Book of Abstracts

XXI Meeting of the Portuguese Society of Animal Pathology

Veterinary Pathology : Beyond Routine Diagnosis

20 and 21 May 2016

Sociedade Portuguesa Patologia Animal

MM Lisboa Instituto de Medicina Molecular

Faculdade de Medicina Lisboa

Abstracts in Conference Proceedings

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Editorial

A Sociedade Portuguesa de Patologia Animal vai realizar este ano o seu XXI encontro científico, a 20 e 21 de Maio, que será acolhido pelo Instituto de Medicina Molecular na Faculdade de Medicina da Universidade de Lisboa.

O tema deste encontro está centrado nas funções de um patologista para além do diagnóstico de rotina. Convidámos oradores portugueses de renome internacional para nos falar da sua área de atuação em áreas tão diversas como patologia experimental, patologia toxicológica, microscopia eletrónica, imunocitologia, etc.

Organizamos um workshop de microscopia eletrónica de transmissão para patologistas, técnicos e investigadores que queiram ter uma primeira abordagem ou para aprofundar conhecimentos numa técnica que reganhou preponderância científica nos últimos anos.

Neste encontro haverá a oportunidade de partilhar e divulgar experiências em patologia animal, discutir casos, assim como conhecer os mais recentes avanços científicos dados nesta área em Portugal.

Será também o momento para entregar o Prémio SPPA para a melhor dissertação de mestrado defendida em 2015.

Aproveite este Encontro para rever colegas e amigos e visitar a cidade de Lisboa na Primavera.

Poderá encontrar toda a informação na página da SPPA www.sppatologianimal.com

Editorial

The Portuguese Society of Animal Patology will celebrate its XXI Meeting in the beautiful city of Lisbon at 20 and 21st of May, at the Instituto de Medicina Molecular, Faculty of Medicine, University of Lisbon.

The theme of this meeting is focused on the duties of a pathologist beyond routine diagnosis. We invited internationally renowned Portuguese speakers to give talks on their areas of expertise, such as experimental pathology, toxicological pathology, electron microscopy, immunocytology, etc.

We organized a workshop of transmission electron microscopy for pathologists, technicians and researchers who want to have a first approach or to deepen their knowledge in a technique that regained scientific predominance in recent years.

At this meeting there will be the opportunity to share your experience and knowledge in animal pathology, discuss cases and learn about the latest scientific advances in this area in Portugal.

These will be also the opportunity to deliver the SPPA Award for best master's dissertation in 2015.

Take advantage of this meeting to review colleagues and friends and visit the city of Lisbon during spring time.

All information is available inwww.sppatologianimal.com

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Program

XXI Encontro da Sociedade Portuguesa de Patologia Animal. Patologia Veterinária: Para além do diagnóstico de rotina

XXI Meeting of the Portuguese Society of Animal Pathology. Veterinary Pathology: Beyond routine diagnosis

20 e 21 de Maio de 2016. Instituto de Medicina Molecular – FM/UL.

20th and 21st May 2016. IMM FM/UL

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11.00h	Moderator: Fernanda Seixas Poster Section Tour (P1-P5)	
11.30h	Moderator: Ricardo Marcos 2nd plenary session- **Afonso Costa** – Toxicologic Pathology – The role of the pathologist in the pharmaceutical industry	Anfit. 57 Piso 01
12.30h	Lunch time	
13.30h	Moderador: Conceição Peleteiro Poster Section Tour (P6-P10)	Anfit. 57 Piso 01
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15.00h	Moderador: Maria dos Anjos Pires 4th plenary session-**Nazaré Cunha**- Immunocytochemistry in diagnostic cytology	Anfit. 57 Piso 01
16.00h	Coffee Break Moderador: Irina Amorim Poster Section Tour (P11-P15)	Anfit. 57 Piso 01
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6-Stromal characterization of feline endometrial adenocarcinomas (FEA) - **Maria dos Anjos Pires** 7-Morphological an immunohistochemical characterization of the cat ovary -**Maria dos Anjos Pires** 8-Comparative study of immunohistochemical expression of TGFβ1 in feline and canine mammary lesions – **Fernanda Seixas** 9-Keratin 8 and 18 expressionin normal uterus, cystic endometrial hyperplasia (CEH) and feline endometrial adenocarcinoma (FEA) - **Maria dos Anjos Pires** 10-In vitro and in vivo model for the study of P-cadherin in feline mammary carcinogenesis - **Ana Catarina Figueira**
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11- Chrysosporium anamorph of Nannizziopsis vriesii (CANV) lesions in bearded dragon (Pogona vitticeps) - **Fernanda Seixas** 12-The histopathological timeframe of Hyalomma lusitanicum infestation development on bovines -**Sara Zúquete** 13-Identification of subcutaneous mite Hypodectes propus (Acarina: Hypoderatidae) in the Northern gannet (Morus bassanus) in Portugal- **Fernanda Seixas** 14-Dirofilaria immitis in South African fur seal (Arctocephalus pusillus pusillus): necropsy and histopathology findings observed in zoological context in Portugal- **Inês Marcelino** 15-Occurrence of blood-sucking mites in ornamental chickens- **Ana Maria Araújo**

Plenary Session

Murine models of human and animal diseases

Paula A. Oliveira

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In this communication, I will address original research performed with laboratory rodents (rats and mice) to study cancer, namely urinary bladder, liver, mammary and skin cancer. These models have been used for either understanding the molecular events underlying cancer development or improving therapy. Although the widespread use of in vitro systems that permit repeating almost all the processes involved in the development of a disease, only the use of murine models enable us to reproduce onset the mechanisms of human and animal diseases. Thereby providing the only system known today to evaluate new drugs or lifestyles for therapeutic intervention. A better understanding of cancer murine models and their uses as well as their limitations may aid future researchers regarding the development and implementation of new targeted therapies and chemotherapeutic agents for human and animal cancer.

