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ABSTRACT BOOK

XXII Congresso AIPVet

PRESENTAZIONI ORALI

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PRESENTAZIONI ORALI

Malattie Infettive Emergenti e Miscellanea

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Generation of *Hypsugo savii* induced pluripotent stem cells by episomal vector reprogramming

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Bats are mammals of great interest due to their response to viral infections and to their ability to host different pathogens without being affected; hence, *in vitro* models such as organoids, could allow to deeply understand the biology of these animals and to further investigate their resilience to different zoonotic agents.
Organoids are a 3D collection of organ-specific cell types, which can be derived from embryonic stem cells (ESCs), adult stem cells (ASCs) and induced pluripotent stem cells (iPSCs). Due to the lack of commercially available veterinary ESCs lines and to the difficulties in the isolation of ASCs, iPSCs obtained by the reversion to pluripotency of somatic cells are a significant starting point for the establishment of organoids in veterinary medicine.
In this study, a *Hypsugo savii* brain cell line was established using a papain dissociation system. Western blot analysis confirmed vimentin expression, and immunofluorescence was performed to further validate cell identity. Cells were reprogrammed through the electroporation of non-integrating non-viral vectors, consisting of episomal plasmids carrying OCT3/4, SOX2, KLF4, c-MYC, LIN28 and NANOG reprogramming factors. Seven days following transfection, cells were counted and plated on matrix-coated cell culture plates. From the day after, a reprogramming medium containing human basic fibroblast growth factor, human leukemia inhibitory factor, CHIR99021, PD0325901, A83-01 and valproic acid was changed every day. Bat induced pluripotent stem cells (biPSCs) colonies appeared at around 11 days following transfection and were manually picked 17 days after electroporation. Pluripotency was confirmed by western blot and immunofluorescence for SOX2, OCT3/4, and NANOG.
In conclusion, in this study we established biPSCs which represent the mean to further develop in vitro models such as organoids, for a better assessment of host-pathogen interactions, focusing on zoonotic infective agents in a One Health perspective.

Use of the Thinprep® 2000 system for Liquid-Based Cytology of urine and body fluids in dogs and cats

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Liquid-based cytology (LBC) is a technique that uses hemolytic and mucolytic solutions to preserve cytological samples. Among the available LBC systems, ThinPrep® is widely used in human medicine, often in combination with Papanicolaou staining, but its application in veterinary cytology remains limited.
This study aimed to evaluate the performance of the ThinPrep®-2000 system for the cytological assessment of urine and body cavity effusions from dogs and cats. A total of 43 samples (22 effusions and 21 urine samples) were prospectively collected from the Veterinary Teaching Hospital-University of Padua between October 2023 and November 2024. ThinPrep® slides were stained with Papanicolaou and May-Grünwald Giemsa (MGG) and compared to cytospin-prepared slides stained with MGG. Three observers (two ECVCP diplomates and one ECVCP resident) evaluated cellularity, background, monolayer distribution, cytoarchitecture, nuclear and cytoplasmic details, assigning a 0-3 score for each parameter. In addition, each observer provided a morphological diagnosis for every sample. Cohen's Kappa test was used for intra- and inter-observer agreement, while the Mann-Whitney test was applied for techniques comparison. In effusion samples, sensitivity, specificity, and diagnostic accuracy for detecting neoplasia and inflammation were also calculated.
Intra-observer agreement was poor to slight when comparing ThinPrep® and cytospin slides, but substantial to almost perfect when evaluating ThinPrep® slides stained with different protocols. Inter-observer agreement was moderate to substantial. Significant differences were observed between the two techniques, but not between staining protocols in the ThinPrep® slides, moreover one observer assigned significantly higher scores to MGG-stained slides, regardless of the technique. Diagnostic accuracy for neoplasia ranged from 85–100%, with specificity >88% and sensitivity 33–100%. For inflammation, sensitivity exceeded specificity, except in MGG-stained slides for two observers.
Overall, ThinPrep® showed different performance compared to the gold standard, possibly due to peculiarities of veterinary cytology, such as training based on a different routine staining method.

Impact of In Ovo Probiotic and Postbiotic Treatments on Intestinal Morphology and Microbial Diversity in Broilers

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The *in ovo* technique, an effective method for early intervention in poultry, permits to administer substances directly into the egg before hatching. This study aimed to assess the effects of *in ovo* administration of two compounds. One-hundred-fifty embryonated eggs were assigned to 3 treatment groups (n=50 each). On day 17.5 of incubation, eggs received into the amniotic fluid 0.05 ml of one of the following preparations, reconstituted in Marek's disease vaccine: Group P, 1 x 10^5 CFU of a probiotic blend (FSG68/22); Group T, 50 CFU of a freeze-dried, tyndalized intestinal extract from healthy adult chickens (Italian patent n. 102021000016055) plus 1 x 10^5 CFU of the same probiotic than P; Group C, vaccine alone, as control. For 45 chickens of each group, considering 3 replicates for each group (15 chicken/replicates), meconium samples were collected immediately after hatchling, and faecal samples were obtained at 42 days of life. Moreover, zootechnical parameters were recorded during the growing cycle, where the chickens were raised at the same environmental and feeding condition. After routinary slaughtering, intestine from 5 chickens/replicate was collected for histopathological evaluation. Across all groups, meconium microbiota was dominated by the *Firmicutes*, particularly *Staphylococcaceae* and *Lactobacillaceae*. Alpha diversity increased in faecal microbiota, with *Firmicutes*, *Actinobacteriota*, and *Proteobacteria* being most prevalent. Group T showed the highest microbial diversity. The most characterising genera were *Lactobacillus* for group C, *Weisiella* for P, and *Corynebacterium* for T. Improved feed conversion ratios ($p=0.0134$) and weight gain ($p<0.0001$) were observed in Groups P and T, along with significantly increased villus height in the duodenum, ileum, and caecum ($p<0.01$). These findings support the efficacy and safety of *in ovo* administration, highlighting its potential for enhancing gut health and preventing early-life infections in poultry.

Clinical and anatomo-pathological findings associated with toxic agents in confirmed non-accidental animal poisoning cases in Northeastern Italy

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Non-accidental poisoning of animals using toxic baits continues to pose serious ethical, environmental, and public health concerns. This study aimed to investigate the association between clinical signs, gross pathological findings, and the toxic agents involved in confirmed poisoning cases. The toxic molecules primarily included metaldehyde, carbamates, anticoagulant rodenticides, ethylene glycol, organochlorines and organophosphates. A retrospective analysis was conducted on 600 cases of suspected animal poisoning reported to the Italian National Web Portal of Animal Poisoning between 2020 and 2021 in the Northeast of Italy. Among these, 219 cases with confirmed toxicological results were selected. The poisoned animals included dogs (47%), cats (36%), red foxes (8%) and birds (2%). Available data regarding clinical signs reported by the referring veterinarians (n=154) and gross lesions observed during necropsy (n=219) were extracted, standardized, and analyzed. Fisher's exact test was applied to assess associations, with statistical significance set at $p < 0.05$. A significant association was determined between poisoning events and the presence of hypersalivation or convulsions. Metaldehyde intoxication was significantly associated with tremors, incoordination, tachypnea, and aggressive behaviour, while ethylene glycol was associated with vomiting and hypothermia. Gross pathological examination revealed that the presence of suspected bait material in the gastrointestinal tract was associated with metaldehyde and carbamate poisoning. Haemorrhagic effusion and hepatic degeneration were significantly associated with anticoagulant rodenticides, while mucosal congestion was linked to metaldehyde. Organochlorine poisoning was associated with hepatic congestion and pulmonary haemorrhages. The findings underline that, while clinical signs often overlap across different toxicants and are generally nonspecific, necropsy evidence may offer more reliable indicators of the causative agent. Nonetheless, toxicological confirmation remains essential for a definitive diagnosis and the identification of the toxicant.

Retrospective survey on chelonian pathology (2006-2024)

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Chelonians constitute a large group of the reptile class in which most species are currently threatened or at risk of extinction. Despite this, there are still few studies investigating the causes of mortality. The goal of this retrospective study was to investigate the causes of mortality and the frequency of pathological findings of different species in Italy, identifying the main diseases involved in the mortality of chelonians of this caseload. One hundred and seven cases, from 7 families, 32 genera, and 36 species, were analysed from the database of the Laboratory of Veterinary Pathology of the School of Biosciences and Veterinary Medicine at the University of Camerino (2006-2024). Main lesions were classified according to organ system and type (inflammatory, chronic/degenerative, metabolic, miscellaneous) and main post-mortem tissue lesions were examined histologically. The main causes of death included chronic/degenerative (58%) especially of an infectious origin (54%) followed by acute inflammation (55%) almost always associated with an infectious disease (93%). The most affected tissues or organs included the hepato-biliary system (64%), urinary system (47%) and gastrointestinal system (30%). All of them, exempt the gastrointestinal system, are mostly affected by chronic/degenerative pathologies, particularly the urinary system. On the contrary, gastrointestinal system is affected mainly by inflammatory conditions. Evidence of an increased incidence of chronic pathologies shows that, in very stoic animals such as chelonians, preventive medical measures are essential in order to detect underlying pathologies at an early stage, especially in those species that hibernate and undergo physiological immunodepressive and metabolic phenomena that may aggravate underlying pathological conditions.

Metabolomic profile analysis of White Striping myopathy in broiler chickens

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White striping (WS) myopathy is an emerging condition of broiler chickens macroscopically characterized by white striation of skeletal muscle. The precise etiology and pathomechanism underlying WS are still to be determined; thus, the overall aim of this work was to analyse metabolomic profiles to identify biological pathways involved in the pathogenesis of WS myopathy. According to the macroscopic severity of the condition, twenty breasts were divided in 4 different groups (5 each for grade 0, 1, 2 and 3). Muscle samples were snap frozen and analysed for histopathology. Moreover, 20 g of muscle were frozen at −80°C for metabolite profiling through ^{1}H-NMR spectroscopy. Multivariate statistical analysis was used to evaluate the metabolite profile according to the presence and grade of WS. Histologically, the main findings in WS were loss of cross striations, multifocal, polyphasic necrosis and a chronic, interstitial, mostly histiocytic inflammation and fibrosis. The severity of necrosis, inflammation and fibrosis was significantly related to the severity of the macroscopic striping. H-NMR data showed that the metabolite profiles in WS were different for each group compared to normal breasts. In WS breast, metabolomic analysis allowed to find 82 differential metabolites closely related to the alteration of several pathways such as alanine aspartate and glutamate metabolism, glyoxylate and dicarboxylate metabolism, arginine biosynthesis and butanoate metabolism. Our findings suggest that the pathologic changes as well as the altered metabolic profiles observed in WS myopathy can occur due to a profound alteration of protein synthesis, energy production, nitrogen metabolism, nitric oxide production and oxidative stress. Despite the current idea that hypoxia is one of the main triggers for the establish of WS myopathy, we believe that the association of histopathology and metabolomic profile may be an open door for a better understanding of this condition.

Helix aspersa maxima: a histopathological and bacteriological survey of a new mass mortality event

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In recent years, heliciculture has gained interest as a sustainable and profitable livestock practice. However, mass mortality events in farmed snails, particularly *Helix aspersa maxima*, remain poorly studied. This study investigates a severe mortality episode in a snail farm in Southern Italy through bacteriological screening and comprehensive histopathological analysis. Microbiological evaluation was performed on ten random living snails from the field and according to ISO standards. While histopathological investigations were carried out on 100 samples of moribund *Helix aspersa maxima* randomly collected from the pens and on 10 samples of healthy *H. a. maxima* collected from outside the pens and used as controls. Tissues were fixed in Davidson solution, sectioned, and stained with Hematoxylin and Eosin and von Kossa techniques. Microbiological evaluation revealed low concentrations of *Escherichia coli*, *Pseudomonas spp.*, *Aeromonas hydrophila*, and mesophilic aerobic bacteria in both snails, while *Salmonella spp.* and coagulase-positive staphylococci were absent. The detected bacterial species, typically part of the snail's normal microbiota, showed no correlation with the lesions observed, suggesting they were contaminants rather than causative pathogens. Macroscopic examination revealed pronounced alterations in the digestive gland (DG), salivary glands (SG), and albumen gland (AG). Histologically, the DG showed calcium cell hyperplasia, necrosis, and fibrosis. Calcium spherule morphology was severely compromised, suggesting impairment of calcium metabolism. SG analysis highlighted cellular dysregulation with an increased presence of immature cytotypes and atrophic changes, while AG tissues displayed degenerative features. Based on these findings, potential pathogenic mechanisms may involve poor management practices such as inadequate nutrition, suboptimal environmental conditions, or overcrowding, which merit further investigation.

Estimation of the Post-Mortem Interval in Wild Boar: Evaluation of Cadaveric Changes and their Interaction with Environmental Factors

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Estimating postmortem interval (PMI) in wild boar cadavers is essential for forensic purposes and epidemiological investigation of re-emerging infectious diseases, such as African swine fever. Postmortem cadaveric changes (PCC) are commonly used to estimate animal PMI. However, their rate of development varies across different environmental microhabitats and seasons. A Mediterranean climate with warm, dry summers and mild winters characterises Southern Italy. This unique climatic condition makes evaluating the PCC rates difficult, leading to possible miscalculation of PMI in animals. In light of these observations, this study aims to 1) examine the decomposition rates of wild boar cadavers in a specific spring microhabitat in the Campania region and 2) investigate the time-dependent histological changes of boar skin and muscle tissues over time. For these purposes, three wild boar cadavers were placed in a forest in the municipality of Montesano Sulla Marcellana in the province of Salerno (SA) between April and June. Cadaveric changes, environmental factors, and macro- and microfauna activity were monitored over time. Furthermore, skin and muscle tissue samples were collected at 1- 4- 15- and 30 days after death for histopathological analysis. The cadavers showed almost complete skeletonisation 30 days after death. Microfauna activity reached a high level 10 days after death. Dogs and Foxes have been identified as the main animals involved in the cadaver's scavenging activity. Skin and muscle tissue showed overall good post-mortem preservation. A specific correlation between the degree of autolysis and time since death was also observed. These results provide a reference basis for better evaluating PCC in wild boars in the Campania region. Furthermore, our study suggests that skin and muscle histological changes could be used as complementary tools for investigating PMI in wild boars.

Animal Poisonings in a Forensic Context: A Retrospective Review of Cases from the Veterinary Pathology Service, University of Padua (2017–2024)

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Intoxication cases reported to the Veterinary Pathology Service of the University of Padua have never been systematically recorded or thoroughly analyzed. For this reason, the present study examined the Simbavet 3.0 management software database, used for archiving necropsy reports, analyzing the cases recorded between 2017 and 2024. The survey was conducted using specific keywords, including "intoxication," "poisoning," and "toxins," with the aim of identifying suspected cases of toxic exposure.

Data analysis revealed nine cases of confirmed fatal intoxication, along with twelve additional cases of suspected intoxication, where a toxicological cause could not be definitively confirmed, although the macroscopic and histological lesions observed were consistent with toxic injury. In particular, these included one case of accidental exposure to Carbofuran in a cat, three episodes of lethal poisoning from carbon dioxide inhalation in dogs, and two cases of death in donkeys attributable to ingestion of *Taxus baccata* L. Finally, two cattle and one alpaca were subjected to standardized *post-mortem* investigations with subsequent analytical toxicological investigation for toxins attributable to *Nerium oleander* L.

The results obtained made it possible to identify several causes of death attributable to exposure to toxic substances, which, in agreement with the scientific literature, represent some of the main etiologies of intoxication in animals. In addition, the study emphasized the importance of a standardized sampling protocol and of a multidisciplinary approach in the diagnosis in veterinary pathology, highlighting the fundamental role of integration between different disciplines, including forensic toxicology and forensic botany. This methodology is confirmed as the most effective in reaching a definitive diagnosis of fatal intoxication in the veterinary field.

Quantifying cerebellar neuronal loss in feline neurological diseases using Deep Learning: a novel approach to reduce subjectivity in evaluation

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A promising application of artificial intelligence (AI) is addressing issues related to subjective evaluations in veterinary pathology, particularly in neuropathology, where assessment of morphological features such as neuronal loss and astrogliosis often depends on operator appraisal. The integration of AI can improve standardization, objectivity, and reproducibility, while also enabling quantitative analyses overcoming human feasibility - for example, providing precise cell counts rather than relying on visual estimations.

We present an AI-based method to quantify cerebellar neuronal loss in some feline neurological disorders. The model was tested on HE-stained sections from five affected cats (including lysosomal storage disease and cerebellar abiotrophy) and five matched controls. The expansion of the dataset is currently underway.

Cells in each sample were identified using the CISCA segmentation framework, a convolutional neural network originally trained on Nissl-stained images from the CytoDArk0 dataset. To enhance its performance on HE-stained Whole Slide Images (WSIs), we applied stain augmentation to the training set and refined CISCA's segmentations by annotating additional HE-stained patches. These patches were carefully annotated to delineate cell contours and distinguish Purkinje cells from other cell types. WSIs were downsampled and normalized to match a reference HE image. Once trained, the CISCA model was applied to our samples. Cells were segmented with CISCA, cerebellar layers annotated in QuPath, and each cell assigned to a layer via morphological reconstruction. Cell density was calculated as the number of detected cells divided by layer area.

The analysis showed a marked reduction in neuronal density in both Purkinje and granular layers of affected cats compared to controls, with no significant differences in the molecular layer. A statistically significant reduction (p-value < 0.1) was observed in the granule cell layer.

This study represents an initial step toward a robust, AI-based quantitative model applicable to various neurological diseases and potentially adaptable across species.

