

II Iberic Meeting of Veterinary Pathology

XVI Annual Meeting of the Portuguese
Society of Animal Pathology

XXIII Annual Meeting of the Spanish
Society of Veterinary Anatomical
Pathology

*Faculdade de Medicina Veterinária,
Lisboa – Portugal, 1st – 3rd June 2011*



Abstracts book

II Iberic Meeting of Veterinary Pathology

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2011



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Fundação para a Ciência e a Tecnologia
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FCT - Fundação para a Ciência e Tecnologia

CECAV – Centro de Ciência Animal e Veterinária

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Royal Canin

Dear colleagues

Today we welcome the II Iberian Meeting of Veterinary Pathology, in Lisbon, Faculty of Veterinary Medicine, and will have as guests Professor Marion Hewicker-Trautwein and Professor Francis Soler Rodríguez.

This meeting brings together the Portuguese Society of Animal Pathology (SPPA) and the Spanish Society of Veterinary Pathology (SEAPV) in a single meeting. This year we focus on two different issues, both very important for our updating as pathologists.

Professor Marion Hewicker-Trautwein, from the University of Veterinary Medicine of Hannover, is a Diplomate of the European College of Veterinary Pathologists and a specialist in canine and feline inflammatory bowel disease, being a world reference in this field, as can be seen by her publications.

Professor Francisco Soler from the Veterinary Faculty, University of Extremadura, is a specialist in Veterinary Plant Toxicology, and will speak to us about clinical pathology of neurotoxic plants and plant toxicology in ruminants.

In parallel with our meeting, a workshop in CISH (Chromogenic In Situ Hybridization) will take place. It will be conducted by Dr Dina Leitão from the External Service of Pathology and Molecular Pathology of IPATIMUP. This molecular biology technique is an asset to our work and can be implemented in veterinary pathology laboratories. These workshops are becoming usual at SPPA meetings, going now in its 3rd edition.

The Faculty of Veterinary Medicine of Lisbon is our host this year, so we want to thank its President, Professor Luis Tavares, for his kindness and his willingness to receive us.

We would like to acknowledge the Organizing Committee, consisting of personnel from the University of Trás-os-Montes and Alto Douro (UTAD), Agrarian School of Viseu (ESAV), University of Évora (UE), Faculty of Veterinary Medicine of Lisbon (FMV), as well as, students from UTAD and FMV.

We also acknowledge the Scientific Committee, composed by staff of the above mentioned institutions; by personnel from the SEAPV, Institute of Biomedical Sciences Abel Salazar, and of the National Veterinary Research Laboratory.

To everyone, thank you very much!

To all participants, thank you for coming and for your participation!

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PROGRAM:

Wednesday, 1st June 2011

16:00 Registration

18:00 Official Opening

18:15 Opening session

"Bowel disorders of cats and dogs". Prof. Institute of Pathology. Veterinary University Hannover

19:30 Wellcome reception (Port of Honour)

Thursday, 2nd June 2011

09:00 Plenary lecture:

"Inflammatory bowel disease of dogs and cats (part one)". Prof. Marion Hewicker-Trautwein.

09:45 First session of oral communications

C1 - CANINE GLIOMA AS ANIMAL MODEL FOR HUMAN GLIOMA: CANCER STEM CELL STUDY FOR DETECTION OF NEW DIAGNOSTIC AND THERAPEUTIC TARGETS

C2 - HISTOLOGICAL MALIGNANCY GRADES IN CANINE MAMMARY TUMORS: RELATIONSHIP WITH CLINICAL STAGE AND HISTOPATHOLOGICAL DIAGNOSES.

C3 - ADHESION MOLECULES EXPRESSION IN METASTASIC AND NON METASTASIC FELINE MAMMARY CARCINOMAS

C4 - HYPER-LDL CHOLESTEROLAEMIA FAVOURS ACUTE LYMPHOBLASTIC LEUKAEMIA DISSEMINATION AND INVASION OF THE NERVOUS SYSTEM

C5 - MORPHOLOGICAL ASPECTS OF FELINE ENDOMETRIAL ADENOCARCINOMAS: A PRELIMINARY STUDY

11:00 Coffee break and posters view

11:30 Second session of oral communications

C6 - INTRAOCULAR TUMOURS DIAGNOSED AT THE PATHOLOGY LABORATORY OF THE FACULTY OF VETERINARY MEDICINE, TECHNICAL UNIVERSITY OF LISBON, OVER A TEN-YEAR PERIOD

C7 - SIX CASES OF CANINE AND FELINE LYMPHOMA WITH ATYPICAL LOCATION

C8 - SUDDEN DEATH IN DOGS AND CATS – A RETROSPECTIVE STUDY OF 213 CASES (2000-2009)

C9 - VETERINARY FORENSIC MEDICINE IN PORTUGAL: IS IT A NECESSITY?