Toxicologic Pathology – The role of the pathologist in the pharmaceutical industry

Afonso Costa laboratório

Non clinical Drug Safety da Boehringer Ingelheim Pharma GmbH & Co. KG

The discovery and development of new drug compounds is a very long, complex, highly regulated, expensive and risky process. It is estimated that only 1 out of 1000 drug candidates reaches market authorization.[1] The costs of developing a single drug can rise to an estimated US $ 1.2 billion.[1] Frequent reasons for discontinuation of drug candidates include toxicity, tolerance, and lack of efficacy or bioavailability in humans.[2] Earlyprediction of safety liabilities can lead to a better selection of drug candidatesand prevent failures in latter stages of pre-clinical or clinical development.[2]Early prediction of liabilities such as hepatotoxicity before trials could save up to US $ 2 billion in development costs per drug, making the development of predictive technologies and new toxicity testing strategies a major priority of pharmaceutical companies.The toxicologic pathologist plays a pivotal role in drug safety and risk management assessments. At all stages from discovery and development, the toxicological pathologist with the combination of clinical and scientific training can contributeto a better understanding of the pathophysiology and mechanisms of adverse toxic eventsand in particular to the assessment of their relevance to humans.[2]

References:

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Immunocytochemistry in diagnostic cytology

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Immunocytochemistry (ICC) is a technique for the detection of cellular antigens in cytologic specimens, using specific antibodiesbound to a chromogen, which are then visualized using a light microscope. The use of ICC is a powerful adjunct to cytological diagnosis, contributing to an increase in diagnostic accurary.[1]

While in human medicine is already part of the routine work in most cytology laboratories, in veterinary medicine ICC has only recently gained a recognized value. In 2016 was published the first consensus statement regarding the use, publication and future directions of immunocytochemistry in veterinary medicine.[2]

The major application of ICC in cytology is the classification of neoplasia, but it can also be used to determine prognosis, identification of etiologic agents and as a marker of cell growth and differentiation. Major advantages over other methods are greater antigen preservation, simultaneous assessment of immunostaining and cell morphology,and minimally invasive sampling.[2]

ICC can be done with different types of specimens, more often fine needle aspirates but also with various types of exfoliated cells, such as effusions and respiratory cytology. Samples may be processed in several ways: smears, cytospins, cell block,monolayered liquid-based (LBC) slides, but it is the proper handling and processing of the specimens that is determinant for the ICC results.[2,3] This technique can be performed in non-stained acetone of formalin-fixed smears, but also in already stained slides.[3,4]

There are several antibodies,with different sensitivities and specificities, available for ICC on animal tissues.Ideally, a panel (2 or more) of markers should be selected and results should always be interpreted together with the routine cytologic staining. A brief list of frequently used antibodies on veterinary ICC includes:cytokeratin (CK) AE1/AE3, CK7, CK14, CK18, CK20 and EMA for epitelial neoplasia; CD1, CD11, CD18, CD90, CD204, E-cadherinand MHCII for histiocytic neoplasia; CD3, CD4, CD8, CD20, CD21, CD45, CD79a, PAX-5 for lymphoid neoplasia; Melan-A for melanocytic neoplasia; S-100, vimentin, desmin, factor VIII, CD31 andmyoglobolin for mesenchymal neoplasia.[2]

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Oral communications

1. Characterization of histopathological lesions in a biological model for environmental studies: *Eisenia andrei Bouché*. Preliminar results.

Vala H.[1,2]*, Rodriguez-Seijo A.[3], Lourenço J.[4], Francisco A.[1], Rocha-Santos T.[5], Costa J.[5], Pereira R.[6]

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Introduction: The strong expansion of the world production of plastics caused a severe accumulation of plastic debris in the environment, which makes them one of the most important contaminants, growing as a global environmental problem. Although the production in Europe has been relatively constant in the last 10 years, world plastic production continues to increase, affecting soil biota and their functions.

Objectives: Thus, in order to evaluate the effects of MP in soil-dwelling organisms, earthworms (*Eisenia andrei Bouché*), were exposed to standard artificial soil mixed with MPs and the authors documented, using microscopic figures, the pathological lesions found in this biological model.

Material and Methods: Eight adult earthworms extracted from soils contaminated with different concentrations of MP (mg/kgdw) with sizes ranging between 250-1000 mm, were fixed in 10% neutral-buffered formalin and processed for routine histopathological diagnosis.

Results and discussion: Contrary to what would be expected, MP were not found throughout the GI tube of earthworms but several lesions were found in the individuals extracted from the soils with high MP concentrations, when compared with control group, namely epithelial intestinal atrophy and evidences of inflammatory responses to this stress agent.

Conclusion: Earthworms have probably avoided the consumption of the biggest MPs. However, evidences point for lesions that were likely caused by the smallest MPs that were likely egested during the depuration phase.

Acknowledgments: CITAB; FCT (UID/AGR/04033/2013 e POCI-01-0145-FEDER-006958). CI&DETS, FCT

Routine histological technique applied to a biological model for environmental studies

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Introduction and aim: Earthworms are an important test organisms used in several standard ecotoxicological tests (e.g. ISO, 2008, 2012; OECD, 2004, 1094) and they are also model organisms to test soil structure and permeability, as well as for important soil services as the degradation organic matter.

Although histopathological changes have been demonstrated to be good biomarkers to assess the exposure of these animals to different physical and chemical stress agents, studies with clear and high quality images describing normal tissue conditions are scarce in the literature, so the aim of this work was to better characterize this biological model.

Material and Methods: Eight adult earthworms exposed to an artificial standard soil (OECD, 2004) for 28 days, were extracted and placed in a plastic box to depurate their gut content, fixed in 10% neutral-buffered formalin and processed for routine histopathological diagnosis.