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Addressing current challenges of emerging therapies: pitfalls in the assessment of irradiated mice in Bone Marrow Transplantation studies

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Bone marrow transplantation (BMT) is the treatment of choice for many leukemias, solid tumors, and metabolic diseases. The field of bone marrow research is highly dependent on *in vivo* experiments, because *in vitro* techniques do not mimic these complicated *in vivo* systems. Whole-body irradiation is one of the most commonly used tools for myeloablation of the recipient's bone marrow. Ionizing radiation causes breaks in the DNA double-strands thus it mostly affects mitotically active cells. The DNA breaks occur in multiple sites, and damage is so severe that the cellular repair systems are unable to fix the affected DNA. Consequently, this damage leads to cell death through either necrosis or apoptosis. The cells of the hematopoietic system and gastrointestinal tract are extremely sensitive to irradiation because they are always mitotically active. With any therapy that has the potential to damage cellular DNA and cause immunosuppression comes the risk of developing secondary neoplasia, a variety of infections, and a decrease in hematopoietic cell populations. Delayed irradiation-related effects can occur in kidneys, lungs and other organs, such as testes, ovaries, eyes, and adrenal glands. These irradiation-related findings need to be identified and properly considered in the evaluation of the results of a study.
Examples of irradiation-associated lesions commonly encountered in GLP toxicology and tumorigenicity studies are shown. We notice significant differences in timing and sensitivity of tissues to irradiation among strains and age of animals as well as irradiation-related procedures (source, irradiator, dose, single or repeated dose). Historical data collection is central for the recognition of confounding effects, such as the development of hematologic or solid tumors and non-neoplastic lesion in irradiated mice, and to provide weight of evidence conclusions for the definition of safety profile of new therapeutic treatments.

Perinatal exposure to short-chain PFASs impairs cognitive function in rats: a potential role of neuroinflammation

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Per- and polyfluoroalkyl substances (PFASs) are a large family of organic compounds used in a variety of industries and are considered contaminants of emerging concern due to their persistence and bioaccumulative potential. This study aimed to evaluate the effects of perinatal exposure to two short-chain PFASs, PFBA and GenX, on neurodevelopment and to explore their potential association with neuroinflammation and neuronal impairment. Female Sprague-Dawley rats received dietary exposure to GenX or PFBA at doses of 0.5 or 5.0 mg/kg body-weight/day, from 30 days before mating and continuing throughout pregnancy and lactation. Adult offspring were assessed for cognitive performance using the Morris Water Maze (MWM). Immunohistochemical analyses were conducted on formalin-fixed, paraffin-embedded (FFPE) coronal hemibrains (Bregma -2.60) from female offspring exposed to the high dose (5.0 mg/kg/day), quantifying the percentage of immunoreactivity (%IR) for Iba1, GFAP, and MAP2 using NIS-Elements software. Exposure to both PFASs impaired spatial learning and memory in adult rats, demonstrated by reduced performance in the MWM probe test (one-way ANOVA, Dunn's test: males – PFBA 0.5 mg/kg, p=0.0147; PFBA 5.0 mg/kg, p=0.0046; GenX 5.0 mg/kg, p=0.00425; females – PFBA 5.0 mg/kg, p=0.0241; GenX 5.0 mg/kg, p<0.0001). In female offspring, PFBA exposure led to an increase in Iba1 %IR in the hippocampus (p=0.0094) and hypothalamus (p=0.0204), while GFAP %IR was elevated in the hippocampus for both PFBA (p=0.0422) and GenX (p=0.0248), and in the parietal cortex for PFBA (p=0.0058). No differences were observed in MAP2 %IR. These findings indicate that perinatal exposure to short-chain PFASs causes long-lasting cognitive impairments and glial activation in several brain areas, suggesting that neuroinflammatory processes may contribute to these deficits.

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

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High-Grade Serous Ovarian Cancer (HGSOC) is the most common subtype of ovarian cancer representing a highly heterogeneous disease with high recurrence, metastatic rate, and mortality due to late-stage diagnosis and drug resistance. First-line treatment involves chemotherapy, but these treatments often result in recurrence and drug-resistance. Progression is associated with the development of Polyploid Giant Cancer Cells (PGCCs). Recent studies indicate that PGCCs exhibit stem cell-like characteristics and may play a key role in HGSOC progression. Since PGCCs numbers correlate with tumor grade, chemoresistance, progression, and prognosis, understanding and targeting PGCCs may improve prognostic tools and therapeutic strategies for HGSOC. This study aimed to quantify PGCCs in a preclinical syngraft mouse model of HGSOC following carboplatin treatment, by manual and Digital Image Analysis (DIA) counting. 46 paraffin-embedded samples of stomach, peritoneum, and pancreas from wild-type C57Bl/6 female mice, intraperitoneally injected with 1 x 10^6 ID8 $p53^{-/-}$ /$PTEN^{-/-}$ cells and treated with vehicle (control) or carboplatin, were retrieved from the Mouse and Animal Pathology Laboratory archives and stained with hematoxylin and eosin. Sections were digitized, and the three largest tumor masses per sample were selected. For each mass, 2 hotspots (one peripheral and one central) were acquired at 20x resulting in 276 images. PGCCs were assessed both manually and through DIA using ImageJ software by categorizing cells by size (small, medium-large, giant). Manual counting revealed higher numbers of PGCCs at the tumor periphery and in carboplatin-treated animals compared to controls. Similarly, DIA revealed increased numbers of medium-large and giant cells at the periphery and in the carboplatin group. These results suggest that carboplatin treatment induces an increase in the number of PGCCs, particularly at the tumor periphery, supporting their potential role in chemoresistance and in tumor progression of HGSOC.

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HER2 STATUS IN CANINE BLADDER AND PROSTATIC UROTHELIAL CARCINOMAS: PRELIMINARY RESULTS

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Urothelial carcinoma (UC) is the most common tumor of the urinary tract in dogs, and it can affect the bladder and prostate. UCs in the bladder are usually high-grade and invasive, leading to death due to metastatic disease; UCs in the prostate are equally aggressive and often metastasize to many organs. Unfortunately, for these tumor types, treatment options are limited. This study aims to evaluate the HER2 protein expression and gene amplification by immunohistochemistry and fluorescence *in situ* hybridization (FISH) to expand therapeutic possibilities for this type of cancer. HER2 expression was assessed following the 2018 ASCO/CAP guidelines. Thirty canine UCs (23 from the urinary bladder and 7 from the prostate) were retrospectively examined and tested by immunohistochemistry and FISH. By immunohistochemistry, 19 cases (63.33%) were scored as positive 3+, 9 cases (30%) were considered equivocal 2+, and 2 cases (6.67%) scored as negative 1, there were no cases scored 0. In particular, 13 out of 23 bladder UCs were 3+ positive (56.52%), and 6 out of 7 prostate carcinomas were 3+ positive (85.71%). FISH analyzed HER-2 in the same cases, and amplification was found in 13 cases (43.33%), of which 7 were from the bladder and 6 were prostatic, 11 were not amplified (36.67%), and 6 could not be evaluated. Fisher's correlation test between immunohistochemistry and FISH results was non-statistically significant with a p=0.104. Of the 19 cases considered 3+ by immunohistochemistry, 10 were amplified, 6 were not amplified, and 3 could not be evaluated. Of the 9 considered 2+, 3 were amplified, 5 were not amplified, and one could not be assessed. The high percentage of cases scoring 3+ by immunohistochemistry (63.33%) and showing amplification by FISH (43%) indicates a rationale for targeted therapies already widely used in human oncology to treat these highly aggressive canine tumors.

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Preliminary assessment of the oncolytic potential of Bovine Herpes virus-4 in canine solid tumor cell lines

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Canine osteosarcoma (c-OSA) and histiocytic sarcoma (c-HS) are aggressive neoplasms characterized by poor prognosis, high metastatic and recurrence rates and limited response to conventional therapies. For these reasons, there is a need in Veterinary Medicine to develop alternative therapeutic strategies. Viral oncolysis could represent such approach. Bovine herpesviruses type 4 (BoHV-4) can be a promising candidate for oncolytic virotherapy due to its safety for dogs and the possibility of wide genome manipulations. This study aims to investigate BoHV-4 oncolytic potential focusing on its ability to infect and induce cytopathic effect. Continuous and primary c-OSA cell lines (D17 and OSA2), continuous c-HS cell lines (DH82) and primary canine cutaneous fibroblasts cell line were infected with BoHV-4 TK deleted, expressing EGFP (BoHV-4-EGFPΔTK) and compared to uninfected controls. Cell lines were infected with different MOI (multiplicity of infection; from 0.1 to 4) and percentages of infected cells over time were evaluated by manually counting five 10x fields. Using flow cytometry the percentage of infected cells (GFP^+), dead cells ($7AAD^+$), and double positive at 48h and 96h, were comparatively quantified. In addition, immunofluorescence was used to investigate specific virus-induced cell death pathways. Activation of apoptotic (Ccas3) and pyroptotic (Gasdermin-D) pathways were evaluated, including the quantification of double-positive cells at 48h and 96h. Results demonstrated that BoHV-4 at 1 MOI successfully infected and induced cell death in c-OSA and c-HS cell lines at 48 and 96h. The almost totality of the cells are successfully infected after 96h. At 96h all infected tumor cell lines showed reduced cell viability compared to controls. In particular, DH82 and OSA2 cell lines showed 37% and 32% of cell death. Fibroblasts had 21% infected cells and about 10% of cell death. These preliminary results show that BoHV-4 has a great oncolytic potential in canine tumors that deserves further investigation.

Influence of Sex, Neuter Status, Breed, Age, and District on Malignant Tumor Development in Cats: a Multicenter Study of 5,289 Histopathologic Diagnoses from Central Italy (2008-2023)

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Animal cancer registries (ACRs) are essential tools in veterinary oncology, providing valuable insights into epidemiological and clinical trends and supporting cross-species comparative research. Despite the significant burden of cancer in cats, the epidemiology of feline tumors remains less explored compared to that of dogs and humans. This study aimed to describe a large dataset of histologically diagnosed feline tumors and to analyze temporal trends and the influence of breed, sex, neuter status, age, and geographical origin on malignancy profiles and tumor distribution.

A modified Vet-ICD-O-canine-1 coding system was applied to 5,289 tumors collected by two pathology-based ACRs in central Italy (2008–2023). Data were analyzed for time trends using the Cochrane-Armitage test, and logistic regression was used to assess the impact of the variables on tumor behavior ("malignant" vs. "benign") and the development of major malignant tumor types.

Out of all tumors, 4,264 (80.6%) were malignant. Fibrosarcomas, adenocarcinomas, squamous cell carcinomas (SCCs), and lymphomas were the most common neoplasms. The risk of malignancy increased by 8% with each additional year of age. Females (OR = 1.39, 95% CI 1.19–1.62) and non-purebred cats (OR = 1.89, 95% CI 1.47–2.38) showed higher odds of malignant tumor diagnosis. While intact status did not significantly affect overall malignancy risk, it was associated with increased adenocarcinoma risk. Significant changes in tumor occurrence were detected over time, including an increase in SCCs and a decline in fibrosarcomas, offering new insights into tumor etiopathogenesis.

This study confirms previous data while identifying novel potential risk factors warranting further investigation. It also provides long-term monitoring of oncological patterns in cats and highlights the importance of standardized and multicenter approaches in identifying feline populations at elevated cancer risk and temporal trends.

Immune microenvironment in canine mammary carcinomas: focus on TILs, TLSs, and HLMs

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The immune tumour microenvironment is a dynamic entity encompassing different immune cell populations. Although macrophages are frequently observed, research has only recently focused on haemosiderin-laden macrophages (HLMs) that may play a role in tumour progression. Tumour-infiltrating lymphocytes (TILs), consisting of T, B and NK lymphocytes, are considered an early stage of tertiary lymphoid structures (TLSs) maturation. In the more mature forms, TLSs are characterised by the presence of follicular dendritic cells and other heterogeneous immune populations. This study aims to assess the presence and distribution of HLMs, TILs, and TLSs in canine mammary carcinomas.

Canine mammary carcinomas (n=154) were evaluated with H&E and Meguro stain to identify HLMs. Immunohistochemistry for CD21 was carried out to detect follicular dendritic cells to recognize TLSs. HLMs and TLSs presence and distribution were evaluated. Stromal TILs were semi-quantified according to the International TILs Working Group guidelines. Peritumoral TILs were assessed by Klintrup score. The chi-square test was used to assess statistical correlations between HLMs, TILs and TLSs and tumour grade and histotypes.

The results showed that HLMs were arranged in conspicuous groups, isolated or in the marginal areas of TIL and TLS, predominantly in barrier-like disposition. CD21-positive cells were located in inflammatory infiltrates with a follicle-like arrangement. The HLMs presence and distribution showed no correlation with tumour grade and histotype. However, a positive correlation was found between stromal and peritumoral TILs and TLSs presence with tumour grade.

The immune tumour microenvironment is gaining increasing attention in canine mammary tumours. The CD21 antibody has proved useful in identifying TLSs. The International TILs Working Group guidelines and the Klintrup score are simple and standardised methods for the semi-quantification of stromal and peritumoral TILs respectively. The TLSs presence is associated with increased tumour malignancy. HLMs could be involved in the tumour microenvironment regulation, representing iron reservoir.

A preliminary report of the specimen-related factors with impact on the Diagnostic Confidence of Italian Veterinary Pathologists.

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Making an accurate diagnosis in surgical pathology is essential, as it can significantly influence how a patient is treated. Nonetheless, the level of diagnostic confidence that pathologists place in their diagnosis can be impacted by several factors. This study is part of an ongoing survey that explores the factors influencing diagnostic confidence in veterinary pathology. This preliminary report focuses specifically on specimen-related factors, highlighting variables that lead to uncertainty in veterinary pathology. Understanding these key sources of doubt can help in creating focused strategies to enhance diagnostic assurance. Data was gathered through an online questionnaire designed using Google Forms and distributed through the mailing list of members of the Associazione Italiana di Patologia Veterinaria (AIPVet), the European Society of Veterinary Pathology (ESVP), and the European College of Veterinary Pathologists (ECVP). After one month of data collection, a total of 32 responses from Italian veterinary pathologists were received. Of these, twenty-two were board-certified pathologists, 7 were non-board-certified pathologists, and 3 were residents. The respondents' professional experience varied: 7 had less than 5 years, 11 reported between 5 and 15 years, and 14 had more than 15 years of experience. Fifteen respondents were generalist surgical pathologists, while 17 were surgical pathologists with additional expertise in specific organ systems. Among the factors identified, small tissue samples, samples suspected of being unrepresentative and poor fixation emerged as the most influential, often receiving the highest ratings for their negative impact on the diagnostic confidence. Less frequently, respondents identified fragmented tissue sample, decalcification issues, lack of hallmark features, and lack of etiological signature features as contributing to diagnostic uncertainty. Overall, the data highlight a consistent pattern in which technical artifacts and intrinsic specimen limitations significantly undermine pathologists' diagnostic confidence. This emphasizes the importance of developing standardized protocols for specimen collection, processing, and sample submission.

Investigation of Canine and Feline Neoplasm Diagnoses through AI-Driven Data Extraction from the University of Padua Histopathological Database (2017–2025)

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In both veterinary and human medicine, the diagnosis and management of neoplastic diseases are critical to ensuring patient health and longevity, while standardized classification and documentation are essential for epidemiological, prognostic, and therapeutic studies. In this context a comprehensive database represents an important tool enabling retrospective analyses on large datasets to identify trends such as tumor prevalence, breed predispositions, and geographic variations. Furthermore, a well-structured database allows comparative pathology investigations since animals can act as sentinels for environmental factors involved in the oncogenesis of specific neoplasms. The application of artificial intelligence (AI) to large-scale datasets is innovative and offers new insights into the epidemiology and diagnostic practices of neoplastic diseases. In this study, we applied AI-based tools to extract and analyze data from the histopathological database of canine and feline tumors of the Department of Comparative Biomedicine and Food Science of the University of Padua in the period between May 2017 and January 2025. Key information regarding animal sex, age, and breed was systematically retrieved in order to investigate tumor prevalence and explore possible correlations with age, breed, and sex, providing valuable epidemiological insights. Beyond patient data, the study also examined the number of pathologists involved in the diagnostic process, uncovering inter-operator variability in diagnostic reporting. Differences in diagnostic terminology and application of diagnostic modifiers were evaluated, highlighting some inconsistencies and lack of standardization. Our findings demonstrate the value and limits of large diagnostic datasets and the potential of AI in successful and relevant data extraction. This approach enhances the value of data collection and provides a point of view regarding database construction and analysis using new innovative technologies for the future.

Mapping Alpha-Synuclein Expression in Canine Neoplastic and Normal Tissues: Focus on Oral Melanoma and Nervous Tumors

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Alpha-synuclein is a small acidic protein predominantly expressed in the brain. Its functions are numerous, from inhibition of apoptosis, antioxidation, neuronal differentiation, to modulation of glucose and calmodulin levels, dopamine synthesis, and vesicle trafficking. Recent studies have also demonstrated that, in humans, this protein supports the survival of primary and metastatic melanoma cells, likely inhibiting the expression of MHC class II proteins and hence anti-tumour immune response.

The expression pattern and mapping of alpha-synuclein has never been evaluated in canine tissue; the aim of this preliminary investigation is to validate a human antibody against alpha-synuclein in the canine species and evaluate its expression by immunohistochemistry on normal and neoplastic tissues.

For this purpose, a selection of 30 canine oral melanomas, 4 gliomas, 4 meningiomas, 4 peripheral nerve sheath tumours, and different healthy tissue samples were tested by immunohistochemistry, after *in silico* validation of the selected antibody.

Results of our study confirmed that alpha-synuclein has in dogs an expression pattern that is similar to what has been reported in human medicine, being particularly expressed in healthy brain samples. Moreover, it is frequently expressed in oral melanomas, where it showed variable expression (>90% of tested cases). Tumors of the nervous system were invariably negative, except for both benign and malignant peripheral nerve sheath tumours.

Alpha-synuclein is an interesting marker that could be exploited in veterinary medicine for what appears to be a sensitive and specific expression. Further studies are needed to support its use both in diagnostic and research environment.