C10 - RECURRENT ANNUAL OUTBREAKS OF SEASONAL BOVINE CONGENITAL DEFECTS IN NORTH OF SPAIN

13:00 Lunch

15:00 Plenary lecture:

"Inflammatory bowel disease of dogs and cats (part two)". Prof. Marion Hewicker-Trautwein. Institute of Pathology. Veterinary University Hannover

16:00 Third session of oral communications

C11 - SEVERE AND UNUSUAL HEPATIC LESIONS ASSOCIATED WITH ALVEOLAR EQUINOCOCCOSIS (*Echinococcus multilocularis*) IN A Gorilla g. gorilla.

C12 - LUNG INFECTION BY *Crenosoma striatum* AND PERFORATED GASTRIC ULCER IN A HEDGEHOG (*Erinaceus europaeus*)

C13 - UNREPORTED NEOPLASIA IN PRAIRIE DOGS (*Cynomys ludovicianus*)

C14 - AVIAN TUBERCULOSIS IN AN EAGLE OWL

C15 - MORPHOLOGICAL EXAMINATION OF PROTEIN INCLUSIONS IN STRANDED CETACEANS

17:00 Coffee and posters view

17:30 Fourth session of oral communications

C16 - THYMIC SQUAMOUS CELL CARCINOMA? DO YOU CONFIRM THIS DIAGNOSIS?

C17 - ABDOMINAL MASS IN AN ASSUMED NEUTERED DOG

C18 - CYSTIC LESION IN THE PHARYNX OF A YOUNG BULLDOG

C19 - CAN ESTROGEN ADMINISTRATION BE A CAUSE OF ACUTE HEMORRHAGES IN DOGS? – A CASE REPORT

C20 - HYDRONEPHROSIS DUE TO SUPERNUMERARY OVARY AND UTERUS

20:00 Congress Dinner

Friday, 3th June de 2011

09:00 Plenary lecture

"Clinical Pathology of neurotoxic plants" Dr. Francisco Soler Rodriguez. Caceres Veterinary Faculty

09:45 Fifth session of oral presentations

C21- PATHOLOGIC AND IMMUNOHISTOCHEMICAL FINDINGS IN RED-LEGGED PARTRIDGES (*Alectoris rufa*) EXPERIMENTALLY INFECTED WITH TWO MEDITERRANEAN STRAINS OF WEST NILE VIRUS (WNV)

C22 - PROGRESSIVE DEMYELINATION, NEURONAL LOSS, AND ASTROGLIOSIS IN CEREBELLUM AND HIPPOCAMPUS OF EXPERIMENTALLY POISONED CATTLE BY INGESTION OF *Solanum bonariense* L.

C23 - CYTOSKELETAL DERANGEMENT IN CEREBELLAR PURKINJE CELLS OF *Solanum bonariense* L. INTOXICATED BOVINES. AN IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY

C24 - PANCREATIC LESIONS AND METABOLIC AGGRAVATION ARE PREVENTED BY LOW DOSES OF SITAGLIPTIN IN A RAT MODEL OF TYPE 2 DIABETES

C25 - INHIBITION OF AFRICAN SWINE FEVER VIRUS REPLICATION BY QUINOLONES: A POTENTIAL WINDOW FOR ANTIVIRAL CHEMOTHERAPY

11:15 Coffee and posters view

11:45 Sixth session of oral presentations

C26 - DEVELOPMENT OF A VITREOUS FLUOROMETRY PROTOCOL WITH A MODIFIED LASER CONFOCAL SCANNING LASER OPHTHALMOSCOPE IN RABBIT EYES TO BE APPLIED IN DIABETIC RETINOPATHY STUDIES

C27 - COX-2 IMMUNOLocalIZATION IN CANINE ENDOMETRIUM DURING THE OESTROUS CYCLE

C28 - FADD AND DAXX MARKERS TO STUDY APOPTOSIS PATHWAYS IN PORCINE PARAFFIN-EMBEDDED TISSUES

C29 - ALPHA AND BETA TUBULIN AS MARKERS FOR ENTERIC NERVOUS SYSTEM IN SIX FARMED TELEOST SPECIES: AN IMMUNOHISTOCHEMICAL STUDY

