image Analysis (DIA), and Artificial Intelligence (AI)-based approaches to quantify PGCCs in a preclinical mouse model of HGSOC. Mice were intraperitoneally injected with 1×10^6 ID8 p53^{-/-}/PTEN^{-/-} HGSOC cells and treated with either vehicle (n=23) or carboplatin (n=23). FFPE intra-abdominal tumor samples from 46 C57BL/6 female mice were analysed. Tissues were stained with H&E, digitized, and the three largest tumor masses per case were selected. Two hotspots per mass (central and peripheral) were acquired at 20× magnification, yielding 276 images. PGCCs were quantified by manual counting and by DIA using ImageJ. Additionally, tumor DNA ploidy was estimated from whole-slide H&E images using an AI-based framework (PloiViT). Carboplatin-treated tumors exhibited a significantly higher number of PGCCs than controls, as consistently demonstrated by both manual quantification and DIA. Similarly, AI-based analysis revealed increased tumor ploidy in carboplatin-treated tumors compared with controls. Concordance among the three methodologies supports the robustness and reproducibility of this multimodal approach for PGCCs quantification. Overall, this integrated strategy highlights chemotherapy-induced tumor polyploidization as a potential adaptive mechanism underlying treatment resistance in HGSOC and provides a reliable framework for PGCCs quantification in translational research.

Funding

This research was funded by “Unione europea-NextGenerationEU, Centro Nazionale ICSC, CN00000013, Decreto MUR n. 1031 - 17/06/2022 (CUP: G43C24001920001)”.

Malignant Transformation of a Benign Bone Lesion into Osteosarcoma with Massive Pulmonary and Renal Micro-metastases in a Dachshund: a Case Report

Luca Spadotto¹, Marta Giacomazzo¹, Giovanni Martino¹, Niccolò Fonti¹, Michele De Luca¹,
Valentina Zappulli¹

1. Dipartimento di Biomedicina Comparata e Alimentazione, Università degli Studi di Padova, Viale dell'Università 16 Legnaro (PD) 35020

Osteosarcoma (OSA) is the most common primary bone tumor in dogs and typically affects large and giant breeds, whereas its occurrence in small-breed dogs is uncommon. Malignant transformation from pre-existing benign bone lesions although rarely described in humans, has not been reported in veterinary medicine. In this work, we report a case of cranial OSA potentially arising from a benign bone-associated lesion with massive pulmonary and renal micro-metastases in a 10-year-old, female neutered Dachshund, which died rapidly of acute respiratory failure.

At post-mortem examination, a focal exophytic nodular lesion of the frontal bone (1.5 cm in diameter) was identified, surrounded by diffuse softening of the surrounding cranial vault. Histologically, the lesion consisted of a fibro-osseous nodule composed of abundant collagenous stroma and poorly mineralized woven bone trabeculae without osteoblastic rimming, diagnosed as fibrous dysplasia. Multifocally arising in continuity with the benign lesion, a highly cellular infiltrative non-encapsulated not well demarcated malignant population was evident. The latter was composed of fusiform to stellate atypical cells with moderate anisocytosis and anisokaryosis, occasional binucleation, and a mitotic count of 2 figures per 2.37 mm². The neoplastic cells were multifocally associated with osteoid matrix production, necrosis, and infiltration of adjacent bone trabeculae, consistent with OSA.

The pulmonary small and medium-sized vessels were characterized by massive neoplastic thromboembolism, sometimes occlusive and infiltrating the perivascular parenchyma as micro-metastases. Associated findings included diffuse severe alveolar damage with massive fibrin interstitial and interalveolar deposits, severe hemorrhages, and type II pneumocyte activation. The same neoplastic emboli were evident within the renal cortical vessels' multifocally associates with infarcts.

This case illustrates the insidious and aggressive metastatic potential of OSA unusually arising from a benign bone lesion, with renal and pulmonary micro-metastatic thromboembolism as the likely cause of acute cardiorespiratory failure and subsequent death.

.

Acute myeloid leukemia in a dog with lymph nodal round cell tumor suggestive of primary myeloid sarcoma: histological and phenotypic characterization

Filippo Spinelli¹, Luca Magna¹, Claudia Calzolari¹, Ester Salsecci¹, Valeria Baldassarre¹, Marco Rondena¹, Ilaria Bronzini¹

¹ San Marco Veterinary Clinic and Laboratory, Via dell'Industria 3, 35030 Veggiano, Italy.