Iron-related proteins in non metastatic and metastatic feline mammary carcinomas: an immunohistochemical study

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Mammary tumors are the third most common neoplasm in female cats, with 80-96% of forms being malignant and presenting high metastasization rates, poor prognosis and low survival rates.

Recent studies have described the phenomenon of iron addition and the role of iron-related proteins in the onset, growth and progression in different animal cancers.

In this study we aim to look into the biological significance of iron-related proteins in non metastatic and primary metastatic feline mammary carcinomas (FMCs) and their lymph node metastasis.

Thirty-eight samples of feline mammary tissue were selected from the histological archives of the Department of Veterinary Medicine and Animal Productions-University of Naples Federico II. Histopathological classification of neoplastic lesions was performed on the recently update of the WHO classification:healthy mammary gland tissue, FMC without lymph node metastasis, FMC with lymph node metastasis, and the tributary lymph node that had the metastasis of the primary FMC.By exploring the immunohistochemical expression of Transferrin Receptor1 (H68.4 anti-mouse TfR1 Thermofisher), Transferrin Receptor2 (polyclonal anti-rabbit TfR2 Antibodies), Ferritin (anti-rabbit FTH1 Antibodies) and PCNA (PC10 anti-mouse Thermofisher), we aim to provide insights in identifying possible novel prognostic biomarkers and/or therapeutic targets. Antibody specificity was confirmed by Western Blot.

Increased TfR1 expression was associated with tumor progression, confirming previous reports, while TfR2 was not expressed in normal mammary tissues but it was strongly expressed in most tumoral cells in all carcinomas: non metastatic, metastatic and their lymph node metastasis. Regarding FTH1, labeling was detected in all epithelial mammary cells of normal tissue samples, while in tumoral samples labeling appeared heterogeneous between and within samples suggesting disparate levels of expression.

Our results suggest an increased uptake of iron from tumoral cells and variable iron storage possibly to sustain cell proliferation.More interestingly, considering the absence of TfR2 labeling in normal mammary tissues and the specific labeling of tumoral cells, we believe that TfR2 could represent a more specific/selective biomarker and therapeutic target in FMCs.

Morphological, molecular and chemical analysis of canine testes living in polluted areas in Campania Region

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In Campania Region an area known as the “Land of Fires”, is currently considered an open-air dump due to the illegal discharges of toxic substances and environmental pollutants. Several epidemiological studies from this area raised significant concern due to the adverse effects on animal and human health and fertility. Our work aimed to evaluate the possible correlations between environmental pollutants such as heavy metals (HMs) and testicular diseases in dogs. 50 puberal male stray dogs living in highly polluted areas in Campania Region were enrolled for the study; following surgical castration, testicular samples (n. 100) were processed for histological, molecular and chemical analyses. Histopathological lesions were graded using the Johnsen scoring system, and 17-β-HSD, P450 aromatase expression was evaluated by immunohistochemistry (IHC) and Western Blot (WB) analyses. Inductively coupled plasma mass spectrometry (ICP-MS) analysis was carried out to assess HMs concentration in testes. Based on histological examination, samples were divided into three groups: A) normal testes (37%); B) testes with moderate to severe degeneration (germinal epithelium atrophy, Sertoli cell loss, peritubular fibrosis and spermatogenic alterations) (54%); C) testicular neoplasm (9%). IHC and WB analyses revealed a significant downregulation of 17-β-HSD expression in testes of B and C groups compared to the A group. Conversely, P450 expression was markedly upregulated in the B and C groups compared to the A group. ICP-MS results showed statistically significant higher doses of Lead, Cadmium, Mercury, Arsenic and Tin in Group C (91%) compared to Group B (67%) and Group A (27%). Interestingly, all neoplastic samples displayed aberrant depleted Uranium accumulation. Our findings suggest a possible correlation between higher doses of HMs accumulation and testicular diseases (both degenerative and neoplastic) in dogs from polluted areas in Campania Region, corroborating the current scientific literature, which suggests how HMs exposure may mimic anomalous estrogenic-like mechanisms leading to testicular diseases.

Definizione del microambiente immunitario e degli immunofenotipi nei carcinomi squamosi del cane mediante immunoistochimica multiplex

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I carcinomi squamosi rappresentano una neoplasia frequente nel cane, con localizzazione prevalente a livello cutaneo e mucosale. Il trattamento varia in base alla sede anatomica e alla resecabilità chirurgica. In assenza di biomarcatori specifici per questa neoplasia nel cane, e considerando l'attuale orientamento dell'oncologia umana verso l'immunoterapia, questo studio ha avuto l'obiettivo di analizzare il microambiente immunitario tumorale e definire specifici immunofenotipi nei carcinomi squamosi cutanei e mucosali del cane. Un ulteriore obiettivo, basato su un nostro precedente lavoro di validazione dell'anticorpo anti-PDL1, è stato valutare l'espressione di PDL1 e la sua eventuale correlazione con l'infiltrato immunitario. A tal fine, sono stati selezionati retrospettivamente 34 casi: 20 cutanei e 14 mucosali. L'immunoistochimica multiplex è stata eseguita con coloratore automatico (Discovery Ultra, Roche Diagnostics) utilizzando anticorpi anti-CD3, -CD20 e -IBA1. L'analisi spaziale dell'infiltrato immunitario ha permesso di classificare i tumori in tre immunofenotipi: immuno-infiammato (infiltrato intratumorale), immuno-escluso (linfociti presenti solo sul fronte di invasione) e immunodeserto (assenza/minimo infiltrato).

Le cellule immunitarie sono state quantificate su vetrini digitalizzati tramite analisi d'immagine computerizzata (QuPath, con machine learning interattivo). L'immunoistochimica per PDL1 è stata eseguita manualmente su 14 casi (11 cutanei e 3 mucosali); l'espressione è stata considerata positiva se >1%.

Dei 34 tumori analizzati, 27 erano immune-infiammati, 5 immune-esclusi e 2 immunodeserti. Tre dei 14 casi valutati per PDL1 risultavano positivi (2 immune-infiammati, 1 immunodeserto), ed erano tutti cutanei.

In conclusione, la maggior parte dei carcinomi squamosi canini presenta un fenotipo immune-infiammato, potenzialmente rilevante poiché in oncologia umana esso è associato a una maggiore risposta all'immunoterapia. L'analisi digitale computerizzata si conferma uno strumento accessibile ed efficace per la caratterizzazione del microambiente tumorale.

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Patologia degli Organismi Acquatici

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Perinatal Pathological Findings in Marine Mammals Kept Under Human Care: A Retrospective Review (1999-2024)

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Perinatal mortality is a major concern in marine mammals kept under human care, with the first days/weeks of life being critical due to physiological immaturity and susceptibility to environmental factors. Among common causes of neonatal deaths, particular attention was given to uncommon conditions such as meconium aspiration syndrome (MAS) and meconium plug syndrome (MPS). This study retrospectively reviews cases from the Mediterranean Marine Mammals Tissue Bank (MMMTB) to investigate causes of death and characterise pathological findings in neonatal and perinatal marine mammals, performed by the pathologists of the Dept. of Comparative Biomedicine and Food Science (University of Padua), between 1999-2024. Standardised necropsy protocols were applied, and causes of death were classified according to ICD-11.

Among all 33 cases of dead marine mammals in the analysed period, nine (cetaceans and pinniped) met the criteria. MAS was confirmed in two bottlenose dolphins (*Tursiops truncatus*, ID-114, ID-123), showing meconium and keratin debris in the airways, bronchial epithelial changes, and complications from secondary bacterial infections. A bottlenose dolphin (ID-343) showed signs consistent with MPS, previously undescribed in marine mammals, histologically characterised by intestinal meconium retention and severe erosive colitis. Three bottlenose dolphins died from infectious diseases: ID-144 had terminal enteritis with *Enterococcus faecalis* overgrowth, suggesting dysbiosis; ID-145 showed subacute enterocolitis with lesions consistent with Gram-negative endotoxaemia; ID-229 died from endotoxic shock likely linked to methicillin-resistant *Staphylococcus aureus* (MRSA) colonisation, confirmed by mecA gene and beta-lactam resistance. Fatal trauma occurred twice: a bottlenose dolphin (ID-186) with cerebral and meningeal haemorrhages, and a harbor seal (*Phoca vitulina*) with systemic haemorrhages including retrobulbar and hepatic foci.

These findings highlight the vulnerability of marine mammals during the perinatal period, where meconium-related syndromes, infections, and trauma are key contributors to early mortality. Improved diagnostics and targeted protocols are essential to improve survival and welfare in managed populations.

Rainbow trout (*Onchorhynchus mykiss*) corneal lesions as an indicator of water quality in TiO_2-Photoelectrocatalysis

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Since sustainability in food production has become a mail concern, recirculating aquaculture systems (RAS) are now available besides the traditional rainbow trout (*O.mykiss*) farming in a continuous water flow system. RAS are land-based aquaculture facilities that minimize water consumption by reusing water after it has been biofiltered to reduce ammonia toxicity. A novel technique combining photoelectrocatalysis (PEC), UV filtration, and biofiltration has recently been developed with the aim of enhancing the quality of recirculating water. In this study we aim to assess PEC efficacy by using corneal damage as an indicator of water quality, in fact cornea is indirect contact with water and the presence of toxic chemicals such as ammonia directly affects its homeostasis, resulting in anatomical changes.

Rainbow trout was reared in six RAS tanks (30 kg/m³ of density) for 28 days: three tanks were equipped with PEC system, three with conventional filtration serving as control (CTR) group. Water quality parameters (ammonia, nitrites, and nitrates) were registered twice a week. Six fish per tank were sacrificed and corneal integrity was assessed by i) morphological evaluation with ia) optical coherence tomography (OCT) and in ib) HE stained glass slides; ii) mucin production evaluation via AB-PAS and HID-AB staining; iii) oxidative epithelial cell damage evaluation with 8-hydroxy-2-deoxyguanosine (8-OHDG) antibody immunohistochemistry.

At the end of the study, ammonia levels were significantly higher in CTR group water (CTR: 1.78±0.20 vs. PEC: 0.96±0.20; $p<0.05$). From a pathological standpoint although oxidative stress (8-OHDG positivity) affected both groups, corneal lesions such as disepithelization and fibrosis were more severe in the CTR group, proving a protective effect of PEC system. No relevant difference was noticed in mucous cell production. Corneal evaluation proved to be a useful bioparameter in water quality assessment and shows the efficacy of PEC in enhancing water quality by preventing ocular damage.

A retrospective histopathological study of disease in marine ornamental fish

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This retrospective histopathological study reviews diseases of marine ornamental fish submitted by clinicians from public aquaria or from private practices to the Veterinary Pathology laboratory at the University of Camerino between 2012 and 2024. A total of 416 fish belonging to 158 different species (381 teleosts and 35 elasmobranchs) were examined. *Hippocampus* was the most commonly submitted genus (29 cases). Case materials included formalin-fixed fish or tissues. Tissues were routinely processed, sectioned at 5 microns, and stained with hematoxylin and eosin (HE). Select tissues were stained with the following special stains: Ziehl-Neelsen (ZN), Fite's acid fast (AF), periodic acid–Schiff (PAS), Brown and Brenn (B&B), Giemsa and Gomori methenamine silver (GMS). Immunohistochemical staining or polymerase chain reaction testing was performed when attempting to identify infectious agents. Based on the morphological and etiological diagnoses obtained from case reports, the cases were classified into different disease groups as: inflammatory/infectious, inflammatory/non infectious, regressive (degenerative, metabolic), vascular and neoplastic. Inflammatory/infectious disease was the most frequently diagnosed pathological process (74%). The predominant etiological agents were bacteria, with a large prevalence of Gram-negative bacteria frequently causing sepsis, branchitis, enteritis and mycobacteria causing variably organized granulomas localized to one or more organs. Histological lesions consistent with megalocytivirus infection were pointed out in two cases of fish died during the quarantine period confirmed by real-time PCR analysis. The most commonly encountered parasitic diseases were cryptocaryoniasis and scuticociliatosis, both corresponding to 22% of the diagnosed parasitic diseases. In order of frequency, infectious diseases were followed by regressive/degenerative processes (17%) and noninfectious inflammatory processes (14%). Noteworthy was the presence of multiple pathological processes in the same case: in 12% of cases a condition of comorbidity was observed. In 52% of the cases in which the co-presence of more than one infection was observed, one of the etiological agents was *Mycobacterium* spp.

Comparison of extracellular vesicles isolated from bottlenose dolphin's (*Tursiops truncatus*) blow with ultracentrifugation or size exclusion chromatography

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Bottlenose dolphins (*Tursiops truncatus)* are possible sentinels for marine health monitoring. Despite the complexity of *in vivo* sampling, cetacean's blow can be an innovative and non-invasive alternative to establish biomarkers for health and environmental monitoring. Extracellular vesicles (EVs) are cell-derived membranous nanostructures studied as promising diagnostic biomarkers in humans and animals. To future apply blow derived-EVs in the assessment of wild dolphins' health, here we characterized EVs isolated from dolphins' blow with two different methods. Blow samples were collected from four bottlenose dolphins kept under human care (UHC) using a six-well plate petri dish. Samples were dissolved in PBS and EVs isolated with ultracentrifugation (UC) or with ultrafiltration (UF) coupled with size exclusion chromatography (SEC). EV-samples were analysed for particle size distribution and concentration with Nanoparticle Tracking Analysis (NTA), for protein expression with Western Blotting (WB) and visualized and measured with Atomic Force Microscopy (AFM). NTA detected particles were in the size range of EVs (40-600 nm) with a variable concentration, higher in UC- ($7.6*10^{11}$ +/- $1,4*10^{12}$ particles/ml) compared to SEC-derived EVs ($9.5*10^{9}$ +/- $1.7*10^{10}$ particles/ml). AFM confirmed these data, showing EV-like structures with a diameter compatible with NTA determinations. At WB, EV-samples were positive to EV-markers (CD9, integrin-beta) and negative to the control Calnexin. Only SEC derived-EVs resulted positive to OmpA, a Gram-negative bacterial membrane protein. In conclusion, we describe the isolation and characterization of EVs from bottlenose dolphin's blow using two different methods. While UC allows to collect more particles, SEC should isolate less EVs in higher purity, allowing here the identification of bacteria derived-EVs. These results suggest the feasibility of the application of blow derived-EVs for the health assessment of free-ranging dolphins. We thank the Italian Ministry of Environment and Energy Security and Costa Edutainment S.p.A. for the possibility of including these animals in the study.

Risposta immunitaria cellulo-mediata in pesci rossi vaccinati con un ceppo attenuato di *Mycobacterium chelonae*

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La micobatteriosi ittica è una malattia cronica causata da micobatteri nontubercolari, per la quale non esistono trattamenti o vaccini approvati. Attualmente la gestione si basa su misure preventive, spesso insufficienti, rendendo necessario lo sviluppo di strategie vaccinali efficaci. In questo studio, è stato valutato l'effetto di un vaccino vivo attenuato basato su un ceppo mutante di *Mycobacterium chelonae* (Δ*M. chelonae*) nei pesci rossi (*Carassius auratus*). Due trial sperimentali sono stati condotti per indagare l'immunogenicità e l'efficacia protettiva del vaccino, con focus su due modalità di somministrazione: via intracelomica e orale. I pesci sono stati successivamente iniettati con i ceppi wild-type (WT) di *M. chelonae* (trial 1) e *M. marinum* (trial 2).
Nel primo trial, 40 pesci sono stati vaccinati con Δ*M. chelonae* (10^6 CFU/pesce) e successivamente esposti a *M. chelonae* WT (10^7 CFU/pesce). L'espressione genica di IL-12 e IFN-γ è stata misurata a 1 e 52 giorni post-infezione tramite qPCR da milza e rene posteriore. Nel secondo trial, i pesci sono stati vaccinati tramite iniezione intracelomica o somministrazione orale con Δ*M. chelonae* e successivamente esposti attraverso iniezione intracelomica a *M. marinum* WT (10^6 CFU/pesce). La mortalità è stata registrata e il carico batterico misurato mediante qPCR da milza e rene.
I risultati hanno mostrato che la vaccinazione con Δ*M. chelonae* ha determinato un incremento significativo dell'espressione di IL-12 e IFN-γ nelle 24 ore post-vaccinazione, suggerendo una risposta immunitaria cellulo-mediata potenziata. Inoltre, la vaccinazione intracelomica ha ridotto significativamente il carico di *M. marinum*, con assenza di mortalità nei pesci vaccinati rispetto al 10% nel gruppo non vaccinato.
Questi dati indicano che il ceppo ΔM. *chelonae* potrebbe costituire una promettente strategia vaccinale contro la micobatteriosi ittica. Tuttavia, la persistenza del vaccino in alcuni soggetti e le limitazioni sperimentali richiedono ulteriori studi per valutarne sicurezza ed efficacia.