Results and discussion: Satisfactory histological sections were obtained. Some difficulties were faced related with microtome sectioning, resulting in artefacts, namely

lines across sections produced by a nick in the cutting edge of the microtome knife cutting tear, motivated by the presence of sand and other solid particles that persisted in the gut of earthworms. Nevertheless, it was possible to obtain representative figures from different earthworm sections.

Conclusion: Routine histological technique was effective for obtaining satisfactory histological sections and the knowledge of the histology of earthworms could be very useful for future application in environmental studies, using this biological model.

Acknowledgments: CITAB; FCT (UID/AGR/04033/2013 e POCI-01-0145-FEDER-006958). CI&DETS, FCT

Topoisomerase IIα expression in canine diffuse large B-cell lymphoma

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Canine lymphomas are one of the most common neoplasms in dogs. These tumors represent a clonal expansion of lymphoid cells with distinctive morphological and immunophenotypical features, with the diffuse large B-cell lymphoma being the most common subtype.

Topoisomerase IIα is a multifunctional nuclear protein and it plays an essential role in cellular proliferation. It is also an important therapeutic target in haematopoietic tumors.

Although several reports showed that expression levels of topoisomerase IIα could be a predictive factor in human non-Hodgkin lymphoma, its importance in canine lymphoma is still unknown. The aims of this study were to evaluate the expression of topoisomerase IIα in canine B-cell lymphomas and to validate its prognostic value.

The immunohistochemical technique was optimised in order to obtain specific staining. Several tests were performed using two primary antibodies: Ki-S1 (MAB4197, Millipore) and #4733 (Cell Signaling Technologies). The selected antibody was Ki-S1.

Thirteen cases of canine diffuse large B-cell lymphoma were analysed and cell counting was performed in the ones with positive staining. Observed staining was divided according to an intensity scale. Correlation levels between topoisomerase IIα expression, Ki-67 proliferation index and survival analysis were performed. Due to the small number of analysed cases, no statistically significant results were obtained.

This study allowed the assessment of topoisomerase IIα staining and quantification methods in canine lymphomas, using immunohistochemical technique. Although the reduced number of cases has limited this work, further studies must be performed in order to assess the predictive value of topoisomerase IIα expression levels.

Spontaneous mammary tumor in a 7-week-old female rat

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Introduction and aims: Development of spontaneous mammary tumors in rats is extremely rare during the first year of life. The present work describes a spontaneous mammary carcinoma in a 7-week-old female rat.

Case description: Thirty-four Sprague-Dawley female rats with four weeks of age were used in an assay of chemically-induced mammary carcinogenesis. After one week of quarantine, animals were randomly divided into five experimental groups and were allowed to acclimate to the lab conditions for two weeks. All procedures followed the National and European legislation, and were approved by National (Approval no. 008961) and University (CE_12-2013) Ethics Committees.

No alterations were found during the quarantine or acclimatization period. However, at fifty days of age, one animal from control group exhibited a movable mass in the left cervical region. The mass grown quickly and the animal health status deteriorated faster. Eight days later, the animal was humanely sacrificed. A complete necropsy was performed. The mass cut surface was reddish in appearance with several cysts filled with a bloody content. The mass was immersed in buffered formalin and routinely processed for light microscopy. It was stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS) and immunohistochemically evaluated by staining with an antibody against estrogen receptor (ER)-α. The tumor was histologically classified as a

high-grade undifferentiated carcinoma originated in the mammary gland. It was negative for PAS staining and positive for ER-α.

Conclusion: This is the first report describing a spontaneous mammary tumor in a Sprague-Dawley rat with less than 10 weeks old. No previous reports had described a spontaneous mammary tumor in such a young rat.

Funding: This work was supported by European Investment Funds by FEDER/COMPETE/POCI - Operational Competitiveness and Internationalization Program, under Project POCI-01-0145-FEDER-006958 and Portuguese Foundation for Science and Technology (FCT), under the project UID/AGR/04033/2013, the project PTDC/DES/114122/2009 and post-graduation grant SFRH/BD/102099/2014.

Pathological study of two cases of mortality in Iberian-lynx (*Lynx pardinus*) re-introduced in Portugal in the years 2015-16

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In Portugal, the Iberian-lynx was considered virtually extinct and classified globally as an "endangered" species. Currently, the Iberian-lynx is being recovered in Portugal, through LIFE+Iberlince reintroduction program (LIFE+10NAT/ES/570), using animals born in captivity.

This study presents two cases of Iberian-lynxes, who died after reintroduction, identified as Case A, (Kayakweru) female, two years, and Case B, (Myrtilis) female one year.

The Case A, presented moderate weight loss, dehydration, incompletely clotted blood, discreet hydrothorax, congested and edematous lung and congestion of liver, spleen and kidneys. The stomach contained undigested lung fragments, from which was held positive drug test for strychnine.

The Case B had very pronounced weight loss, near cachexia, with no signs of food in the stomach. Histopathology interstitial pneumonia, necrotic enteritis, and lymphoid hypoplasia in the spleen and mesenteric lymph nodes were seen together with degeneration and necrosis of liver and kidneys. Virology was positive for feline panleucopenia virus.

We conclude that, in Case A, death was due to poisoning by strychnine and in Case B to infection by feline panleucopenia virus.

Among the animals introduced in Portugal/Spain these were the first known case of strychnine poisoning and the first recorded death by feline panleucopenia.

The anatomic-pathologic diagnosis proves to be a valuable tool in determining the cause of death of the Iberian lynx, as well as in the process of reassessment of control measures in order to reduce appropriately mortality from other Lynxes, thus increasing the success of recovery project of the species.

Liver and spleen infiltration with round cells in a cat

Peleteiro M.C.