Myeloid sarcoma (MS) is a rare extramedullary neoplasm of granulocytic origin described in both human and veterinary medicine. Diagnosis is challenging due to morphologic overlap with other round cell tumors and the lack of standardized diagnostic protocols. Primary MS with leukemic progression has only recently been reported in dogs (Ubiali et al., 2025). CD34, a marker of hematopoietic stem and precursor cells, is crucial for the diagnosis of hematopoietic neoplasia, confirming an immature hematopoietic phenotype. The aim of this study is to describe a case of acute myeloid leukaemia (AML) in a dog with a primary lymph nodal MS and assess the potential diagnostic role of CD34 immunohistochemistry (IHC). A 10-year-old male mixed-breed dog was presented with generalized lymphadenomegaly. Histopathology of retromandibular and popliteal lymph nodes revealed a round cell neoplasm, initially interpreted as large cell lymphoma. Neoplastic cells lacked lymphoid IHC markers (CD3, PAX5, CD79a, MUM1, granzyme B), indicating a null phenotype. Mild leukopenia was the only clinicopathologic abnormality. Two months later, complete blood count revealed pancytopenia and circulating atypical cells, suggesting leukemic progression. Flow cytometry showed expression of CD45, CD11b and CD34, while lymphoid markers were negative, confirming granulocytic origin. ICC demonstrated positivity for myeloid markers CD11c and CD18. Alkaline phosphatase staining further supported the myeloid lineage. MHC II, MPO, and MAC were negative in both ICC and IHC. CD34 IHC was negative, in both neoplastic cells and internal control. Overall findings supported a diagnosis of primary MS with progression to AML. This case contributes to the limited veterinary literature on canine MS and highlights the importance of considering non-lymphoid hematopoietic neoplasms when round cell tumors lack lymphoid marker expression. Although CD34 IHC was not informative in this case, the antibody clone has been reported to work on formalin-fixed tissues and could be further optimized for diagnostic application.

Flow cytometric assessment of Reactive Oxygen Species in canine erythrocytes: a preliminary study on possible clinical applications

Stranieri Angelica¹, Martini Valeria¹, Gilardoni Roberta¹, Paltrinieri Saverio¹

¹Department of Veterinary Medicine and Animal Science, University of Milan, Lodi, Italy

Reactive oxygen species (ROS) are highly reactive molecules involved in oxidative stress. Erythrocytes (RBCs) are particularly susceptible to oxidative damage. Erythrocyte oxidation can impair oxygen transport, contributing to secondary damage in peripheral tissues. Quantification of intracellular ROS in RBCs could therefore provide useful information on the systemic oxidative status of the organism. A flow cytometric assay based on the oxidation of 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) has recently been validated to measure ROS in RBCs of healthy dogs, but its clinical application has not yet been investigated.

The aim of this study was to apply the flow cytometric DCFH-DA test to RBC of dogs collected during the routine activity of a veterinary diagnostic laboratory, in order to evaluate its possible practical application. Sixty-two blood samples (from 15 healthy dogs and 47 sick dogs) were included. The RBC suspensions were stained with DCFH-DA and analyzed by flow cytometry to determine the Median Fluorescence Intensity (MFI) and the derived parameters, MFIRatio and MFI%, using unstained cells and cells oxidized in vitro as negative and positive controls, respectively. Results from healthy and diseased dogs (subgrouped based on the submitting hospital ward) were statistically compared.

Both MFI and MFI% were significantly higher in sick than in healthy dogs, indicating an increase in intracellular ROS. Higher values were observed in samples submitted from surgery and oncology wards and in subjects with acute onset of disease. No significant associations were observed between ROS-related parameters and the acute phase proteins C reactive protein (CRP) and paraoxonase 1 (PON-1).

This study represents the first application of flow cytometry to quantify ROS in canine RBC in clinical settings and suggests that the test is applicable in laboratory practice and could have clinical relevance, particularly in veterinary oncology. Further prospective studies on specific diseases are needed to confirm its diagnostic value.