Patologie granulomatose nei pesci d'allevamento in Sardegna

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L'acquacoltura costituisce una risorsa economica strategica per la Sardegna, con una produzione annuale di circa 13.000 tonnellate di peso vivo, di cui il 17% rappresentato da specie ittiche (orata, spigola, cefalo, anguilla) e l'83% da molluschi bivalvi. Negli ultimi anni, tuttavia, il settore è sempre più esposto al rischio di malattie infettive, in particolare alle patologie granulomatose, responsabili di rallentamenti nella crescita e di un incremento della mortalità nei pesci allevati. Il presente studio ha analizzato la prevalenza e le potenziali cause di tali patologie nelle principali specie ittiche d'allevamento in Sardegna (Italia centro-orientale). Sono stati esaminati 180 pesci adulti: orate (*Sparus aurata*, N=60) e spigole (*Dicentrarchus labrax*, N=60) provenienti da due impianti di acquacoltura intensiva, e cefali (Mugilidae, N=60) provenienti da due impianti estensivi lagunari. Da ciascun soggetto sono stati prelevati sei organi (cervello, cuore, fegato, intestino, milza, rene), per un totale di 1.080 campioni, sottoposti ad analisi istopatologiche, microbiologiche e molecolari. I risultati hanno evidenziato la presenza di granulomi in tutte le specie esaminate, con una prevalenza significativamente più alta nei cefali allevati in ambienti estensivi (93%), rispetto a orate (42%) e spigole (30%) provenienti da sistemi intensivi. In una minoranza dei cefali (5%) è stato isolato *Mycobacterium chelonae*, mentre le analisi PCR (*16S rDNA*, *hsp65*, *ureC*, *pbp1A*) e qPCR (*atpE*) su tessuti freschi hanno rilevato *Enterovibrio spp.* e *Photobacterium damselae* in due esemplari. Tuttavia, nella maggior parte dei casi, le lesioni granulomatose risultavano associate alla presenza di parassiti trematodi e di organismi del subphylum *Myxozoa* (Cnidaria) rispettivamente nel 90% dei cefali, nel 76% delle orate e nel 78% delle spigole. I dati suggeriscono un'eziologia prevalentemente parassitaria e indicano come le buone pratiche gestionali adottate negli impianti intensivi possano contribuire a contenere le infezioni batteriche.

The oldest dolphin in Europe: comparative pathology in a 59 year-old bottlenose dolphin from under human care

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Cetaceans are long-lived mammals that are exposed to similar environmental and biological pressures as humans, and yet remarkably little is known about their ageing process. We therefore studied an over 59-year-old wild-born bottlenose dolphin (*Tursiops truncatus*) female kept under human care that died of natural causes and was submitted to a complete and standardized *post-mortem* examination at the Dept. of Comparative Biomedicine and Food Science. Diagnostics included histopathology, bacteriology, and PCR for common infectious agents, and immunohistochemistry of the auditory brain cortex using amyloid-β-42 antibody (1:700, mOC64, Abcam, UK). Antibody against Herpesvirus simplex 1 (HSV1, 1:75, ab9533, Abcam) was used on kidney, skin lesion, lymph-nodes, and brain tissues.

Forensic findings included: 1) multifocal ulcerative skin lesions; 2) miliary microabscesses in the liver; 3) moderate-severe renal degeneration; 4) mild-moderate multifocal-coalescing gastritis in the first (erosive-ulcerative) and second (lymphocytoplasmatic) stomachs, with presence of laurel leaves and viscous/oily black substance in the first stomach and esophagus; 5) fatty atrophy and severe lymphodepletion of pulmonary and prescapular lymph-nodes; 6) hypotrophic spleen; 7) tecoma of the right ovary and left periovarian cyst; 8) anthracosis of the lungs and pulmonary lymph-nodes; 9) multifocal metaplastic mineralization of bronchial and bronchiolar epithelia; 10) mild multifocal satellitosis and edema in the brain cortex; 11) mild-moderate fibrosis of the kidney and heart; 12) worn teeth. Findings 3-12 are consistent with the dolphin's advanced age.

Systemic presence of *Streptococcus canis*, and Common bottlenose dolphin gammaherpesvirus 1 strain Sarasota in the ulcerative skin lesion and kidney suggest a chronic, primary viral infection that, along with age-related immunosuppression, predisposed the dolphin to fatal septicemia. Disseminated amyloid-β-42 dense-core and fibrillar plaques were present in the auditory cortex.

This case provides a baseline for studying cetacean ageing and suggests that Alzheimer's-like lesions and herpesvirus infection may indicate a viral contribution to neurodegeneration, as in humans.

STUDY OF A SARCOMA MODEL INDUCED BY TROUT GRANULOMATOUS VIRUS (TGV) IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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In both humans and animals, viruses can contribute to the development of tumors with various mechanisms, including the induction of chronic inflammation that can promote a microenvironment conducive to neoplastic transformation.A recently discovered Nidovirus, Trout Granulomatous Virus (TGV), has been identified in Israel. This virus has been found to be the causative agent of a granulomatous hepatitis observed in farmed rainbow trout. TGV-induced liver granulomas exhibit central necrosis and a robust chronic-active inflammatory reaction, with pre-dominance of lymphocytes, epithelioid macrophages, giant cells and capsular fibroblastic proliferation. The present study analysed 14 liver samples from TGV-infected rainbow trout, with the aim to assess the oncogenic potential of the virus. In 30.76% of cases, granulomatous capsule fibroblasts demonstrated pleomorphic transformations, polynucleation, typical and multipolar mitoses and myofibroblast-like morphology. As previously observed in human malignant fibrous histiocytoma, cells with myofibroblast morphology can originate from activated fibroblasts or macrophages. Immunohistochemical investigation revealed the expression of mutated p53 protein, elevated levels of PCNA, Ag-NORs, and expression of E-cadherin within capsular fibroblasts. These findings suggest a potential neoplastic origin of capsular fibroblasts compared to fibroblasts of more recent, well-differentiated granulomas. The expression of mutated p53 was detected in 53.84% of cases, exhibiting a strong correlation with proliferation markers (PCNA, AgNORs) and number of mitoses ($r > 0.9$). In addition, the degree of inflammation demonstrated a moderate correlation with the degree of apoptosis in capsular structures ($r = 0.61$).The results suggest that TGV may have oncogenic potential for trout and that the virus-induced granulomatous lesions may, over time, transform into fibrosarcoma, similarly to what has been observed in feline vaccine-associated sarcoma, which involves early p53 mutation. Therefore, it can be hypothesized that a persistent inflammatory response to the virus may induce a fibrotic connective tissue response and, in some cases, neoplasia.

Impact of aquatic and air pollution exposure on lung health in *Stenella coeruleoalba* and in *Tursiops truncatus*

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Pneumoconiosis is a chronic inflammatory reaction brought about by inhaling various types of mineral particles. Anthracosis is a pneumoconiosis caused by humans and terrestrial animals inhaling carbon particles and other pollutants. To the authors' knowledge, there has been only one previous report of anthracosis in dolphins. Our study evaluated whether *Stenella Coeruleoalba* and *Tursiops Truncatus* are negatively impacted by air pollution due to extra-aquatic respiration. This study was conducted on 43 dolphins stranded along the coast of Campania. A necropsy examination was performed, and lung samples were taken. A complete histological study was performed for each pulmonary specimen to evaluate the morphological changes. Fourteen cases were selected and then subjected to histochemical staining to characterise the accumulation of exogenous material. The results showed that the positive subjects mostly belonged to *Stenella Coeruleoalba's* species, were mainly adult females, and were beached along the coasts of Naples. The degree of anthracosis was variable (43% was mild, 29% was moderate, and 28% was severe). All the lung samples were negative with PAS and Perls stains. Fibrosis around the areas of dust accumulation was evaluated with the Masson Trichrome stain and graded as mild in 9 cases (65%), moderate in 3 cases (21%), in the absence of severe fibrosis. Two samples (14%) were unremarkable. Immunohistochemical examinations are underway to characterise the inflammatory infiltrate, oxidative stress, immune response, and tissue remodelling. Finally, our study has shown that even marine mammals are subjected to atmospheric pollution, and the effect of airborne dust on the lungs can be responsible for chronic inflammation and probably affect the organ's functionality over time. Therefore, this study opens the way to other research perspectives aimed at understanding the pathophysiology of the lesion and the strategies for safeguarding the species from the effects of environmental pollution.

RISPOSTA INFIAMMATORIA E IMMUNITARIA DELLA TROTA IRIDEA (*O. MYKISS*) ALL'INFEZIONE SPERIMENTALE CON *L. GARVIEAE:* OSSERVAZIONI IN LINEE SELEZIONATE

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La lattococcosi ittica, un'infezione batterica sistemica a rapido decorso provocata da *L. garvieae* (ora anche *L. petauri/L. formosensis*), provoca ingenti perdite in trota iridea (*O. mykiss*). Oltre al trattamento antibiotico e alla prevenzione con presidi vaccinali efficaci, una prospettiva di controllo dell'infezione è rappresentata dalla selezione genetica e dall'impiego di riproduttori resistenti. Lo studio illustrato in questo contributo è integrato nel progetto PRIMA "SUPERTROUT", e si prefigge di fornire un profilo dei fattori di immunità/infiammazione in linee di trota selezionate, in seguito all'esposizione al patogeno (challenge). La sperimentazione ha previsto l'infezione sperimentale di trote suscettibili (S) e resistenti (R) mediante iniezione intracelomatica di *L. garvieae*, il monitoraggio del decorso post-infezione, supportato da analisi batteriologiche, immunologiche e biomolecolari, con particolare riferimento all'espressione di 12 geni associati al processo infiammatorio e all'immunità. L'esito della prova *in vivo* è stato l'avvento di una infezione iper-acuta, caratterizzata da una diffusione setticemica dei batteri entro 3 giorni dal challenge. Non sono state osservate differenze di mortalità tra soggetti S ed R. Le analisi sierologiche finalizzate a valutare la modulazione di alcuni parametri di immunità aspecifica, quali proteine e IgM totali, attività del lisozima, delle antiproteasi, battericida e perossidasica, hanno evidenziato variazioni significative solo a carico dei primi due parametri, che risultavano ridotti nei soggetti S passando da 1 a 3 giorni post-challenge. La maggior parte dei geni hanno subito una modulazione differenziale tra 1 e 3 giorni post-infezione. Questo effetto è stato più marcato per i geni IL-8, SAA, Cox-2, CATH -1 e -2, Hep. Confrontando gli individui S con quelli R, la risposta al patogeno sembra essere diversa per quanto riguarda IL-8, MHCII and Casp3. Questo profilo evidenzia per la prima volta alcuni meccanismi molecolari alla base dell'interazione tra la trota e il patogeno *L. garvieae,* con particolare attenzione alla fase acuta della risposta.

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POSTER

Post-mortem histological adipocytic changes in canine cadavers - a preliminary study

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Physical and chemical changes that occur after death in the body constitute a fundamental component of forensic pathology, offering valuable information regarding the post-mortem interval (PMI). Post-mortem histological changes of adipocytes remains an underexplored theme in forensic studies, especially within veterinary pathology. This study characterised the post mortem histological alterations in canine adipose tissue and evaluated their potential utility for PMI estimation. A retrospective analysis of canine post-mortem examinations occurred in 2019 at the department of Veterinary Pathology, University of Liverpool was performed, where cadavers' preservation method (refrigeration or freezing), known or estimated time of death (< 24h, 24-72h, 72h-1 week, >1 week), body condition, and season of death were recorded. From each case, HE sections from subcutis, pericardium, heart, bone marrow, small and large intestine, and peri-adrenal gland adipose tissue were re-evaluated for detecting adipocytic changes. These data were then assessed to see whether significant associations between adipocytic changes and other variables were detected. One-hundred and twenty-five post-mortem cases were included. Three post-mortem histological changes in adipocytes were observed: intracytoplasmic accumulation of yellow to dark brown acicular pigments (YelPig), intra-cytoplasmic accumulation of eosinophilic amorphous material (EosMat), and adipocere (AC - cytoplasmic radial eosinophilic structures with crystalloid appearance). YelPig, EosMat and AC were detected (at least in one organ) in 45.6%, 36% and 16% of the cases, respectively. Dogs dead for > 1 week showed significantly more of all of the adipocytic changes compared to dogs dead for < 24 hours. Dogs dead in Summer showed significantly more YelPig and AC compared to dogs dead in Winter. Frozen cadavers showed significantly more adipocytic changes compared to refrigerated ones. These findings underscore the relevance of post-mortem adipose tissue changes in the forensic evaluation of canine remains, with novel insights for the forensic veterinary pathologists community.

Grave colangioepatite cronica da epatolitiasi e coledocolitiasi massiva in un cavallo

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La calcolosi delle vie biliari è una condizione rara negli equini. Interessa principalmente soggetti di età superiore ai 10 anni ed è caratterizzata dalla presenza, anche simultanea, di liti all'interno dei dotti biliari intraepatici (epatoliti), dei dotti biliari extraepatici (coleliti) e del dotto epatico comune o coledoco (coledocoliti). La litogenesi è condizionata da un aumento della concentrazione biliare di bilirubina non coniugata, per ipersecrezione dagli epatociti o deconiugazione enzimatica in corso di sepsi biliare, favorita da preesistente colestasi da corpi estranei, parassiti o stenosi di varia natura. La presenza di calcoli comporta stasi biliare, in seguito alla quale si sviluppano colangite e colangioepatite cronica con fibrosi progressiva e comparsa di ittero, anoressia, piressia, coliche intermittenti e, nei casi avanzati, encefalopatia epatica.

Si riporta il caso di un pony Camargue femmina di 20 anni, soppresso per insufficienza epatica, sindrome colica e alterazioni neurologiche in cui una biopsia epatica aveva evidenziato grave colangioepatite neutrofilica cronica con marcata fibrosi a ponte e steatosi. All'esame necroscopico si apprezzavano subittero e moderata distensione meteorica di stomaco e grosso intestino. Il fegato era diffusamente sclerotico e di colore grigio-giallo. Il dotto epatico comune era marcatamente dilatato e ostruito da un voluminoso calcolo (11x5 cm, 140 grammi) che sporgeva parzialmente in duodeno, di colore giallo arancio con struttura concentrica in sezione. I dotti biliari intraepatici erano estremamente dilatati e contenevano bile di aspetto catarrale e numerosissimi calcoli di dimensioni variabili, fino a 2 cm. Il soggetto presentava inoltre una neoformazione labiale e masse multinodulari nella regione perianale identificate come melanoma con interessamento linfonodale.

Il caso sottolinea l'importanza di includere la coledocolitiasi tra le diagnosi differenziali nei pazienti equini con segni di insufficienza epatica e segni neurologici, in quanto la diagnosi tardiva in presenza di fibrosi epatica avanzata preclude la possibile efficacia di interventi terapeutici.

Translational Research for Systemic Amyloidosis: The TeRESA Network

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Systemic AA amyloidosis is a rare protein misfolding disorder affecting humans and a variety of different animal species. Recent studies have highlighted an unexpected high prevalence of the disease in cats from three shelters in Italy, for which the hypothesis of a possible horizontal transmission in a confined environment has been raised. The cooperation between researchers together with the results obtained, led to the creation of a pathology research group composed of experts from different fields, who believe that studying systemic AA amyloidosis with a 360-degree approach could help improve the understanding of the disease, thus enhancing diagnostic and prognostic accuracy, as well as therapeutic approaches. These goals led to the creation of a new translational research network named TeRESA (Translational Research for Systemic Amyloidosis) which aims to deepen the knowledge on systemic AA amyloidosis in animals. TeRESA involves researchers from different academic institutions (University of Milano, Padova, and Uppsala) and from the Istituto Zooprofilattico Sperimentale of Piedmont, Liguria and Aosta Valley (IZSPLV), veterinarians of the Anicura Institute of Novara, and physicians of the Amyloidosis Research and Treatment Center of Pavia. To date, the results obtained by the network have led to the publication of six scientific papers in peer-reviewed journals, shared findings at several scientific seminars, and enrolled two dedicated PhD students. Currently the network is carrying projects on the monitoring of the prevalence of systemic AA amyloidosis in cat shelters focusing on the discovery of innovative indicators for clinical use, with potential implications in the human healthcare field as well. Furthermore, the network aims to update veterinary clinicians on the disease by providing them with tools for an early diagnosis and better management of the condition. It also seeks to disseminate its findings through publications, conferences, and workshops, and to encourage collaborations with other researchers, helping to bridge the gap between veterinary and human medicine.

Identificazione mediante immunofissazione degli isotipi anticorpali o di componenti monoclonali in cani con leishmaniosi

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L'immunofissazione permette di marcare proteine sieriche dopo migrazione elettroforetica ed è solitamente utilizzata per identificare, prima che appaiano evidenti gammopatie, l'isotipo anticorpale responsabile di paraproteinemie in corso di neoplasie linfoplasmacellulari. Non è noto se nel cane, come nell'uomo, esistano componenti monoclonali associate a forme infettive. Inoltre, non sono stati definiti gli isotipi anticorpali associati a leishmaniosi canina, nella quale si riscontrano marcate alterazioni elettroforetiche. In questo studio, per delineare eventuali associazioni tra isotipi presenti, titolo anticorpale e tracciato elettroforetico, sono stati analizzati con immunofissazione campioni di siero prelevati da 15 cani con leishmaniosi, con diverso titolo anticorpale e diversa presentazione elettroforetica, e da 4 sieronegativi, 2 con gammopatia (con sintomi respiratori ed infezione urinaria, rispettivamente) e 2 senza gammopatia (clinicamente sani).

In tutti i cani inclusi nello studio erano presenti positività policlonali per IgG e catene leggere lambda, più marcate in caso di gammopatia. Due sieronegativi, uno con e uno senza gammopatia, mostravano anche positività policlonali per IgM e IgA, rispettivamente. In 14 sieropositivi si sono rilevate le seguenti ulteriori positività: in un caso, con gammopatia monoclonale in elettroforesi, una banda monoclonale di IgG e catene lambda; in 11 casi, 9 con picchi beta, bande IgM policlonali; in 1 caso, senza alterazioni elettroforetiche, una banda IgM ristretta e debole positività per catene kappa; in 5 casi, tutti con picchi beta, 3 dei quali anche IgM-positivi, bande IgA policlonali. Le alterazioni immunoelettrofetiche erano presenti indipendentemente dall'entità del titolo anticorpale.

Tali risultati evidenziano una notevole eterogeneità negli isotipi anticorpali dei cani con leishmaniosi, senza una chiara associazione con il titolo anticorpale o il pattern elettroforetico. Il riscontro di occasionali componenti monoclonali e la presenza di isotipi come IgM o IgA, solitamente associati a patologie acute o mucosali più che a malattie croniche e sistemiche come la leishmaniosi, meritano di essere approfonditi.