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The present case refers to a consented necropsy performed in a cat with no known clinical history.

Identification – Feline, sterilized female, estimated age - 9 years, undetermined breed.

The necropsy revealed:

Discrete emaciation; severely hypertrophic liver with round borders and a myriad of milimetric whitish foci dispersed throughout the whole organ; the same type of foci could be seen in the spleen, together with a larger nodule 2.5 cm in diameter; hypertrophy of the mesenteric lymph nodes; severe atrophy of the left kidney; congestion and edema of the lungs.

Histopathology revealed:

Marked infiltration of the liver and spleen with round cells, with acidophilic finely granular cytoplasm, and small eccentric nucleus. Very few hepatic cells remain in the liver. In the lumen of blood vessels in both organs, the same round cells can be seen.

The mesenteric lymph node showed edema and lymphoid cell hypoplasia with no evidence of follicles. No microbial agents were identified.

What is your diagnosis?

Fine-needle aspirate cytology of a mass posterior to the eye

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A 15-year-old neutered female dog of undefined breed presented with clinical signs on the left eye: exoftalmy, third eyelid protusion, ocular pain, dilated episcleral vessels and purulent conjunctival secretion. The patient also felt pain on opening the mouth. During the ecography, a mass posterior to the left eye was identified. A complete blood cell count (CBC) in the ADVIA 210 hematology analiser and a serum chemistry profile were performed, as well as fine-needle aspirate cytology of the mass behind the ocular globe.

The CBC revealed mild normocytic normochromic anemia, apparently not regenerative after blood smear evaluation. Biochemistry profile indicated mild urea elevation (81.2 mg/dL; Reference Interval [RI] 15.0-68.5 mg/dL), as well as ALT (184 UI/L; RI 10-90 UI/L) and ALP (694 UI/L; RI <120 UI/L).

The cytology showed sparse to moderate cellularity with a proteinaceous background. There was a mixed population of cells distributed in groups, each one of the same cell type, occasionally forming sequential layers containing: cells with high relation nucleus: cytoplasm and cleaved nucleus with dense chromatin; cells with abundant cytoplasm containing numerous needle-shaped melanin granules and round central or peripheral nucleus; cells with abundant eosinophilic cytoplasm and round nucleus; and large cells with slightly basophilic cytoplasm with indefined boundaries, with central nucleus of reticular chromatin and evident nucleolus. All of these cell populations were relatively monomorphic without obvious signs of cytologic atypia. There were also frequently lysed cells.

The animal was treated with anti-inflammatory and antibiotic and the mass receded.

To date the dog presents no clinical signs of ocular pathology.

Multiple myeloma: atypical presentation with cutaneous metastases

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A 9-year-old Golden Retriever was referred with polydypsia-polyuria, weight loss and lameness. On physical examination, inguinal papules and regional lymphadenopathies were found.

The first diagnostic workup included fine-needle aspiration (FNA) of papules, complete blood count and biochemical analysis. The dog had bicytopenia (moderate normocytic normochromic anaemia and severe thrombocytopenia) and FNA presented round cells (some binucleated) with 10-20 µm diameter and, occasionally, with cytoplasmatic eosinophilic border. Granular eosinophilic material and lymphoglandular bodies were observed in the background. Round atypical cells were also detected in buffy-coat smears. The cytological features were consistent with disseminated round cell neoplasia, suggestive of multiple myeloma.

Biopsy of papules, cytology of bone marrow and popliteal lymph node were carried out. Additionally, cell blocks (cytotube technique) of buffy-coat and bone marrow were performed. Bone marrow was characterized by a large proportion of plasmacytoid cells, including flame-cells. These atypical cells were observed in lymph node. Histopathological examination of papules revealed multifocal infiltrates of round cells with high pleomorphism and nuclear atypia, negative to CD3, PAX-5, CD18, and with intense staining for MUM-1. Immunolabeling for MUM-1 was also present in buffy-coat and bone marrow cell blocks. The imaging study revealed osteolytic lesions in axial skeleton and diffuse changes in liver and spleen. A final diagnosis of multiple myeloma was made.

A case of T-cell-rich B-cell lymphoma in a dog

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A sample was received in the Laboratory of Histology and Anatomical Pathology from an incisional biopsy of a gingival mass located in the rostral mandible. It was sampled from a female mongrel dog that presented with hyporexia and oral pain.

The sample had 1cm of diameter consisted of white and brown tissues.

The histological appearance had no identifiable tissue from the anatomic area and presented with proliferation of round cells arranged on solid nests that invaded blood vessels (which have vasculitis and thrombosis) as well as the nerves and the adjacent tissues that were crushed in the surgical margin. The tissues also showed necrosis and a low mitotic index.

The cells had the morphology of small lymphocytes and immunoblasts. It was noted as well the presence of many linfoglandular bodies.

The samples were immunostained for lymphocytes (monoclonal anti-CD3 and polyclonal BLA.36, Dako) and macrophages (monoclonal MCA 387, Serotec). The immunostaining was markedly positive for T lymphocytes (CD3), small cells and regular in their shape and size, present in the periphery of the lesion. There was also positivity to B lymphocytes as the larger and aberrant cells with atypical nuclei. Macrophages were present in small agglomerates.

The phenotypic view was rated as T-cell-rich B-cell lymphoma. The animal is undergoing chemotherapy.