Late-onset Krabbe-like leukodystrophy in an adult Azawakh dog

Giulia Vadori¹, Luca Spadotto¹, Cristian Falzone², Ginevra Brocca³, Massimo Castagnaro¹

¹Comparative Biomedicine and Food Science, University of Padua, Legnaro (PD), Italy

²Diagnostica Piccoli Animali, Zugliano (VI), Italy

³Fish & Shellfish Pathology Consultant, Glasgow, UK

Lysosomal storage diseases are a large group of inherited or acquired metabolic disorders affecting both mammals and birds. They result from defects in lysosomal metabolism, commonly due to mutations in genes encoding lysosomal acid hydrolases. Globoid cell leukodystrophy (GLD, Krabbe disease) is an autosomal recessive disorder caused by deficient activity of lysosomal hydrolase β -galactosylceramidase (GALC), resulting in the accumulation of toxic metabolites, particularly galactosylsphingosine (psychosine), and severe demyelination of the central and peripheral nervous systems with axonal degeneration, astroglial activation and infiltration of globoid macrophages within the white matter.

Here we describe a case of Krabbe-like leukodystrophy in an adult Azawakh dog.

A 15-year-old female Azawakh dog presented with progressive neurological signs including right-sided drifting, right head tilt, pelvic limb ataxia with a broad-based stance, proprioceptive deficits and reduced withdrawal reflexes in all four limbs. Electrodiagnostic testing revealed findings consistent with polyneuropathy. Magnetic resonance imaging revealed mild dilation of the cerebral cortical sulci and ventricular system, consistent with mild cerebral atrophy.

Due to continuous deterioration, the dog was euthanized and submitted for necropsy. Gross examination revealed bilateral depressions of the frontal and parietal lobes. Histopathological examination of the central nervous system (CNS) revealed severe diffuse leukodystrophy characterized by extensive myelin loss and white matter rarefaction associated with marked gliosis and numerous perivascular cuffs of foamy macrophages. These macrophages showed moderate-to-strong PAS positivity, resembling classical globoid cells.

Although the condition has not been previously reported in adult dogs, the observed lesions were considered indicative of globoid cell leukodystrophy. Ancillary testing, including lipid profiling of the CNS and genetic analysis of the GALC gene, would be required for definitive confirmation.

This case highlights an unusual presentation of Krabbe-like leukodystrophy in an adult Azawakh dog, similar to cases reported in humans, suggesting that GLD-like pathology may occur beyond the typical canine juvenile presentation.

Oxytocin receptor expression in canine malignant tumors: an exploratory study

Alessandra Verdi¹, Paola Roccabianca^{1*}, Damiano Stefanello¹, Elisa Maria Gariboldi¹, Roberta Ferrari¹, Luigi Auletta¹, Alessandra Ubiali¹, Valeria Grieco¹, Camilla Recordati¹, Chiara Giudice¹, Silvia Dell'Aere^{1*}

¹ Department of Veterinary Medicine and Animal Sciences – DIVAS, University of Milan, Lodi (LO)

*These authors contributed equally

In human oncology, the oxytocin receptor (OXTR) is implicated in tumor biology, exerting context-dependent and opposing effects. In veterinary oncology, its role remains poorly characterized, with studies limited to canine mammary tumors. This exploratory study assessed OXTR expression in canine apocrine gland anal sac adenocarcinoma (AGASAC), non-visceral soft tissue sarcoma (STS), jaw/appendicular osteosarcoma (OSA), oral/cutaneous malignant melanoma, and oral/cutaneous squamous cell carcinoma (SCC), to evaluate its relevance as a potential therapeutic target.

Formalin-fixed, paraffin-embedded samples from 53 canine tumors and 1 canine normal tissue microarray were analyzed. Immunohistochemistry was performed using an anti-human OXTR antibody (AbCam, ab217212, 1:100, 1h RT), validated in dogs. Expression was assessed in neoplastic cells, cancer-associated vessels (CAVs), cancer-associated fibroblasts (CAFs), and extracellular matrix (ECM).

All normal tissues variably expressed OXTR, except liver, spleen and pancreas. OXTR expression was detected in 51 of 53 selected canine tumors (96%). Neoplastic cells expressed OXTR in 89% of cases, with diffuse, intense staining observed in SCC, melanoma, and STS. CAVs expressed OXTR in 77% of tumors; CAFs were positive in 51% of tumors, predominantly in SCC. ECM was mostly negative. The AGASAC had limited positive neoplastic cells but consistent CAVs expression, whereas corresponding nodal metastases had preserved to reduced OXTR expression in neoplastic cells and increased expression in CAVs.

Overall, OXTR expression was observed in neoplastic cells and tumor microenvironment, expanding current knowledge of OXTR distribution in selected canine malignant tumors, and supporting further investigation on OXTR as a prognostic marker or therapeutic target in larger canine tumor cohorts.



XXIII congresso AIPVet
23-25 Aprile 2026
San Benedetto del Tronto

Sponsored by:



veterinary sciences