Hospital-Acquired *Klebsiella pneumoniae* Infections in Veterinary Facility: a Case Study

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Hospital-Acquired Infections are well known in humans, whereas limited data are available in the veterinary field. These are infections usually associated with antibiotic-resistant bacteria, including Methicillin-Resistant Staphylococci, Extended-Spectrum Beta-Lactamase (ESBL)-producing Enterobacteria, Vancomycin-Resistant Enterococci*, Pseudomonas aeruginosa*, etc. We report four clinical cases, two dogs and two cats hospitalized for surgical procedures over a period of five months, all of them developed ESBL-producing *Klebsiella pneumoniae* infection likely acquired during their stay. The microorganism was isolated from urine and infected surgical wounds of patients.

To assess potential contamination within the clinic, bacteriological exams were carried out on surfaces and respiratory devices to isolate suspected ESBL-producing bacteria. Since operating room and hospitalization area undergo deeper cleaning procedures, including ozone treatment, it is likely that contamination occurred in the pre-surgical room. In this room, some devices that do not come into direct contact with the patients were sanitized/replaced less frequently.

Several resistant bacteria were isolated: *Acinetobacter* spp., *Pseudomonas* spp., *Enterobacter* spp., and *Klebsiella pneumoniae*. *K. pneumoniae* isolated from breathing respiratory circuit samples showed a resistance profile comparable to that of strains responsible for infections and present ESBL producing characteristics.

These results suggest that surfaces and equipment in veterinary facilities, even those not in direct contact with patients, represent a potential source of infection for hospitalized animals. More specifically, bacteria carried in aerosol particles may be present at various levels of devices and could evade disinfection. The presence of ESBL-producing bacteria and other pathogens highlights the critical need for rigorous sanitization protocols within veterinary facilities. Additionally, considering the pathogenic potential of these bacteria, continuous monitoring of their spread is essential to ensure both animal and public health. Furthermore, implementing hygiene protocols and periodic microbial surveillance in veterinary facilities could significantly reduce the risk of hospital-acquired infections, improving patient outcomes and overall biosecurity measures.

Quadri infiammatori associati ad una epidemia da *Mycobacterium avium complex* in gatti di razza abissina

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Le micobatteriosi sostenute da *Mycobacterium avium complex* (MAC) non sono comuni nel gatto ma colpiscono spesso gatti di razza abissina, a suggerire una loro maggiore suscettibilità all'infezione da MAC. Non sono però stati ben definiti i quadri infiammatori che caratterizzano l'infezione in questa razza, potenzialmente caratterizzata da risposte infiammatorie anomale perché predisposta a sviluppare amiloidosi ereditaria. In questo report vengono descritti i quadri infiammatori ematici e/o tissutali di 11 gatti abissini (5 deceduti, e 6 sottoposti a terapia antibiotica dopo la diagnosi), provenienti dallo stesso allevamento, che hanno mostrato linfo-, epato- e/o splenomegalia, a volte associata a sintomi gastroenterici o respiratori e/o a versamento.

In 7 gatti sono stati rilevati gravi quadri infiammatori sierici rappresentati da aumenti delle proteine di fase acuta (rispetto all'intervallo di riferimento la AGP risultava più alta di 2-5 volte e la SAA di 2-30 volte) e da iper-α_2-globulinemia e gammopatia policlonale in elettroforesi. In 7 gatti la citologia degli organi colpiti ha mostrato flogosi piogranulomatosa: in due casi erano rilevabili, nei macrofagi, strutture colorate negativamente riferibili a micobatteri. La presenza di MAC negli aspirati è stata confermata mediante Ziehl–Neelsen (ZN) o PCR in 4 casi. In uno dei due casi sottoposti a necroscopia, gli esami istologici e immunoistochimici hanno rilevato lesioni granulomatose ZN positive contenenti macrofagi e linfociti T e rari linfociti B. Le sezioni sono risultate negative per Rosso Congo. L'infezione da coronavirus felino, potenzialmente responsabile di quadri simili, è stata esclusa mediante RT-qPCR sugli aspirati e immunoistochimica nei tessuti.

Tali riscontri indicano uno stato infiammatorio intenso, a differenza di report precedenti su micobatteriosi feline, apparentemente non associato ad amiloidosi. Questi risultati indicano che in caso di quadri infiammatori marcati va sospettata anche l'infezione da MAC, e supportano l'ipotesi, da approfondire attraverso indagini genetiche, della particolare suscettibilità di razza a queste infezioni.

Background Clinical and Anatomical Pathology findings in SDRG Rats

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The SDRG rat, derived from the Sprague-Dawley genetic background and carrying a double knockout of the Rag1 and IL-2Rγ genes (Rag1-/- Il2rγ-/-), presents significant deficiencies in B, T, and NK cells. These characteristics make it an attractive model for preclinical studies, particularly in applications like tissue transplantation, humanization research and evaluating Advanced Therapy Medicinal Products.
Existing data on spontaneous background findings in this strain—especially concerning aging animals—remains limited. This investigation aims to address this gap by evaluating clinical and anatomical pathology findings in a cohort of 39 male and 43 female SDRG rats, aged between 10 and 26 weeks. The study encompasses evaluations such as hematological and clinical chemistry assessments, bone marrow smear analysis, blood immunophenotyping, necropsy, and histopathological examinations.
Hematological profiles of SDRG rats revealed markedly low lymphocyte counts compared to Sprague Dawley (SD) rats, though a slight increase was observed with age. Compared to SD rats, both sexes had higher neutrophil, monocyte, and eosinophil counts, increased urea and cholesterol, lower albumin and total protein, and higher GLDH concentrations. Compared to SD rats, Additional findings included extended prothrombin times in males, while sporadic elevations in ALT, AST activity and triglyceride levels were noted among females.
Immunophenotyping confirmed the absence of B and T lymphocytes (CD4+ and CD8+) and NK cells, alongside increased monocyte levels. Histopathological assessments highlighted lymphoid hypoplasia and inflammatory findings in the kidneys and lungs, with urinary bacterial infections contributing to single mortality. Age-related conditions, including rodent progressive cardiomyopathy, chronic nephropathy and early reproductive senescence in females, were also observed.
These findings underscore the SDRG strain's potential as a robust model for non-clinical evaluations of immunotherapeutic and cell-based products, providing an initial reference into background features of this immunodeficient model that might help interpretation of experimental results.

Spontaneous cutaneous papillomas in two athymic nude mice

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Mouse papillomas are exceedingly rare benign epithelial tumors associated with infection by *Mus musculus* papillomavirus (MmuPV1). Despite being extensively studied as models for viral oncogenesis, immune responses, and malignant transformation under experimental conditions, spontaneous outbreaks of murine papillomas are exceptionally rare and have been only infrequently reported. Two Hsd:Athymic Nude-Foxn1^{nu} mice (Envigo)—one subcutaneously inoculated with an ovarian carcinoma patient-derived xenograft (PDX) and the other with a human lung carcinoma cell line—developed raised cutaneous lesions and subsequently underwent complete necropsy. Organs were processed routinely for histopathological evaluation, and immunohistochemistry for papillomavirus L1 antigen detection was performed on the cutaneous lesions. Both mice developed focal exophytic cutaneous lesions, located dorsally near the interscapular region, with one lesion presenting as ulcerated. Histological examination revealed hyperplastic epithelial fronds supported by thick fibrovascular stalks. Hypergranulosis and multifocal intranuclear inclusions were observed within the epithelium, although classic koilocytotic changes were absent. Immunohistochemistry for papillomavirus L1 antigen was negative. Additionally, both mice demonstrated multifocal renal tubular degeneration and regeneration with tubular intranuclear viral inclusions, and in one case, a concurrent multicentric lymphoma was identified. Altogether, the findings document 2 cases of cutaneous papillomas occurring alongside renal parvoviral inclusions and, in one case, lymphoproliferative disease. Further analyses are needed to definitively confirm or exclude the role of MmuPV1 in papillomas induction.

Drug-induced Polyploid Giant Cancer Cells in a Preclinical Model of High Grade Serous Ovarian Cancer: Histopathological Characterization

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Chemotherapy represents the primary therapeutic approach for several tumors including high-grade serous ovarian cancer (HGSOC), the most common subtype of human ovarian tumor. [2, 3, 6, 9] While a significant proportion of neoplastic cells succumb to treatment, a reservoir of cancer cells may survive chemotherapy, acquiring mutations that enhance drug-resistance, tumor invasiveness and metastatic potential. [1, 4, 8] Among these resilient cells are polyploid giant cancer cells (PGCCs), characterized by a large size and a polyploid genome. Recent studies indicate that PGCCs exhibit stem cell-like characteristics and may play a key role in HGSOC progression, potentially serving as both a prognostic marker and a therapeutic target in this disease. [5, 7, 10] The aim of the study was, therefore, to retrospectively characterize PGCCs and their relationship with chemotherapy in a murine model of HGSOC. Paraffin blocks of stomach, peritoneum and pancreas from 46 wild type C57BL/6 female mice intraperitoneally injected with 2 x 10^6, ID8 $p53^{-/-}$/$PTEN^{-/-}$ cells of HGSOC, treated with either intraperitoneal injection of PBS (23 mice) or carboplatin (23 mice) and sacrificed 40 days after inoculation were retrieved from the archives of the mouse and animal pathology laboratory (MAPLab) and stained with hematoxylin and eosin. A semiquantitative grading system was developed to assess tumoral burden, growth pattern predominance, necrosis, inflammation, aberrant mitoses and PGCCs number and location. No statistically significant differences in tumor necrosis, inflammation, or aberrant mitoses were observed between PBS and carboplatin-treated mice. PGCCs were statistically more abundant at the periphery of the tumor masses compared to their centres, and carboplatin treatment, while effectively reducing overall tumor burden, was statistically associated with an increased number of PGCCs. Furthermore, PBS-treated mice demonstrated a greater propensity for infiltrative growth along the pancreatic surface. Overall, carboplatin therapy decreased tumor mass but was associated with an enrichment of PGCCs in the HGSOC model.

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AI per la diagnosi macroscopica delle patologie polmonari negli ovini

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Le patologie polmonari infettive, parassitarie o neoplastiche sono considerate tra le principali cause di perdita economica negli allevamenti ovini, incidendo negativamente sull'accrescimento e sulla produttività. Una diagnosi precoce è quindi essenziale per ridurre la diffusione delle malattie e ottimizzare la gestione sanitaria del gregge. In quest'ottica, lo screening sistematico dei polmoni in sede di macellazione può rappresentare una soluzione efficace, offrendo una valutazione immediata e accurata dello status sanitario polmonare.

In questo lavoro, proponiamo un sistema di classificazione real-time basato su AI e nominato SPIRO (Sistema di Predizione e Indagine per le patologie Respiratorie Ovine) progettato per distinguere macroscopicamente il tessuto polmonare sano da quello affetto da patologie infiammatorie o neoplastiche negli ovini. La piattaforma utilizza reti neurali convoluzionali (CNN) integrate con tecnologie di edge computing, impiegando dispositivi come NVIDIA Jetson Nano e Raspberry Pi 5 per un'inferenza rapida e *in loco*. Abbiamo analizzato un dataset di 1.160 immagini macroscopiche JPEG, arricchito mediante tecniche di data augmentation (rotazione, zoom, ribaltamento) e testato quattro diverse architetture CNN: EfficientNetB0, VGG16, VGG19 e ResNet50 con l'obiettivo di classificare i polmoni in quattro categorie diagnostiche: sano, infiammazione, neoplastico e non determinabile macroscopicamente.

Tra i modelli testati, EfficientNetB0 ha fornito le migliori prestazioni in termini di precisione (P), recall (Rec) e F1-score (F1), mostrando una notevole robustezza nella rilevazione delle condizioni polmonari, particolarmente per le patologie neoplastiche (P=0.87, Rec=0.92, F1=0.89). Nonostante i risultati promettenti, sono necessari ulteriori sviluppi, quali la validazione dei dati tramite esami istopatologici e l'ampliamento del dataset con campioni più diversificati, soprattutto per i casi diagnostici più complessi.

In conclusione, l'automatizzazione dell'esame macroscopico dei polmoni ovini attraverso soluzioni AI-based potrebbe velocizzare e standardizzare la diagnosi offrendo uno strumento implementabile in campo, e favorendo l'acquisizione di dati di incidenza finalizzati allo sviluppo di sistemi integrati di gestione sanitaria nell'allevamento ovino.

Investigation of Ketamine-induced apoptosis in the brain of Sprague Dawley newborn rats

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Ketamine, an NMDA receptor antagonist, is a dissociative anesthetic widely employed as a general anesthetic for pediatric patients undergoing surgical procedures. It is characterized by rapid onset, short duration of action and swift recovery profile. However, ketamine-induced neurodegenerative effects in the developing brain have been reported in both *in vivo* and *in vitro* studies. Neuronal and oligodendrocytic cell death has been described, while no significant effects on microglia and astrocytes were previously reported. This study applied double immunofluorescent staining to evaluate neuronal and glial apoptosis in a neonatal rat model of prolonged sedation, aiming to identify the CNS cell types affected by ketamine neurotoxic effect. Sprague Dawley rats [Crl:CD (SD)] at postnatal day 7 were intraperitoneally injected with 20 mg/kg of ketamine or vehicle five times at 90-minute intervals and were sacrificed two hours after the final administration. Brains were fixed in 4% paraformaldehyde, paraffin-embedded, sectioned, and immunostained with the apoptotic marker Cleaved Caspase-3, in combination with neuronal and glial markers (NeuN, Iba-1, GFAP, and Olig2). Additional staining with Cleaved Caspase-9 and γH2AX was performed to assess whether ketamine induced apoptosis via mitochondrial and DNA damage pathways. A significant increase in the number of Cleaved Caspase-3-positive cells was observed following ketamine treatment in the cortex and hippocampus. Furthermore, colocalization of apoptotic with cell-specific markers demonstrated cell death in neurons, oligodendrocytes, microglia, and astrocytes. Apoptotic cells were also positive for Cleaved Caspase-9 and γH2AX further supporting the involvement of the mitochondrial apoptotic pathway. These findings suggest that ketamine may act as an inducer of oxidative stress, affecting not only neurons but also glial cells, ultimately leading to DNA damage and apoptosis through the mitochondrial pathway.

Development and histopathological characterization of an air-liquid interface model of goblet cell hyperplasia for in vitro screening of new therapeutic compounds

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In vitro models and new approach methodologies (NAMs) are essential tools in modern research, offering ethical, cost-effective, and disease-relevant alternatives to traditional animal testing. Detailed histopathological characterization of these models is critical to validate their pathophysiological relevance, ensure reproducibility, and accurately interpret experimental outcomes. The aim of this pilot study was to establish and characterize an air-liquid interface (ALI) culture of normal human bronchial epithelial (HBE) cells treated with IL-13, modeling airway goblet cell hyperplasia, a feature of several chronic respiratory diseases. HBE cells were seeded onto 24-well plate-fitting transwell inserts and cultured at ALI until complete differentiation, then treated with scalar doses of IL-13 (30, 10, 3, 1 ng/mL) to induce a goblet cell hyperplasia phenotype. Untreated cells served as negative controls, while co-treatment with an anti-IL-4R/IL-13R antibody was used as positive control to inhibit IL-13 effects. Secreted cytokines (eotaxin-2 and eotaxin-3) were measured in the basal medium by ultra-sensitive immunoassay, while gene expression of *CCL24, CCL26, POSTN, FOXA3 and MUC5AC* was analyzed by RT-qPCR. Each sample was routinely processed for histopathology and immunohistochemistry for Mucin 5AC, followed by semiquantitative scoring of morphological features and quantitative image analysis on digital slides. Histological evaluation revealed a concentration-dependent increase in the epithelial thickness, along with increased goblet cell number compared to the untreated control and formation of mucous-filled cysts in samples treated with IL-13 at concentrations ≥3 ng/mL. Anti-IL-4R/IL-13R antibody treatment almost completely reversed the hyperplastic phenotype. Immunohistochemistry results correlated with *MUC5AC* gene expression analysis and inflammatory cytokines showed a concentration-dependent increase, as measured by both MSD and RT-qPCR. This study successfully established and validated an in vitro ALI model of IL-13-induced airway goblet cell hyperplasia, providing a robust platform for investigating chronic respiratory disease mechanisms and screening therapeutic compounds.

Evaluation of the cellular response to osmotic stress in porcine intestinal epithelial cells (IPEC-J2) through tight junction protein regulation

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The intestinal epithelium acts as a crucial barrier between the intestinal lumen and the host tissues, and plays an essential role in nutrient absorption, immune defence, and overall, in animal health. Intestinal epithelial cells constantly face physiological changes, including fluctuations in osmolarity across intestinal segments or in the concentration of solutes, thus regulating water distribution and fluid balance, which ultimately influences tissue health. Maintaining this balance is critical for epithelial morphology, function, and systemic health. Hyperosmotic conditions, often triggered by bacterial components and other stressors, can alter absorption, induce severe diarrhoea, and impair growth in young animals. This study investigates the regulation of tight junction proteins (TJp) upon osmotic stress in intestinal epithelial cells. The IPEC-J2 cell line, derived from the jejunum of precolostral piglets, was cultured in transwells under hyperosmotic conditions (500 mOsm/L) induced by mannitol or sucrose at different time-points (4 hours, 24 hours, and 4 days). Morphology, permeability (transepithelial electrical resistance, TEER), cell viability, nitric oxide production, gene expression and protein localization (qPCR and immunohistochemistry) of TJp (Claudin-4, Occludin, and ZO-1) were evaluated. The results showed that mannitol-induced hyperosmolarity led to a significant reduction of cell viability, an increase in nitrite release and a significant reduction of TEER starting from 4 hours, which remained low for the following time-points, accompanied by a reduction in TJp gene and protein expression. In contrast, sucrose-induced hyperosmolarity promoted increased cell viability, significantly increased TEER, and stable nitrite release compared to control over time. Claudin-4, Occludin, and ZO-1 expression was increased in sucrose-induced hyperosmolar environment. TJ gene expression and protein amount were significantly increased in sucrose-treated cells. The results highlight that the type of solute induces different cellular responses, particularly influencing the functionality of the epithelial barrier, and underline the importance of osmotic balance in maintaining intestinal epithelial integrity.