CASE REPORT: MEDULOBLASTOMA NUM CÃO COM TRÊS ANOS

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A three year-old male Labrador dog was brought to the Veterinary Teaching Hospital with complaints of dysphagia, vomiting, cough and lameness of the right hind limb. At physical examination Horner's Syndrome was diagnosed and hypertrophy of the right popliteal lymph node was noted. Cytology revealed numerous round cells with scant cytoplasm and dense nucleus, suggestive of lymphoma. Biopsy of the same lymph node identified invasion of most of its structure by compact cells with round to spindled nucleus; immunohistochemistry was negative for CD3 and CD20, excluding the lymphoma hypothesis. One week later the animal suffered a convulsive episode and the cranial CT scan revealed a multifocal lesion, involving the optic chiasm and the cerebellum´s left hemisphere. The deterioration of the clinical condition led the owners to opt for euthanasia. At necropsy lymphadenomegaly of the left retropharyngeal and iliacs was detected. A 2x1cm lardaceous mass was involving the optic chiasm and a hemorrhagic friable mass occupied almost all the cerebellum´s left hemisphere. Histologically, the encephalon's masses and the lymph nodes presented the same tumoral tissue as the right popliteal, corresponding to a proliferation of spindle to round shaped cells with hyperchromatic nucleus, arranged in solid lobules separated by a scant stroma, characteristic of an embryonal tumor. The immunohistochemical study was positive for synaptophysin and negative for GFAP, with 100% of positive cells to Ki67. These results allowed us to diagnose this neoplasia as a medulloblastoma with neuroblastic and not glial (astrocytic) differentiation, with meningeal and lymphatic dissemination

Establishment of antibody panels and histochemical techniques in routine tumour diagnosis in Veterinary Pathology

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In Portugal, Veterinary Pathology is developing rapidly, and in recent years we assist to the emergence of private laboratories and the restructuring of universities, polytechnics and public laboratories.The Portuguese Society of Animal Pathology, through its actions and its associates has been keeping the discussion among its peers in order to standardizethe criteriaof description, classification and evaluation of cases which are the subject of our daily work.

One of the last challenges is associated with the use of routine histochemical techniques and immunohistochemistry, in an effort to establish standardized panels for

tumour diagnosis, which could eventually reduce each analysis cost. For this purpose a simple survey was built, in which all collaborators answered questions about the markers used for carcinoma, sarcoma and round cell tumour diagnosis, as well as general questions related with the subject. We obtained twenty-one answered to the questions, from public and private laboratories. In general, in most cases immunohistochemical and histochemical methods are used for diagnosis. The wide spectrum cytokeratins are universally used to confirm carcinoma, and vimentin for sarcoma. The CD3 marker is used by all laboratories to identify T lymphocytes. For the diagnosis of B-cell lymphoma, the marker used is not consensual. In each laboratory there are different markers for more specific situations and only two labs perform PCR techniques for diagnosis. These data will be presented to promote extended discussion, namely to reach a consensus when different markers are used.

Poster presentations

1. Cyclooxygenase-2 expression during urinary bladder carcinogenesis in ICR mice

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Cyclooxygenase-2 (COX-2) is considered a promising molecular marker not only for urinary bladder cancer detection, but also for prediction of disease progression and prognosis. The present study investigated the expression of COX-2 in mouse urothelial preneoplastic and neoplastic lesions induced by the oral administration of N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN).

The study comprised two groups of ICR animals: group 1 (control) and group 2 (which received BBN over the course of 12 weeks). Urothelial lesions were analyzed by haematoxylin and eosin staining. COX-2 immunostaining was assessed according to its distribution and intensity.

In negative control mice COX-2 expression was restricted to umbrella cells in the urothelium and scattered leukocytes in the lamina propria, assuming a diffuse cytoplasmic pattern. Dysplastic lesions showed multifocal to coalescing COX-2 positivity, with positive cells in all epithelial layers. The single invasive carcinoma showed multifocal positivity in 10-25% of cells.

Altered COX-2 expression may be indicative of tumour progression in BBN induced urinary bladder cancer.

2. Ptaquiloside from bracken (Pteridium spp.) inhibits tumour-infiltrating CD8+ T cells in HPV-transgenic mice.

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Bracken (Pteridium sp.) is a globally-distributed weed, widely present in pastures. Exposure to bracken toxins like ptaquiloside is hypothesized to increase the risk of papillomavirus-related upper digestive cancers. Ptaquiloside is thought to be immunotoxic, facilitating the development of viral lesions. We used a human papillomavirus type 16-transgenic (K14-HPV16) mouse model to study the effects of ptaquiloside on tumour-infiltrating CD8+ T lymphocytes, which are critical players against virus and tumour cells.

HPV16+/- mice were orally administered ptaquiloside (0.5mg/mouse/week) during 10 weeks and euthanized at 30 weeks-old, together with matched untreated controls.

Skin samples were enzymatically digested and CD8+ T cells analysed for CD107a and CD44 surface expression.

Ptaquiloside-exposed HPV16+/- animals showed a significantly ($P < 0.05$) reduced percentage of CD8+CD107a+ and CD8+CD44+ T cells compared with untreated HPV16+/- animals and a trend towards reduced CD8+ T cell infiltration. Histologically, 100% of ptaquiloside-treated mice showed diffuse epidermal dysplasia, compared with 50% untreated mice.

These findings indicate that ptaquiloside exerts an immunosuppressive role by decreasing CD8+ T cell memory activation and degranulation in HPV-induced lesions. Given the key role of CD8+ T lymphocytes against papillomavirus-induced lesions, this effect is likely to contribute for viral persistence and tumour progression.

3. Extraskeletal sarcomas: Retrospective study of 5 years (2011-2016)

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The extraskeletal sarcomas (ESS) are mesenchymal neoplasms characterized by the production of bone and cartilage, without association to the skeletal system or periosteal tissue. These are rare neoplasms in dogs, and even more rare in felines. They are also uncommon in humans. The biological behavior is usually aggressive, with high rates of local recurrence and metastasis.

The histopathology reports of confirmed extraskeletal sarcomas diagnosed between January 2011 and March 2016 were reviewed. Data were collected from the laboratory computer system (Clinidata XXI of Maxdata®). The main analyzed features were: species, gender, breed, age, tumor location and diagnosis.