Applications of humanized mice in regulatory submissions of cell-based therapeutic medicinal products

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The development of cell-based therapeutics presents unique challenges in preclinical research, particularly in accurately predicting human responses. Humanized mice, traditionally used for proof-of- concept studies, are now recognised for their potential in addressing safety questions within the regulatory framework and are being employed more and more frequently in toxicity, tumorigenicity, and biodistribution studies. Pathologists, depending on the primary endpoints of the *in vivo* studies, evaluate the efficacy of cell-based candidates in reducing tumor size in patient-derived xenograft mice or improving the pathophenotype in disease models; assess cell distribution, engraftment, and persistence within the host; evaluate administration site reactions and the toxicity profile in both target and non-target tissues; and contribute to the identification of potential unregulated or dysregulated cell proliferation, using cell morphology in conjunction with cell-specific identification markers. Pathologists must also address methodological challenges associated with the use of immunodeficient and humanized mice in long-term studies, such as low survival rates, the confounding role of transplant-related pathology and lesions induced by conditioning regimens, limited availability of comprehensive background data, and variability associated with different cell donors. Italy is home to several pioneering stem cell research institutions and companies, offering numerous opportunities for advancements in cell-based medicinal products. As the field continues to evolve, pathologists with a specialised experimental pathology training are at the forefront of driving progress in stem cell therapy.

Digital quantification of placental lesions and their association with neonatal mortality in dogs

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Placenta is a temporary organ supporting foetal development through physiological exchanges between maternal and foetal circulation. Exchanges occur in the placental labyrinth, a specialized surface with a complex anatomical structure comprising a network of maternal and foetal vessels and highly specialized cells. Lesions affecting the human placental exchange surface impair foetal development and have been associated with pregnancy complications and neonatal mortality. The use of image analysis and deep learning has enabled precise quantification of human placental lesions predictive of adverse foetal outcomes. Contrarily, studies investigating and quantifying placental lesions by digital tools are mostly missing in veterinary medicine. This study aims to quantify placental lesions by digital image analysis and assess their association with neonatal mortality. Placental samples (34) from healthy bitches undergoing c-sections were collected, 27 from healthy puppies and 7 from newborns deceasing within seven days postpartum. Infectious causes were excluded basing on bitch and puppies' clinical evaluation. Placental tissues were fixed and processed routinely, 5-micron sections were stained with H&E. Whole slides were scanned and analysed using QuPath software to measure areas of more frequent lesions such as necrosis, haemorrhages and oedema. Most extended lesion was necrosis, and binary logistic regression demonstrated a statistically significant correlation between the reduction of functional placental surface and neonatal mortality ($p = 0,034$). These findings suggest that a reduction of placental labyrinth's surface greater than 20% plays a decisive role in neonatal outcomes and highlight the potential of digital analysis as a tool for the study of placental lesions.

Histological evaluation of synthetic scaffolds loaded with pancreatic BxPC3 tumor cells and treated with Hadrontherapy (HT) and Magnetic Fluid Hyperthermia (MFH)

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Pancreatic cancer has poor prognosis, and since a complete surgical remove is often not possible, it is usually treated with radiotherapy (RT). However, innovative strategies, including Hadrontherapy (HT) and Magnetic Fluid Hyperthermia (MFH) have recently been introduced. MFH involves the injection of magnetite nanoparticles (MNPs) into the tumor, followed by the application of an alternating magnetic field to locally increase temperature and damage cancer cells. This study aimed to evaluate the effects of HT and MFH on human pancreatic BxPC3 tumor cells cultured within synthetic scaffolds. Scaffolds were fabricated with CELLINK-Bioink synthetic matrix and bio-printed with $2x10^6$ BxPC3 cells, with or without MNPs (100 µg/ml), and treated with HT (0 and 4 Gy) and/or MFH (42°C, 30 minutes) 48h post-printing (n = 3 per tested condition, for a total of 18 scaffolds). Scaffolds were formalin-fixed 15 days after irradiation, paraffin-embedded, and 4 µm sections were stained with Hematoxylin-Eosin (H&E) for histological evaluation. Digital Image Analysis (DIA) was performed using QuPath to automatically quantify the number of cell clusters and cells per cluster. Statistical analysis was performed using Mann-Whitney and Kruskal-Wallis tests. The histological evaluation revealed the presence of viable cells arranged in clusters (suggestive of clonogenic capacity) primarily located at the periphery of the scaffolds. Differences emerged in the Kruskal-Wallis test but were not retained after correction for multiple comparisons. Nevertheless, a reduction in both the number of cell clusters and the number of cells per cluster was observed following HT and MFH treatment compared to untreated controls. These findings suggest that HT and MFH are effective in reducing the viability of BxPC3 pancreatic cancer cells in a 3D synthetic scaffold model. Furthermore, this model of synthetic scaffold proved to be valid as an alternative model to conventional 2D *in vitro* systems for evaluating therapeutic efficacy of HT and MFH.

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

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High-Grade Serous Ovarian Cancer (HGSOC) is the most prevalent subtype of epithelial ovarian cancer, often associated with high rate of recurrence and metastasis leading to poor prognosis. Within the heterogeneous cellular populations of HGSOC, Polyploid Giant Cancer Cells (PGCCs) have emerged as a clinically relevant population, potentially linked to prognostic factors and therapeutic strategies and have been shown to be induced by chemotherapy treatment. This study aimed to immunohistochemically characterize PGCCs in a preclinical HGSOC model, with the goal of identifying specific markers to facilitate PGCCs detection in tissue samples and investigate potential treatment-related effects on markers expression. Formalin-fixed paraffin-embedded (FFPE) tissue blocks from the stomach, peritoneum, and pancreas of 4 wildtype C57BL/6 female mice were analysed. These mice had been intraperitoneally injected with 1×10^6 ID8 $p53^{-/-}$/$PTEN^{-/-}$ HGSOC cells and treated with either vehicle (n = 2) or carboplatin (n = 2). Tissues were stained using a panel of antibodies (based on bibliography and assumed to identify PGCCs), including Vimentin, wide-spectrum Cytokeratin (CKws), E-cadherin, N-cadherin, γH2AX, Cleaved-Caspase-3, and LAMP-2. The staining was evaluated based on subcellular localization (nuclear, cytoplasmic, membranous) and semi-quantitatively graded as absent (0), weak (+), moderate (++), or strong (+++), in both tumor cells and PGCCs. None of the markers tested were specific for PGCCs, as all stained both tumor cells and PGCCs. However, Vimentin (and to a lesser extent, CKws and γH2AX) showed increased expression in carboplatin-treated samples compared to controls, in both cell populations. No significant differences were observed between treatment groups for the other markers. These findings suggest that carboplatin treatment influences the expression of certain markers in HGSOC cells. However, none of the markers tested demonstrated specificity for their identification. Further tests with other antibodies are therefore necessary to identify specific markers for PGCCs.

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A case of extracoelomic paravertebral (sacrococcygeal) subcutaneous nephroblastoma in an elderly dog.

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Nephroblastoma originate from the poor differentiation of the metanephric blastema and typically present as a unilateral renal mass that affects young, large-breed dogs. In human, nephroblastoma (Wilms tumour - WT) is the most common renal tumour affecting children under 5 years of age. Both in adult dog and in human the tumour is exceedingly infrequent (0.2 per million/year for the WT).

Extra renal location is occasionally described both in juvenile human and dog, generally arising from intradural, extraspinal ectopic nephrogenic rests with possible vertebral column, genitalia or inguinal involvement. We report herein a nephroblastoma apparently arising from heterotopic tissue outside the renal developmental tract in a 12 years-old neutered female Labrador Retriever, in absence of neurological signs and without evidence of renal or spinal involvement. The tumour was a firm, not painful and not ulcerated 15 cm in diameter mass, with smooth surface. At gross examination it was soft with a homogeneous lardaceous appearance. Microscopically it was an expansive and infiltrating, poorly demarcated, not capsulated subcutaneous mass and consisted in abundant nephrogenic tissue arranged in irregular tubules with multifocal glomeruloid structures and admixed with undifferentiated mesenchymal cells (blastematous cells). Immunohistochemical positive staining for VIM, panCK, and WT-1 confirmed the diagnosis.

After removal, the dog was submitted for abdominal x-rays and ultrasound, with no visible alteration; few weeks afterwards a local recurrence occurred.

In young woman a subset of WT with similar characteristics have been described together with spinal defect as spina bifida, dyastemomyelia or dysraphysm; despite the primary site of this rare nephroblastoma in an elderly dog have not yet been established (further analysis are ongoing), no congenital spinal defects have been observed. The aformentioned tumor were diagnosed as an extracoelomic paravertebral (sacrocoggygeal) subcutaneous nephroblastoma. To the best of our knowledge, this is the first case report in veterinary medicine.

Malignant Granulosa Cell Tumor in a female kitten

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Granulosa cell tumor (GCT) is the most common sex cord–stromal tumors in animals; in the cat it occurs prevalently in older individuals (3-16 years), although it can occasionally develop in juvenile cats (<1 year). Similarly to cats, a juvenile form has been documented in young women (JGCT), characterized by different pattern of hormone production.

GCT in cats is usually associated with clinical signs, particularly repeated estrus and cystic endometrial hyperplasia, swelling of the abdomen, anorexia, emesis, polydipsia and polyuria.

This case report investigates the macroscopic and microscopic findings of a GCT in a 4.5 month old female DSH kitten, euthanized for pulmonary oedema and with an ovarian 4x4 cm neoplasm, ascites and mammary gland swelling.

Macroscopically, the tumor had a smooth nodular surface characterized by prominent cystic and haemorrhagic lesions. Histologically, the ovarian parenchyma was diffusely replaced by a well-demarcated, unencapsulaeted, densely cellular neoplasm composed of granulosa cells arranged in a macrofollicular and cord-like pattern, interspersed with cystic areas within a haemorrhagic collagenous stroma. Neoplastic cells displayed mild anisocytosis and anisokaryosis, with rare mitoses.

The diagnosis of GCT was confirmed by the immunohistochemistry (IHC): positive for inhibin α, negative for cytokeratin 7, HBME and 3β-H-dehydrogenases.

JGCTs are well documented in human medicine, including cases in neonates, whereas in cats they are rarely reported and poorly characterized. This case highlights the rare occurrence of a granulosa cell tumor in a juvenile cat and contributes to the limited veterinary literature on this topic. Further case reports are crucial to enhance the understanding of the clinical and pathological characteristics of these tumors in young animals.

Another good reason to not over-interprete gross pathology: a puzzling diagnostic case

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A 16-year-old dog was presented with altered hind limb gait. Clinical signs progressively worsened with the dog being unable to walk on all 4 limbs. The neuroanatomical localization was cervico-thoracic and thoraco-lumbar spinal cord. Radiographs of these regions showed small lytic lesions in numerous vertebrae as well as enlargement and lysis of the spinous process of the second thoracic vertebra. The most likely diagnosis was a neoplastic condition. The dog was humanely euthanized. A full necropsy was performed. No remarkable gross lesions were found besides within bones. 90% were located along the spinal column and the rest at the femoral head and tibial crest. Lesions where round, multifocal to coalescing, white-ish to very hematic and appeared like they were punching holes in the vertebral bodies and/or in the spinous process. An initial, preliminary diagnosis of XX was made based on the gross pathology, with YY and ZZ as likely differential diagnosis. Before placing the samples in formalin, different cytological specimens obtained by scraping of the lesions were obtained and stained with May-Grunvald stain technique. The cytological diagnosis was entirely in disagreement with the gross diagnosis. The particularly hematic gross lesions and the malignant features of the mesenchymal cells present in the cytological specimen moved the diagnosis towards YY as a first likely diagnosis and ZZ still in the differential list, whereas, XX was completely excluded from the diagnosis list. HE-stained slides were obtained from the formalin fixed-paraffin embedded samples. Based on histology, the final diagnosis of

–sarcoma was made. Surprisingly, was none of the 3 that were present in the differential diagnosis list. Histochemical stains like Alcian blue was used to confirm the diagnosis. No immunohistochemistry has been performed as of today.

Many key information and the 3 initial differential diagnosis (XX, YY, and ZZ) as well as the final diagnosis (-sarcoma) are omitted on purpose to not influence or bias the reader when she/he will see the pictures in the poster. If you want to know more about this curious case, just come and pay a visit to the poster.

Neuropathology of Canine Hemangioblastoma

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Hemangioblastoma is a benign, slow growing, highly vascular human neoplasm (WHO grade I). The tumor most commonly arises in the cerebral hemispheres and cerebellum, where it is more frequently observed in patients with von Hippel-Lindau disease. Only 7.5 to 25% of all hemangioblastomas occur in the spinal cord, and cases of extraneural localization are exceedingly rare.

In veterinary medicine, hemangioblastoma has only been described in the central nervous system of dogs and in the skin of a lamb. Cases of canine hemangioblastoma have been reported in the spinal cord (5 cases), forebrain (1 case), and brainstem (1 case). Dogs treated surgically had a good prognosis.

Our study aimed to characterize the neuropathological features of 6 cases of canine spinal cord hemangioblastoma and one case of sciatic nerve localization. The dogs were 4 males, and 3 females aged between 6 and 12 years. The histological staining panel included H&E, Luxol Fast Blue, and Goldner trichrome staining; immunohistochemistry included the following markers: NSE, VIM, factor-VIII, GFAP, inhibin-α, carbonic anhydrase IX (CAIX), and Ki67.

The tumor was composed of numerous, haphazardly arranged capillaries lined by plump endothelium and interstitial fusiform to stellate stromal cells with slightly eosinophilic cytoplasm, occasionally containing small lipid vacuoles. Mitotic activity was low. Frequently, surrounding the capillaries were moderate to prominent perivascular aggregates composed of plasma cells and fewer lymphocytes. Immunohistochemically, the stromal cells were strongly immunolabeled with NSE and were negative for factor-VIII in all cases. Inhibin-α was negative, whereas stromal cells expressed CAIX in most cases.

Although the predominant location of canine hemangioblastoma is the spinal cord, its morphological and immunohistochemical features are comparable to the human counterpart. This is the first report of peripheral nerve hemangioblastoma in animals.

Uropigial gland keratoacanthoma related to an avian herpesvirus infection in a saker falcon (*Falco cherrug*)

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Viral infections play a significant role in the development of neoplasms, both directly (oncoviruses, such as *Retroviridae* and *Herpesviridae*) by altering host cell DNA and disrupting tumour suppressor genes and oncogenes; and indirectly by causing chronic inflammation or immunosuppression. Our case refers to a 24-year-old female saker falcon (*Falco cherrug*), which was presented with a hyperkeratotic lesion associated with the uropygial gland. Initially, a conservative medical approach was preferred over complete surgical excision, but due to the recrudescence, a surgical excision of the uropygial gland was carried out. However, during the procedure, the bird developed a cardiorespiratory arrest. Gross pathology and histological examinations were performed revealing a compact cystic mass with atypical proliferation of the keratin and squamous cell layers, consistent with an infiltrating keratoacanthoma. Additional histopathological findings included necrotizing hepatitis, splenic focal necrotic lesions and white pulp depletion, pyogranulomatous nephritis, pulmonary oedema, and infiltrative chronic-active enteritis. Amphophilic intranuclear inclusion bodies were observed in the epidermis basal layer of the uropygial tumour, and in the hepatocytes, suggesting a herpesvirus infection. Performing an immunohistochemical examination with mouse monoclonal anti-Marek Disease Virus (MDV) antibody, and rabbit anti-Infectious Laryngotracheitis Virus (ILTV) glycoprotein E polyclonal antibody, was confirmed the presence of avian alpha-herpesvirus antigens in the examined tissues. The anti-ILTV antigen detection was positive for avian herpesvirus in the uropygial gland mass, liver and kidneys, while the anti-MDV antigen detection was negative. The viral immunosuppressive induction, but also a chronic viral integration in host-cells, may have contributed to the neoplastic proliferation of the uropygial gland's epithelium. To the authors' knowledge, this is the first reported case of uropygial keratoacanthoma in a bird of prey.

Characterization of fibroblasts in BPV1-associated equine sarcoids

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Equine sarcoids are the most commonly diagnosed skin tumors in horses, marked by locally invasive growth and a high recurrence rate following surgical removal. These neoplasms are associated with δ bovine papillomavirus (BPV) types 1, 2, and 13, and are characterized by activated fibroblasts capable of invading and degrading the extracellular matrix. To date, few studies have explored their phenotypic characteristics beyond matrix metalloproteinase activity. To further investigate the characteristics of sarcoid fibroblasts, HIF-1α, αSMA and S100 protein were investigated. A retrospective study was performed on 33 specimens, 23 equine sarcoids and 10 healthy specimens. Histopathological evaluation was performed and the presence of BPV-1, -2 and -13 DNA was assessed by qPCR in all samples examined. ISH was used to detect E6/E7 oncogene mRNA expression. IHC was performed to evaluate the expression of HIF-1α, αSMA and S100. All tumour samples were positive for BPV-1 DNA and showed expression of the E6/E7 oncogenes. Control samples were all negative for BPV. IHC results showed that 80% of the fibroblasts expressed intense cytoplasmic S100, while all samples were negative for αSMA. In addition, 80% of sarcoids showed moderate to strong cytoplasmic staining for HIF-1α in >60-70% of fibroblasts. Based on their histological features, anatomical localization and lack of αSMA expression, these S100-positive spindle cells are interpreted as activated fibroblasts. Although S100 is commonly associated with neural lineages, its expression in fibroblasts under pathological conditions has been reported. The absence of αSMA indicates they are not myofibroblasts, suggesting a non-canonical activation state. The strong HIF-1α expression supports the presence of a hypoxic microenvironment, which may promote fibroblast activation and tumor persistence without inducing myofibroblastic differentiation. These findings suggest that BPV-associated sarcoid fibroblasts may be adapted to a hypoxic tumor niche, highlighting the role of viral oncogenes and hypoxia in sarcoid pathogenesis.

Malignant mesothelioma of the peritoneum and pleura in a wild alpine red deer (*Cervus elaphus*)

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Malignant mesothelioma is a rare neoplasm originating from the mesothelial lining of coelomic cavities. Reports in wild animals are scarce, with only three cases documented in cervids – *Dama dama*, *Cervus alfredi*, and *Cervus nippon yesoensis*. This report describes the first case in a wild, adult female red deer (*Cervus elaphus*). The animal was euthanized in the Belluno province (Northern Italy) due to poor clinical condition (lateral recumbency with cervical extension). The carcass was examined by the official veterinary authority, and representative organs were submitted to the Istituto Zooprofilattico Sperimentale delle Venezie for macroscopical and histopathological evaluation. Gross findings included abundant serohemorrhagic effusion in the thoracic and abdominal cavities and several whitish, firm, lardaceous nodules and masses in the peritoneum – on the liver, diaphragm, spleen, omentum – and pleura – on the lung and diaphragm – and infiltrating mediastinal lymph nodes. Histological examination revealed a densely cellular, infiltrative proliferation of pleomorphic neoplastic cells ranging from fusiform/stellate cells arranged in bundles, to epithelioid cells in nests, consistent with a biphasic mesothelioma. Mediastinal lymph node architecture was completely effaced. Anisocytosis and anisokaryosis were severe, bi- or multinucleated neoplastic cells were common, and the mitotic count was elevated. Foci of colliquative necrosis were frequent. Multifocal neoplastic lymphovascular emboli were present, particularly in the liver and diaphragm. Immunohistochemistry revealed diffuse expression of pan-cytokeratins (CK AE1/AE3), multifocal positivity to vimentin (V9), and, less frequently, to the mesothelial cell marker HBME-1, consistent with a mesothelial origin. The primary site of origin was undetermined due to the widespread distribution of the lesions; however, the predominant abdominal involvement suggests a primary peritoneal origin. This case highlights the importance of systematic post-mortem surveillance and integrated pathological approaches in identifying rare neoplasms in free-ranging wildlife.

Metastatic intrahepatic cholangiocarcinoma in a captive puma (*Puma concolor*)

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Cholangiocarcinoma (CCA) is a rare aggressive neoplasm arising from the intra-hepatic or extra-hepatic biliary epithelium, with limited reports in large felids. A 16-year-old captive puma (*Puma concolor*) presented with weight loss, abdominal distension, acute vomiting and diarrhea. Due to the critical clinical picture and poor prognosis, euthanasia was chosen. Necropsy revealed extensive sero-hemorrhagic cavitary effusions and multiple white-yellow neoplastic masses in the left hepatic lobes. Metastases were detected in the peritoneum, pericardium, kidney, spleen, and spermatic cord. Cytologic smear from the pericardial effusion revealed the presence of cohesive clusters of cuboidal epithelial cells. Histologically, the neoplasm was composed of tubular and acinar structures embedded in a desmoplastic stroma. Immunohistochemistry revealed diffuse staining with pan-cytokeratins (CK AE1-AE3) and CK7, while CK20 staining was multifocal and less intense. Lymphovascular invasion was evident, particularly associated with diaphragmatic metastatic nodules. Intestinal samples tested positive for Feline Coronavirus (FCoV) by Real-Time RT-PCR, while mesenteric lymph nodes were negative, suggesting an infection confined to the intestine. Although primary hepatobiliary tumors are rare in domestic animals, cholangiocarcinoma seems to be the most common non-hematopoietic hepatic tumor in domestic cats. Neoplastic diseases are a significant cause of morbidity and mortality in captive wild felids, likely due to their longer life expectancy compared to their free-ranging counterparts. However, the higher exposure to pollutants, diverse infectious agents, and genetic alterations may act as predisposing factors to the development of neoplastic disease.

Molecular and Histopathological characterization of equine sarcoids from Southern Italy

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Equine sarcoid is the most common cutaneous tumour in horses and other equids. Although non-metastatic, it is locally invasive and often recurs, causing significant animal welfare issues.
δ Bovine papillomaviruses (BPV-1, BPV-2, BPV-13) are recognized as the main causative agents. BPV infection typically begins in the epidermis, where it may remain latent, with subsequent detection of viral material in subepidermal fibroblasts, where full cellular transformation occurs. Despite the established association between sarcoid clinical subtypes and BPV infection, limited data exist about the relationship between histopathological features and BPV genotypes. This study aimed to identify BPV DNA (BPV-1, BPV-2, BPV-13, and BPV-14), in normal and pathological skin lesions (sarcoid and non-sarcoid tumours and non-neoplastic conditions) from horses raised in Southern Italy and to assess by histopathology the main histological features of sarcoids to evaluate any correlations with BPV genotypes. BPV DNA was detected in 76% of sarcoid samples, with BPV-1 being the most prevalent (61%), followed by BPV-2 (27%) and BPV-13 (20%). BPV was also detected in non-sarcoid tumours and inflammatory lesions, but not in normal skin. No samples tested positive for BPV-14. Histological examination revealed typical sarcoid features, including severe epidermal hyperplasia with rete peg formation, dermal fibroblast proliferation arranged in a "picket fence" pattern, and variable extracellular matrix deposition. BPV-1 was associated with more severe fibroblastic atypia and abundant matrix production, while BPV-2 and BPV-13 were linked to milder histological changes. No statistically significant correlation was found between specific BPV genotypes and histopathological patterns. Further studies with larger sample sizes are needed to provide insights into the prevalence and potential pathogenic roles of different BPV genotypes in equine sarcoids, which may help in the development of new immunotherapeutic approaches.

The matryoshka effect. A case of collision tumors: melanoma and squamous cell carcinoma in the prepuce of a horse

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A 20-year-old Camargue gelding was referred to the veterinary hospital due to the presence of multiple neoplasia in the perianal, preputial and paragenital regions. Following surgical removal of prepuce a and portion of penis, a reconstructive plastic surgery was performed to restore the non-resected portion of the penis, which allowed to complete return of urinary function.
The preputial area was inflamed and showed multiple superficial erosions, while the distal penile region showed 3 neoplasia ranging from 7 to 15 cm of diameter.
Prepuce, a portion of 30 cm of penis and adjacent lymph nodes were sectioned and fixed in formalin, embedded in paraffin, and stained with routine HE, melanin bleaching and immunohistochemistry for Cytokeratin AE1/AE3, Vimentin and MelanA.
Gross pathology dissection of the distal penile region neoplasia revealed extremely melanized areas inside or adjacent to firm and whitish areas. Lymph nodes were partially invaded by melanized tissue. Morphological and immunohistochemical analysis revealed a collision tumor composed of two malignant populations, epithelial and melanocytic, with metastasis of the neoplastic melanocytes to the regional lymph node. The epithelial component consisted of trabeculae and islands of well-differentiated squamous epithelium immunoreactive to cytokeratins AE1/AE3. The melanocytic component had a varying degree of pigmentation of polygonal and spindle-shaped cells, growing in nests or densely packed aggregates and immunolabelled with melanoma-associated antigen (melan A).
Collision tumor is a neoplastic lesion where two or more distinct cell populations, each representing a separate tumor type, are present within the same lesion, separated by a clear boundary. While relatively uncommon, collision tumors have been reported in horses in ovary, nervous system, and digestive tract. To the authors' knowledge, this is the first description of a penile collision tumor involving malignant melanoma and squamous cell carcinoma in the horse.

Valutazione interlaboratorio della riproducibilità diagnostica istologica nei tumori degli animali domestici: risultati del Ring Test 2024 del CEROVEC

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L'uniformità diagnostica tra laboratori è essenziale per garantire l'affidabilità dei risultati istopatologici nella medicina veterinaria. Il presente studio, organizzato dal Centro di Referenza Nazionale per l'Oncologia Veterinaria e Comparata (CEROVEC), ha avuto l'obiettivo di valutare la capacità dei laboratori partecipanti nel riconoscimento istologico di lesioni neoplastiche benigne, maligne e non neoplastiche negli animali da compagnia, nonché la concordanza tra lettori, mediante un circuito interlaboratorio (Ring Test) standardizzato.
Il Ring Test del 2024 ha coinvolto 20 patologi afferenti a 11 strutture diagnostiche italiane, principalmente della rete degli Istituti Zooprofilattici Sperimentali. Ogni partecipante ha esaminato 36 campioni istologici digitalizzati, colorati con ematossilina-eosina, provenienti da casi diagnostici reali (29 canini, 7 felini). Le diagnosi sono state espresse come benigno (B), maligno (M) o non neoplastico (N). Il valore assegnato per ciascun caso è stato determinato come valore modale (giudizio di maggioranza, GM). La concordanza qualitativa è stata misurata con l'indice kappa
La percentuale media di risposte accettabili per lettore è stata superiore all'80%, con punte massime del 100%. L'indice kappa rispetto al GM ha mostrato valori tra 0.67 e 1.00 (IC95% da 0.46 a 1.00), evidenziando una buona riproducibilità inter-lettore. L'accordo medio per diagnosi morfologica (ICD-O) è risultato compreso tra 0.60 e 0.76. I dati indicano un livello di concordanza diagnostica generalmente soddisfacente, ma con margini di miglioramento nella classificazione morfologica dettagliata.
Il Ring Test 2024 conferma l'affidabilità delle letture istopatologiche nella rete dei laboratori veterinari italiani, soprattutto per la classificazione generale (B/M/N). Tuttavia, la variabilità osservata nella codifica morfologica suggerisce l'importanza di ulteriori momenti formativi condivisi e l'adozione di criteri diagnostici standardizzati. Lo studio, organizzato annualmente dal CDR, rappresenta uno strumento utile per il monitoraggio della qualità diagnostica e il rafforzamento dell'armonizzazione interlaboratorio.

Carcinoma squamocellulare cutaneo in ovini di razza sarda e papillomavirus: relazione plausibile?

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Le infezioni da papillomavirus sono considerate fattori di rischio per lo sviluppo di carcinomi squamocellulari cutanei (cSCC) in varie specie animali e nell'uomo, agendo sinergicamente, tramite l'inibizione di fattori oncosoppressivi, con i danni al DNA indotti dall'esposizione ai raggi UV. Nell'uomo, la bassa frequenza di rilevamento dei papillomavirus nei cSCC è stata attribuita alla mancata integrazione del DNA virale in quello dell'ospite e alla conseguente perdita del virus durante la proliferazione neoplastica. Tra i papillomavirus noti nell'ovino, OaPV3 e OaPV4 sono stati identificati per la prima volta in Sardegna in pecore di razza sarda in associazione a un carcinoma squamocellulare e un fibropapilloma, suggerendo il loro coinvolgimento nella patogenesi neoplastica cutanea.

In questo lavoro, è stata indagata la presenza di papillomavirus in cSCC di ovini provenienti da 6 allevamenti in Sardegna. Durante la macellazione, sono state campionate 32 neoformazioni cutanee da 29 ovini di razza sarda. Le lesioni sono state processate per le analisi istopatologiche e conservate a -80°C per le indagini di biologia molecolare.

L'esame istopatologico ha evidenziato la presenza di 32 cSCC (mammella = 25; padiglione auricolare = 7; regione cutanea nasale = 11), dei quali 9/32 sono risultati microinvasivi con dimensioni <1 cm. Le indagini molecolari non hanno rilevato la presenza di DNA virale nei campioni esaminati.

Dopo l'estrazione del DNA dai tessuti, i campioni sono stati analizzati tramite PCR per i geni E6 di OaPV3 e OaPV4 e nested PCR per il gene L1 di OaPV3.

Questi risultati sottolineano i limiti della diagnostica dell'infezione da papillomavirus nei cSCC dell'ovino, analogamente a quanto riportato nell'uomo. Sono in corso ulteriori indagini per rilevare la presenza di papillomavirus su lesioni preneoplastiche e, tramite tecniche di next-generation sequencing, per caratterizzare la popolazione virale associata a tali lesioni, al fine di indagare la relazione tra papillomavirus e cSCC nella specie ovina.

Metastatic melanoma in a Seychelles giant tortoise (Aldabrachelys gigantea hololissa): Case report

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The occurrence of melanomas in reptiles, compared with those reported in homeothermic species, is low. In reptiles, there are several pigmented cells, including melanophores (or melanocytes), iridiophores, and xanthophores. Among these, melanocytes are the most frequently associated with tumor development. The purpose of this study is to present a case of metastatic melanoma in a Seychelles giant tortoise kept under human care in a zoological garden in northeastern Italy.

In March 2025, an adult male of Seychelles giant tortoise (*Aldabrachelys gigantea hololissa*) housed in Parco Natura Viva (Italy) was found dead. The clinical history reported by the veterinary personnel of the park reported only lethargy and depression of the animal. The following day, a complete standardized necroscopy was performed at the laboratories of the Department of Comparative Biomedicine and Food Sciences of the University of Padua (Italy). Gross examination revealed multifocal spherical nodules, varying in size, infiltrating the hepatic parenchyma; on cut section, the masses appeared dark brownish. Some of the larger nodules presented multiple cavitary spaces and encapsulated cystic cavities. Metastatic nodules exhibiting the same features were also present in the lungs and in the left testicle, while lesions with irregular and undefined margins were evident in the trachea, stomach, and duodenum. Histological evaluation revealed spindle cell elements with undefined cytoplasmic boundaries and cytoplasm containing brownish pigment granules, referable to melanin. The nucleus is oval, central, with margined chromatin and a prominent nucleolus. Features of anisokaryosis and anisocytosis are severe, while mitoses are rare.

To our knowledge, this is the first report of malignant metastatic melanoma in *Aldabrachelys gigantea hololissa*. No possible primary tumor site was detected during the external examination; however, the eyes could not be evaluated because the head was preserved for museological purposes, given the biological and educational importance of the specimen.

Platelet-derived growth factor receptor beta (PDGFRβ) as a potential druggable target in canine apocrine gland anal sac adenocarcinoma (AGASAC)

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Apocrine gland anal sac adenocarcinoma (AGASAC) is a highly aggressive tumour in dogs, often developing nodal metastasis in early stage. PDGFRβ is a tyrosine kinase receptor involved in proliferation, metastasis, invasion, and angiogenesis. Several tumours overexpress PDGFRβ, and tyrosine kinase inhibitors (TKIs) have shown promising results in treating AGASAC by improving clinical signs or increasing survival time. However, the scientific basis for this response has not been substantiated by an evaluation of PDGFRβ expression in AGASAC. This study assessed PDGFRβ expression in different AGASAC histotypes to confirm its usefulness as a druggable target for adjuvant therapies.

Tissues from 3 normal anal sacs and 51 canine AGASACs were assessed histologically and immunohistochemically with a polyclonal anti-human PDGFRβ antibody made in rabbit validated in dogs. Included AGASAC histotypes were mixed with solid prevalence (26/51), solid (11/51), mixed with tubular/rosette prevalence (9/51), tubular/rosette (4/51), and neuroendocrine-like (1/51). Normal anal sacs expressed PDGFRβ in ≥75% of epithelial cells, while 42/51 tumours were PDGFRβ positive in 5-100% of neoplastic cells, with 25/42 of the positive tumours expressing PDGFRβ in ≥75% of neoplastic cells.

Positivity was observed in 18/26 mixed-solid, 10/11 solid, 9/9 mixed-tubular/rosette, 4/4 tubular and 1/1 neuroendocrine AGASACs.

The observation of PDGFRβ expression supports the use of TKIs for the treatment of AGASAC, at least for cases with high expression. Further studies should investigate PDGFRβ expression in nodal metastases to support the use of TKIs as adjuvant therapy in metastatic cases.

Analisi preliminare per l'identificazione e la localizzazione di biomarcatori dello stato di salute e di risposta allo stress ambientale in ostrica piatta (*Ostrea edulis*)

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L'ostrica piatta (*Ostrea edulis*) è mollusco bivalve della famiglia Ostreidae, originario dell'Europa. In passato il suo areale di distribuzione era piuttosto ampio e si estendeva dalla Norvegia al Marocco nell'Atlantico, nell'intero bacino del Mediterraneo e nel Mar Nero. Tuttavia, diversi fattori tra cui l'eccessivo sfruttamento della risorsa, il deterioramento degli habitat e l'intensificarsi di episodi di mortalità, dovuti alla presenza di patogeni, hanno portato a un progressivo declino delle popolazioni selvatiche e allevate.

Nell'ambito del progetto Interreg Italia-Croazia (MARINET) ci si propone di verificare la fattibilità dell'allevamento delle ostriche piatte in Italia e Croazia tramite l'impiego di tecnologie classiche e innovative, testando diverse tecniche di allevamento in vari siti produttivi.

La presente indagine rappresenta un approccio preliminare per monitorare lo stato di salute e la risposta ad eventuali stress ambientali di *Ostrea edulis*, tramite metodica immunoistochimica (IHC).

Per l'attuazione della ricerca, campioni di ostrica piatta sono stati fissati in Davidson e Bouin e processati per l'istologia. Lo studio si è focalizzato sull'individuazione di markers cellulari che indicano: risposta a stress termico come Heat shock protein 70; stress ossidativo come Nitrotirosina, 4-idrossinonenale e Malondialdeide; risposta a xenobiotici come citocromo P450 1 A (Cyp1A); infiammazione come inducible Nitric Oxide Synthase (iNOS) e ciclossigenasi 2 (CoX-2). Inoltre, sono stati anche testati indicatori di apoptosi come Caspase-3 e di attività antimicrobica come Lisozima.