Over a period of 5 years, 7 cases of ESS have been diagnosed, 5 in dogs and 2 in feline species.

In dogs, there were 4 female and 1 male with an average age of 11.2 years, with tumors located in abdominal region (n=2) and subcutaneous (n=5). In cats, we found 2 cases of subcutaneous tumors, both were females, with 10 and 11 years, respectively. Clinical data of these cases did not report a history of trauma or inoculation or evidence of bone involvement.

In literature, there are few cases described in both dogs and cats, the affected animals are usually geriatric with no apparent sex or breed predisposition.

In this five years period, we conclude that the ESS are extremely rare neoplasms, particularly in cats. Our sample is in agreement with the literature in terms of epidemiological data, however we found more cases at cutaneous sites rather than visceral locations.

4. OvisOme: an updated database for Ovis aries

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Introduction: The Omics sciences are part of the research and diagnostic routines in human health. However, their application in veterinary sciences is still sparse, albeit the increasing number of proteomics studies published, especially regarding farm animals. The amount of information accumulated by these high throughput techniques, makes the existence of specialized databases fundamental. These databases are essential to store, annotate and make available to the scientific community, all the information gathered by the different omics studies, so that researchers can use it to understand the physio pathological mechanisms underlying sheep diseases, as well as to develop new and improved diagnostic, prognostic and therapeutic strategies.

Objective: The aim of this work is to present the OvisOme database and to demonstrate how it can be used to understand the molecular mechanisms underlying sheep disease.

Methodologies: OvisOme compiles all proteins identified by proteomics studies of Ovis aries. The proteins are annotated as to the sample characterization, the proteomics techniques used and all the data the authors refer regarding the donor sheep's health.

Results: The database currently has 1451 proteins, associated to 8 diseases and 10 breeds. When compared to other proteomics databases, the OvisOme stores and displays more information than other databases not specific for sheep, such as UniProt.

Conclusion: OvisOme is a valuable tool for the study of the molecular mechanisms underlying sheep health and disease.

5. A case of Myxosarcoma in a Chilean Flamingo (*Phoenicopterus chilensis*) of a Zoologycal Collection "Quinta dos Plátanos

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This was a case study reported in a Chilean Flamingo (*P. Chilensis*), from a private zoo collection, "Quinta dos Plátanos", in Abrantes – Portugal, during October of 2015. There isn´t still a great knowledge of cancer disease in avian medicine specially regarding diagnostic and treatment, and for so reporting cases like this, can be of extreme importance. Myxosarcomas are even more uncommon and can occur in different regions of the body, rich in conjunctive tissue.

It was a 3 year old female flamingo, with microchip number 967000009551840, which presented a mass located over the first finger joint of the left wing, with about 6 cm of diameter, exophytic, ulcerated, highly vascular and with a soft consistency. The first approach was to surgically remove the mass, sending it conserved in 10% formalin solution, to the Pathology Laboratory of the FMV-ULisbon, for analysis by the routine histopathology technique. The diagnostic was myxosarcoma. After the first surgery the tumor developed again, so the decision was to amputate the left wing at the level of the humeral-radio ulnar joint.

Malignant tumors are not very common in flamingos (Phoenicopteridae), though retrospective studies indicate that they have similar prevalence when compared with other birds. This specific type of tumor, myxosarcoma, has a low metastasis rate, though it may recidivate after surgery.

This may be the first case of myxosarcoma in a Chilean flamingo's wing (*P. Chilensi*), and for so it is an original and so far a successful case.

6. Stromal characterization of feline endometrial adenocarcinomas (FEA)

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Feline adenocarcinoma of the endometrium (FEA) are more common than literature describes, and can even be present in young animals. The descriptions of these lesions have increased in recent years aiming the improvement knowledge behavior as well the prognosis prediction. The tumour stroma is an important component in cancer behaviour and aggression.

In the current work it was used tree markers: vimentin and desmin which are intermediate filaments of mesenchymal cells and alpha-actin that is a constituent of microfilaments, associated with the smooth muscle.

It was used samples of normal uterus and FEA belonging to the archived of Laboratory of Histology and Anatomical Pathology of UTAD. Normal samples were separated in two stages of oestrus cycle (estrogenic phase and progestogen phase). Immunolabeling was evaluated based on its intensity.

Vimentin positivity was shown on epithelial cells in normal uterus, as well on endometrial stroma. The FEA stroma intensity was greater than in the normal endometrium.

Desmin and α-actin have almost overlapping staining, with weak labelling of the stroma and strong muscle positivity. Blood vessels were strongly and consistently marked by α-actin and desmin shows intensely staining of myometrium than stroma and blood vessels. Some tumours cells are inconsistently positive for these two markers.

The invasion of muscle layers its one important feature in FEA. We note that desmin is the best marker for identification of this invasion that will be usefully in more complex cases of FEA diagnosis.

7. Morphological an immunohistochemical characterization of the cat ovary

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In the ovary, the alternation in dominance between follicles and corpora lutea translates into a cyclical dynamics of sex hormones. The understanding of the morphology and immunohistochemical staining of the intermediate filaments was the aim of this study, which used 53 representative sections of ovaries, grouped according to them being either in the follicular or luteal phase of the estrous cycle. This phase was determined based on the morphological aspect of the ovary and uterus; the estrogenic phase being represented by the presence in the ovary of follicular growth and nonspecific glands in the endometrium and the progestagenic phase having corpora lutea in the ovary and endometrium glands at its maximum expression.

Using immunohistochemical technique we studied the expression of three markers using monoclonal antibodies: vimentin, a marker for mesenchymal cells, and cytokeratins 7 (CK7) and 20 (CK20), markers for epithelial cells. Quantification of the staining was done according to the intensity and percentage of positive cells.