Alcuni markers (Caspase-3, iNOS, Cyp1A, Cox-2) hanno rivelato immunopositività specifiche in alcuni tessuti del mollusco (branchie, ghiandola digestiva e sistema digerente). Questi risultati, ancora preliminari, e che richiederanno una validazione con tecnica di western blotting, potranno servire come base conoscitiva per il monitoraggio dello stato di integrità delle popolazioni selvatiche/allevate e per il miglioramento delle tecniche di allevamento dell'ostrica piatta, al fine di garantirne il benessere e aumentare la produttività.

Neoplasia epatica primaria con diffusione metastatica in *Mustelus mustelus* partenogenetico (Carcharhiniformes: Triakidae)

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Le neoplasie sono raramente riportate negli elasmobranchi, con una prevalenza documentata tra lo 0,4% e lo 0,5%. Questo dato potrebbe indicare una minore suscettibilità oncogenica nei condroitti oppure derivare da una scarsa diagnosi delle neoplasie, in relazione alla frequenza con cui vengono segnalate in queste specie. Tra le neoplasie, quelle epatobiliari risultano particolarmente rare, con soli tre casi descritti negli squali. In questo contributo descriviamo un caso di una neoplasia epatica maligna con diffusione metastatica in una femmina di *Mustelus mustelus*, precedentemente identificata come individuo partenogenetico facoltativo (Esposito *et al*., 2021), presso l'Acquario di Cala Gonone (Sardegna, Italia).

All'esame macroscopico, il fegato era caratterizzato dalla presenza di neoformazioni biancastre multifocali, delimitate, delle dimensioni variabili da 0.5 cm a 10 cm e di consistenza aumentata. Lesioni analoghe erano presenti a livello della milza e del miocardio. All'esame istologico, il parenchima epatico era multifocalmente sostituito da una neoplasia scarsamente delimitata, non capsulata e a crescita infiltrativa, densamente cellulare e costituita dalla proliferazione di cellule di forma prevalentemente poligonale disposte in nidi e cordoni, con nucleo eccentrico, cromatina dispersa e scarso citoplasma. Erano evidenti atipie cellulari marcate, anisocariosi e anisocitosi. Il parenchima epatico adiacente mostrava necrosi e un aumento degli aggregati melanomacrofagici. Le lesioni spleniche e cardiache presentavano morfologia sovrapponibile, con evidenza di infiltrazione neoplastica del miocardio.

Il quadro istopatologico era compatibile con una neoplasia epatica scarsamente differenziata con metastasi spleniche e cardiache. Secondo le attuali conoscenze, questo rappresenta il primo caso riportato di tale neoplasia in *M. mustelus* e il primo in assoluto documentato in uno squalo partenogenetico. Sono attualmente in corso ulteriori indagini immunoistochimiche finalizzate alla caratterizzazione immunofenotipica della neoplasia. Il caso evidenzia la necessità di potenziare lo *screening* neoplastico nei condroitti ed in particolare nelle specie minacciate e allevate in cattività, per favorire diagnosi precoci e interventi mirati.

InsectFish: histopathological evaluation of hepatopancreas and proximal intestine of Gilthead seabream (*Sparus aurata*) fed *Hermetia illucens* meal

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Aquaculture production has increased significantly, with fish meal being a key ingredient in aquafeeds, but its production negatively impacts the environment. Insects could represent a sustainable and nutritious alternative. In this study, we analyzed the liver and gut histomorphological features of Gilthead seabream (*Sparus aurata*) fed an experimental diet with a partial replacement (10%) of fish meal with *Hermetia illucens* meal (IF) and compared it to a control group fed a diet containing only fish meal (CTRL). A total of 132 *S. aurata* specimens were fed the two diets for 60 days, with a 24-hour fasting period before slaughter. Hepatopancreas and proximal intestine samples from 24 fish (12 for each dietary treatment) were collected for hematoxylin-eosin histopathological evaluation and subjected to semiquantitative scoring. Morphometric analyses on whole slide images of the gut were performed to assess mean villous height, width, and muscular layer thickness, while goblet cell density was automatically quantified from Alcian blue-stained sections using *QuPath* software. Differences between groups were assessed by non-parametric tests (for scores) and t-tests (for morphometry and cell density). In both groups, the gastrointestinal system and liver were well preserved with no obvious gross morphological changes. In the IF group, the villi were significantly higher ($p = 0.01$) and narrower ($p = 0.006$) compared to the CTRL, with better preserved mucosal fold organization. No significant differences were observed between the two treatments in hepatocyte morphology, intraepithelial and lamina propria inflammatory infiltrates of the proximal intestine, or in the goblet cell density. Results indicate that the insect meal did not negatively affect the gastrointestinal system or the liver of Gilthead seabreams. On the contrary, these findings suggest that insect meal is not only safe but may also promote gut health by increasing absorptive surface area.

Immunohistochemical investigations of Amyloid beta in specimens of bottlenose dolphin (*Tursiops truncatus*) and striped dolphin (*Stenella coeruleoalba*)

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In the study of neurodegenerative diseases (NDDs) like Alzheimer's disease, there's a lack of an animal model that represents the full spectrum of human neuropathological features. Cetaceans are potential candidates as they spontaneously develop amyloid-β plaques and neurofibrillary tangles in the brain. Moreover, little is known about correlation between these lesions, age and comorbidities in these species. Several amyloid-β-42 antibodies have been used in previous studies in cetaceans, yet their reliability and comparability remains unclear.

The study therefore compared the sensitivity of two amyloid-β-42 antibodies — clone mOC64 (1:700, AB_2818982, Abcam, Cambridge, UK) and clone H31L21 (1:1000, AB_2532306, Invitrogen) — to detect amyloid-β deposits. Parietal cortex sections obtained from two bottlenose dolphins (*Tursiops truncatus*), three striped dolphins (*Stenella coeruleoalba*) and four dogs (*Canis lupus familiaris*) were used. The different expression of amyloid-β was then evaluated in relation to age and comorbidities in a larger group of individuals (30 bottlenose dolphins and 13 striped dolphins) with clone mOC64. For both analyses, immunohistochemistry was performed with Ventana Benchmark semi-automatic stainer. In each run, a positive and blank control were used. Histoscores were compared to systematically analyze immunoreactivity.

Results from the first analysis showed that mOC64 antibody interacts better with perineuronal deposits, which weren't highlighted by H31L21 in adjacent brain sections in the same individual. This led to the conclusion that mOC64 is more efficient in detecting amyloid-β plaques, in which the main isoform is amyloid-β-42. In the analysis of the larger cohort, amyloid-β plaques were found only in older dolphins affected by viral (Dolphin morbillivirus, herpesvirus) or bacterial (*Staphylococcus sp.*) comorbidities. These results provide additional evidence to the hypothesis that cetaceans may develop a neuropathology similar to Alzheimer's disease. Future studies should investigate more individuals and brain areas, in order to support their potential role as models for human neurodegenerative diseases.

Effetto della temperatura ambientale sulla suscettibilità all'infezione sperimentale con RGVNN in due famiglie di *Dicentrarchus labrax*

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La Viral Nervous Necrosis (VNN), nota in passato come encefalopatia e retinopatia virale (VER), è una delle patologie più devastanti dell'acquacoltura, che causa un'elevata mortalità e ingenti perdite economiche. L'agente eziologico è un virus a RNA del genere *Betanodavirus*, appartenente alla famiglia Nodaviridae. Recentemente sono stati condotti diversi lavori sulla possibile resistenza innata della spigola selvatica europea al virus, con minori segni clinici e un particolare aumento della sopravvivenza all'infezione.

L'obiettivo di questo studio è stato quello di valutare la potenziale resistenza di due diverse famiglie di spigola europea all'infezione sperimentale del ceppo RGNNV anche in relazione a diverse condizioni di temperature ambientali.

In particolare, la patogenicità del VNNv è stata valutata sperimentalmente in 400 esemplari di spigola europea (*Dicentrarchus labrax*) egualmente divisi tra due diverse famiglie (F1 e F2) provenienti da un avannotteria Italiana, infettati con il ceppo di *Betanodavirus* RGNNV e mantenuti a due diverse temperature dell'acqua (25°C e 28°C). Al fine di valutare l'avvenuta infezione sperimentale il virus è stato isolato dai tessuti degli animali infetti, poi inoculato su cellule SSN-1 monitorate regolarmente per lo sviluppo di effetti citopatici (CPE). Le attività sperimentali si sono svolte presso Centro di Ittiopatologia Sperimentale dell'Università degli Studi di Messina con autorizzazione Ministeriale n. 516/2023-PR.

I risultati hanno indicato una associazione tra la temperatura dell'acqua e il decorso della malattia, con un'insorgenza più rapida della sintomatologia negli esemplari mantenuti a temperature più elevate. Le differenze nel decorso dell'infezione sono state correlate anche alle due diverse famiglie di *D. labrax*, che potrebbero essere correlate a una diversa suscettibilità genotipica, incoraggiando così futuri studi genomici per selezionare genotipi resistenti a VNNv da utilizzare nel settore dell'acquacoltura.

Rimangono necessarie ulteriori ricerche per migliorare l'efficacia delle strategie di prevenzione, per gestire efficacemente la prevedibile emergenza sanitaria, anche in relazione al cambiamento climatico e per selezionare genotipi resistenti.

Espressione di marcatori immunoistochimici nei granulomi di ombrina (*Argyrosomus regius*) affetta da SG

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L'immunoistochimica costituisce una metodologia essenziale per l'analisi delle patologie e per la diagnostica in campo veterinario. Tuttavia, il suo impiego nelle specie acquatiche è frequentemente limitato dalla scarsa disponibilità di anticorpi opportunamente validati. Sulla base di precedenti evidenze sperimentali, che hanno valutato la cross-reattività degli anticorpi anti-panCytokeratin (clone AE1/AE3) e anti-vimentina (clone V9) mediante analisi Western Blot (WB) e immunoistochimica (IHC) su tessuti di pesci sani, il presente studio ha avuto l'obiettivo di analizzare l'applicabilità degli stessi anticorpi su tessuti di ombrina boccadoro (*Argyrosomus regius*) affetti da Granulomatosi Sistemica (SG). L'analisi WB ha confermato la reattività dell'anticorpo anti-panCytokeratin sui tessuti cutanei di *A. regius,* evidenziando una banda a circa 50 kDa. Tale evidenza è stata ulteriormente confermata dall'indagine immunoistochimica, la quale ha permesso di localizzare un intenso segnale citoplasmatico nello strato di cellule epitelioidi costituenti il granuloma. Al contrario, l'anticorpo anti-vimentina (clone V9) non ha evidenziato alcun segnale nelle cellule fusate che delimitano il granuloma. L'espressione della citocheratina nelle cellule epitelioidi del granuloma suggerisce un fenotipo epiteliale e rafforza l'ipotesi di un processo di transizione epitelio-mesenchimale, fenomeno già documentato nei granulomi indotti da micobatteri in *Danio rerio*. Tale transizione, mediata da segnali infiammatori quali TGF-β e varie citochine, potrebbe costituire un meccanismo adattativo nei teleostei, denotando una considerevole plasticità fenotipica del sistema immunitario dei pesci. Questa caratteristica, se confermata, assumerebbe una valenza evolutiva nell'ottimizzazione delle barriere difensive contro l'insorgenza di patogeni in contesti di infiammazione cronica. Infine, l'assenza di reattività all'anticorpo anti-vimentina potrebbe essere interpretata come un limite insito nella specificità del clone V9 o di una diversa regolazione dell'espressione delle proteine dei filamenti intermedi nei pesci rispetto ai mammiferi. Ulteriori studi saranno necessari per una migliore caratterizzazione della tipologia cellulare presente nei granulomi e per la validazione di nuovi marcatori utili nell'ambito dell'immunodiagnostica comparata.

Suscettibilità dei biofilm di *Mycobacterium* spp. ai disinfettanti comunemente utilizzati in acquacoltura

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La micobatteriosi è una malattia batterica comunemente riscontrata in acquacoltura, causata da diverse specie del genere *Mycobacterium*, alcune con potenziale zoonotico. Attualmente, non esistono vaccini né trattamenti efficaci per la micobatteriosi nei pesci, e le principali misure di controllo prevedono l'eliminazione degli stock infetti e la disinfezione degli impianti. Sebbene alcuni studi abbiano investigato la suscettibilità di *Mycobacterium* spp. ai disinfettanti, la maggior parte si è concentrata sulle forme planctoniche, trascurando la formazione di biofilm, che risultano generalmente più resistenti e difficili da eradicare.
Questo studio propone di valutare la capacità di formazione di biofilm di diverse specie di *Mycobacterium*, precedentemente isolate da focolai di micobatteriosi in pesci, e di valutarne la resistenza a disinfettanti comunemente impiegati nei sistemi acquatici. A tal fine, la formazione di biofilm è stata valutata tramite il saggio MBEC ®, testando le specie *M. chelonae*, *M. salmoniphilum*, *M. arcueilense* e *M. marinum* a 25°C in agitazione. I biofilm maturi di ciascun ceppo sono stati esposti rispettivamente a povidone-iodio e ipoclorito di sodio (ciascuno per 30 minuti), perossido di idrogeno e Virkon® Aquatic (ciascuno per 15 minuti), e alcol etilico al 70% (15 e 30 minuti). I risultati hanno evidenziato una variabilità nella suscettibilità ai disinfettanti tra le diverse specie micobatteriche. *M. marinum* si è mostrata sensibile a tutti i disinfettanti testati, mentre *M. arcueilense* è risultata suscettibile alla candeggina, al povidone-iodio e all'alcol al 70% (dopo 30 minuti di trattamento). Tuttavia, nessuno dei disinfettanti testati, alle concentrazioni comunemente raccomandate in acquacoltura, è stato efficace contro i biofilm di *M. chelonae* e *M. salmoniphilum*.
Questi risultati preliminari indicano che la formazione di biofilm varia significativamente tra le specie di *Mycobacterium* e sottolineano l'urgenza di sviluppare disinfettanti più efficaci per il contenimento della micobatteriosi negli impianti di acquacoltura.

Immunohistochemical and RNASeq analyses on brains of European sea bass (*Dicentrarchus labrax*) exposed to underwater shipping sounds

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Despite being considered a pollutant the effects of noise on the marine animal brain, the organ that ultimately processes all sensory information and behavioral reactions, is understudied. As part of the DIagnostic framework to Assess and Predict tHe impact Of underwater NoIse on mArine species (DIAPHONIA) JPI oceans project, we studied the brains of European sea bass (*Dicentrarchus labrax*) that underwent single exposures to synthesized underwater boat engine sounds from the SATURN project. Controlled exposures in tanks lasted one hour at five increasing levels of intensity ranging between 110 and 150 dB RMS re 1 µPa.

The octavolateralis area of the brain from control (ambient noise controls approximately 90 dB re 1 µPa, 0.1 % of maximum SPL), medium (level 3, 10 % of maximum SPL), and high-intensity (level 5, 100 % of maximum SPL) exposed fish were evaluated using immunohistochemistry against apoptotic (pro-apoptotic protease factor 1; diacylglycerolkinase-ζ), neuroinflammation (amyloid-β oligomer 42), and oxidative stress (inducible nitric oxide synthase) markers.

Additionally, 8-9 biological replicates per exposure level were selected for transcriptomic analyses (total: 42 brains) and sequenced by BMKGene using an Illumina platform. An open-source nf-core RNASeq pipeline was used for raw sequencing data processing and gene counting, and differentially expressed genes between exposed and control groups were assessed using iDEP2.01 software against the dlabrax2021 reference genome.

We observed individual, but no clear group differences using to the above-mentioned apoptotic and neuroinflammatory markers and corresponding genes. Principal component analyses showed no significant clustering. Preliminary gene ontology analyses included a range of biological processes; however, none were consistently up-/down-regulated with increasing sound exposure. This analysis suggests that this sound exposure did not have an evident effect on the assessed brain areas. Further DIAPHONIA analyses will integrate the findings from the brain to other organs and behavior in the same fish.

Assessing the Reliability of Herpesvirus Detection in Formalin-Fixed, Paraffin-Embedded Cetacean Tissues Using Immunohistochemistry and PCR

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Cetaceans are susceptible to A*lpha-* and *Gammaherpesviruses*, pathogens that may increase mortality and the risk of stranding. Despite growing reports of herpesvirus infections, reliable forensic detection methods for these viruses in cetaceans remain unvalidated.

This study aimed to assess a herpesvirus (HV) detection protocol inspired by Sierra et al. (2022), who demonstrated the utility of immunohistochemistry (IHC) and nested PCR in detecting HV in FFPE tissues of marine mammals. Their method pairs IHC screening using anti-HSV1 antibodies with PCR confirmation.

We applied this protocol to 36 FFPE tissue samples from nine bottlenose dolphins (*Tursiops truncatus*), two striped dolphins (*Stenella coeruleoalba*), and one fin whale (*Balaenoptera physalus*), previously classified as HV-positive based on PCR results from frozen tissue or FTA cards.

IHC was performed with a rabbit polyclonal HSV1 antibody, using an EnVision FLEX Mini Kit, and appropriate positive and negative controls were included.

Furthermore, DNA was extracted from 25 of the IHC-tested tissue samples, chosen between those previously reported to contain herpesviruses by PCR or IHC, using two different commercial kits (Qiagen AllPrep® and Macherey-Nagel NucleoSpin®) and analysed via pan-herpesvirus nested PCR (VanDevanter et al., 1996). Real-Time PCR targeting the GAPDH gene as an internal control was employed to evaluate DNA extraction.

IHC revealed immunoreactivity in five animals in lymphoid tissue, CNS, liver, spleen, kidney, heart, and other tissues, consistent with previous reports. However, nested PCR failed to detect herpesvirus DNA in all the selected samples, despite successful DNA extraction confirmed by Real-Time PCR.

These findings suggest that DNA extraction from FFPE tissues is not adequate to validate HSV1 antibodies in cetaceans, possibly owing to nucleic acid degradation, biomolecular assay sensitivity and focality. Further research employing alternative approaches, such as in situ hybridization and cetacean virus-specific primers, is warranted to enhance herpesvirus detection in cetacean FFPE tissues.