Blood vessels, theca cells, stroma of ovaries and corpora lutea showed higher intensity staining for vimentin, in contrast to the granulosa cells. The CK7 showed a strong positive staining in the surface epithelium, while the other structures manifested less intense staining. The CK20 showed a very weak positive staining in the surface epithelium and higher intensity label in gin other structures. We found that, in general, it was during the estrogenic phase that greater staining intensity in most structures was

observed, with the corpus luteum being the structure which demonstrated more reactivity across all markers used.

8. Comparative study of immunohistochemical expression of TGFβ1 in feline and canine mammary lesions

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Breast cancer is the most common type of cancer in women, both in developed and in developing countries. In cats, breast tumors account for 12% of all tumors and 17% of the tumors in females. In female dogs, spontaneous mammary tumors represents 25 to 50% of all neoplasia in this species. TGFβ1 regulates processes such as division, differentiation, motility, adhesion and cell death in almost all tissues and some studies suggest that it functions both as a tumor suppressor and as tumor promoter. The aim of this work is to contribute to the biopathological characterization of breast tumors in cats and dogs, more precisely regarding the expression of TGFβ1 during the development of these tumors.

Immunohistochemical expression of TGFβ1 was evaluated in the epithelium and stroma of 99 samples of feline mammary tissue and in 116 samples of canine mammary tissue. In epithelial cells of feline mammary tissues, most of the histological groups showed high expression of TGFβ1. On the contrary, in the stroma, the majority of samples from the different histological groups showed low expression of TGFβ1. Regarding canine tissues, the expression of TGFβ1 in epithelial cells was low in most of the samples, regardless of the histological group. In the stroma, statistically significant associations were achieved ($p < 0.0001$). We verified that, probably, it is not the amount of TGFβ1 available in the medium that promotes tumor progression, but most likely is the way it interacts with its receptors or other existing factors in the microenvironment.

9. Keratin 8 and 18 expression in normal uterus, cystic endometrial hyperplasia (CEH) and feline endometrial adenocarcinoma (FEA)

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Keratin-18 (CK-18) and CK-8 are recognized epithelial markers for histopathology diagnostic. This CK forming one of the pairs of intermediate filaments which constitute the cytoskeleton of epithelial cells. CK-18 is associated with the flexible support of various cytoplasmic structures, and has been associated with various cellular processes such as apoptosis, mitosis and signalling, and several metabolic pathways. The changes of expression are associated with metabolic and structural changes, and will be associated with aggressiveness of certain cancers.

The CEH of the cat may be associated with an early stage of uterine cancer, in particular with FEA, which are most common to the literature refers.

The aim of this study was to evaluate by immunohistochemistry the expression of CK-8 and CK-18 in the cat oestrous cycle and in HQE and FEA.

It was used archival material from the Laboratory of Histology and Anatomical Pathology of UTAD, eight uterus in estrogenic phase and 9 in progestagenic phase, 10 with CEH and 26 with FEA.

There was a non-significant decrease in CK-18/8 expression in CEH related to a normal uterus. The expression of these CK in FEA also showed loss of positivity when compared with the endometrium in estrogenic and progestagenic stage.

This study allowed us to conclude that the loss of expression of these CK 8/18 in FEA and CEH of the cat may be associated with cell dedifferentiation and most severe lesions. Nevertheless, follow-up studies are needed to improve these findings.

10. In vitro and in vivo model for the study of P-cadherin in feline mammary carcinogenesis

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Mammary cancer in cats have high recurrence and metastatic potential. Abnormal expression or function in the major molecules of the cadherin-catenin adhesion complex have been related to breast cancer development and associated to cell migration, invasion and metastatic dissemination. In feline mammary tumours, cadherins´ role is still poorly known. Therefore we seek for suitable in vitro and in vivo model systems to study the leading role of P-cadherin in feline mammary carcinogenesis. Major molecules from the cadherin-catenin complex (E- and P-cadherin) were evaluated in a feline metastatic mammary carcinoma cell line (FMCm), by Western blot analysis, immunofluorescence, imunoprecipitation and in situ proximity ligation assay. The FMCm cell line tumourigenic and metastatic capacity were assessed by orthotopically inoculation of a cell suspension in the mammary fat pad of athymic nude mice (N:NIH(S)II-nu/nu). Mice xenografts, as well as metastatic lesions, were evaluated immunohistochemically for cadherins expression. The FMCm cell line expressed E- and P-cadherin. The FMCm cell line revealed to be high tumourigenic and showed a great metastatic capacity as the orthotopic inoculation in nude mice lead to the formation of primary and metastatic lesions in all mice. Those lesions expressed P- and E-cadherin. FMCm cell line can be proposed as a useful model for in vitro and in vivo studies of P-cadherin in feline mammary carcinoma progression.

11. *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) lesions in bearded dragon (*Pogona vitticeps*)

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Bearded dragons (*Pogona vitticeps*) kept in captivity suffer a sometimes fatal dermatological condition known in the pet trade as ‘‘yellow fungus disease” or “flesh eater fungus”. The Yellow fungus disease is caused by the keratinophilic microfungus *Chrysosporium* anamorph of *Nannizziopsis vriessi* (CANV). These fungi cause superficial and deep mycoses, and as fungi destroys the skin, the normal colouration of the skin of the infected reptiles’ changes and may become yellowish, brownish, or even black in later stages. Infections with *N. vreisii* are contagious and often fatal if not treated. Over the last years, this fungus has been shown to be an emerging pathogen in captive reptiles, especially in bearded dragons and iguanas, but has also been identified in wild reptiles. Human infection was also confirmed.

Herein, authors describe histopathological lesions observed in Bearded Dragon infected with *Chrysosporium* anamorph of *Nannizziopsis vriesii*.

12. The histopathological timeframe of *Hyalomma lusitanicum* infestation development on bovines

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Ticks are competent vectors of both animal and human pathogens. Hard ticks secure their meals by attaching to a host for several days. Even though some animals are capable of acquiring some resistance to ticks after several infestations, tick attachment to naïve cattle often result in skin lesions because host inflammatory reaction is not enough to induce efficient responses capable of leading to ticks skins detachment.

In order to characterize the recruitment of local inflammatory responses, two *Holstein-Frisea* females were infested with laboratory reared *Hyalomma lusitanicum* adult ticks. Ticks remained secured inside tissue sleeves, which were glued to the bovine dorsum. Sampling occurred at different time points throughout the complete period of infestation, from the attachment of unfed ticks to the detachment of fully engorged (fertilized) females. Skin biopsies (8 mm) were fixed in tamponed formaldehyde (3.7%), embedded on paraffin blocks and later cut. Histological sections had 3 µm and stained by hematoxylin & eosin coloration. The microscopic observation of the biopsies allowed skin lesions identification. Both superficial and deep dermatitis, as well as interstitial and follicular, with diverse diffuse and perivascular patterns were found. An infiltration with predominance of inflammatory cells (neutrophils and eosinophil cells) was registered. Some lymphoid cells were identified as well as rare mast cells.

In conclusion, significant differences were found in samples obtained from control and the infested animal, as well as among those obtained in different phases of tick engorgement allowing us to document different pathological changes due to this long rostrum tick.

13. Identification of subcutaneous mite *Hypodectes propus* (Acarina: Hypoderatidae) in the Northern gannet (*Morus bassanus*) in Portugal

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An adult female Northern gannet (*Morus bassanus*) was rescued in Esposende, northern Portugal, after swallowing two fishing hooks, but died shortly after emergency surgery. At necropsy, a large number of perfectly individualized cyst-like structures were found in the subcutaneous tissue of the abdominal region, apparently without associated inflammatory reaction. Those structures were identified as heteromorphic deutonymphs (hypopi) of the subcutaneous mite *Hypodectes propus* (Astigmata: Hypoderidae), based on their size and additional morphological characteristics. The mites were 1.38 mm in length and 0.57 mm in width on average, and presented distinct typical coxal apodemes in the anterior part. To the best of our knowledge, this is the first report of the subcutaneous mite *H. propus* in the Northern gannet in Portugal.

14. Dirofilaria immitis in South African fur seal (Arctocephalus pusillus pusillus): necropsy and histopathology findings observed in zoological context in Portugal- Marcelino I.[1,2*], FlanaganC.[1], Silva N.[1], Madeira de Carvalho L.[2], Correia J.[2], Alho A.M.[2]

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Dirofilariosis is an emerging zoonotic vector-borne disease, with a major impact on companion and wild animals. Despite the importance of cardiopulmonary parasites, studies conducted regarding *Dirofilaria immitis* in pinnipeds are few, being reported only in two species: California sea lion (*Zalophus californianus*) and common seal (*Phoca vitulina*).

A survey of cardiopulmonary parasites was performed in the collection of 19 pinnipeds belonging to Zoomarine – Mundo Aquático S.A., which is located in the Algarve. In parallel, *D. immitis* adult nematodes were found in the right ventricle and pulmonary artery in three necropsies of South African fur seals (*Arctocephalus pusillus pusillus*). Generalized congestion in the lungs, liver and kidneys was noted. Additionally, lesions of exudative pneumonia in red hepatization phase as well as, interstitial pneumonia, emphysema, hemorrhagic lung lesions and also catarrhal bronchitis were observed. The animals had lesions associated with the presence of the parasite but not to a great extent. This was not the cause of death, but a number of factors that were due to old age and immune status of the animals.

To the authors' knowledge, this is the first worldwide report of *D. immitis* in *A. p. pusillus* and the first report of this nematode and associated lesions in a pinniped population from Portugal. Considering that *D. immitis* is a worldwide emerging zoonotic parasite and extremely important in domestic and wild animal health, this work will increase awareness of the disease in pinnipeds, so that veterinarians adopt control measures against the vectors and perform preventive therapy in animals kept in zoological contexts.

Occurrence of blood-sucking mites in ornamental chickens

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Domestic and ornamental birds can be parasitized by hematophagous Dermanyssoid mites belonging to Dermanyssidae and Macronyssidae families. They are responsible for decrease of productivity, can be vectors of diseases and interfere with bird's welfare, and other hosts including humans can be parasitized, being responsible for skin problems and transmission of zoonoses. Morphologically, *Dermanyssus gallinae* closely resembles *Ornithonyssus sylviarum*, both are responsible for numerous economic losses in the poultry industry but they differ in their life cycles. Nymphs and adults of *D. gallinae* remain on the hosts to feed, spending most of his life cycles in the cracks and crevices of the bird's facilities.

On March of 2016 was observed a high infestation by mites in ornamental chickens from a farm located in center of Portugal. The birds had weight loss, intense itching, and erythematous dermatitis, depluming, behavioral changes and decrease of egg production. Infestation by Dermanyssoid mites was observed in 10 birds of Paduana and Old English breeds and in the nests of Japan Silky. Mites were collected directly from the body of birds and in the facilities. The diagnosis was made by optical microscopy. Specimens collected have been identified as "Dermanyssoid mites" because there was difficulty in identifying *D. gallinae* and *O. sylviarum*. On the body of the birds was observed presence of eggs, larvae, nymphs and adults and in the nests was observed mostly adults.

Treatment of birds was carried out individually with the topical application of fipronil (Frontline®), disinfestation of facilities and nests through a flamethrower ("fire broom") and placement of diatomaceous earth in bathing places of birds.

After two weeks of daily observation of birds and facilities no mites was observed. The improvement of general condition and eggs production was regularized.