C30 - QUANTITATIVE AND QUALITATIVE EVALUATION OF THE INDUCIBLE ISOFORM OF NOS EXPRESION IN TURBOT (*Psetta maxima*) INFECTED WITH *Enteromyxum scophthalmi*

C31 - LOCALIZATION OF VACCINE ANTIGEN AND HUMORAL IMMUNE RESPONSE IN TURBOT (*Psetta maxima*) VACCINATED AGAINST FURUNCULOSIS

13:00 Lunch

14:30 Plenary lecture

“Other important pathologies on plant toxicology”. Dr. Francisco Soler Rodriguez. Caceres Veterinary Faculty

15:30 Seventh session of oral presentations

C32 - LOCALIZATION OF VACCINE ANTIGEN AND HUMORAL IMMUNE RESPONSE IN TURBOT (*Psetta maxima*) VACCINATED AGAINST FURUNCULOSIS

C33 - SKELETAL MALFORMATIONS IN EARLY STAGES OF DEVELOPMENT OF SENEGALESE SOLE (*Solea senegalensis*, KAUP 1858) REARED AT DIFFERENT DENSITIES

C34 - IMMUNOHISTOCHEMICAL ASSAYS WITH COMMERCIAL ANTIGEN-PRESENTING CELL ANTIBODIES IN TURBOT (*Psetta maxima* L.)

C35 - PRELIMINARY STUDY OF INJURIES ASSOCIATED WITH MYCOBACTERIOSIS IN HORSE MACKEREL (*Trachurus trachurus*)

C36 - DESCRIPTION OF A LYMPHOMA OUTBREAK IN CULTURED TENCH (*Tinca tinca*)

16:45 Coffee and posters view

17:00 Teaching Veterinary Pathology Session

Prof^a Elena Mozos, Prof^a Conceição Peleteiro, Prof^o Aniceto Mendez, Prof^a Fátima Gartner, Prof^a Maria dos Anjos Pires

17:45 Closing Ceremony Annual

18:00 Annual Meeting SPPA

Annual Meeting SEAPV

Invited speakers

BOWEL DISORDERS IN DOGS AND CATS

Hewicker-Trautwein, Marion

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The lecture mainly summarizes the histomorphological features of non-neoplastic lesions, i.e. intestinal polyps, and neoplastic changes in the small and large intestine of dogs and cats. Tumorlike proliferative lesions and different alimentary tumors are explained by following the WHO International Histological Classification of Tumors of Domestic Animals with special emphasis on hematopoietic tumors, i.e. malignant alimentary lymphoma, and gastrointestinal stromal tumors and their differential diagnoses. The characteristics and the classification of feline alimentary lymphoma (B-/T-cell lymphoma, high-/low-grade tumor) are explained. The significant histological findings in feline low-grade alimentary lymphoma (LGAL) in comparison to its most important differential diagnosis, i.e. lymphoplasmacytic enteritis (LPE) are summarized. For mesenchymal alimentary tumors the classification system for smooth muscle tumors and gastrointestinal stromal tumors (GIST) in dogs suggested by Maas et al. (Vet. Surg. 2007, 36: 302-313), which is based on histopathological and immunohistochemical findings, and its prognostic relevance is described.

INFLAMMATORY BOWEL DISEASE OF DOGS AND CATS

Hewicker-Trautwein, Marion

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In the two parts of this lecture a survey is given on chronic idiopathic gastrointestinal diseases known under the term inflammatory bowel disease (IBD). The contents include the classification of the different IBD forms (lymphoplasmacytic, eosinophilic, granulomatous) occurring in the small and/or large intestine based on histopathological findings including variants of IBD in special breeds of dogs (Basenjis, Soft-coated Wheaten Terriers) and histiocytic ulcerative colitis in Boxer dogs. The ultimate diagnosis of IBD depends on the histopathological findings in intestinal biopsies. The principles of collection of biopsies (endoscopic and full-thickness samples) and of standardized histopathological evaluation of biopsies according to Day et al. (J. Comp. Pathol. 2008, 138, suppl. 1) are explained. An important differential diagnosis to lymphoplasmacytic enteritis (LPE), especially in cats but also in dogs, is diffuse alimentary lymphoma. The histopathological characteristics of LPE and lymphoma are explained. The current knowledge and hypotheses about the pathogenesis of IBD in dogs and cats including genetic, immunological and environmental (bacteria and dietary antigens) factors are reviewed.

CLINICAL PATHOLOGY OF TOXIC PLANTS

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Among the animal diseases, poisoning by toxic plants is a problem to be considered when the animal production is based mainly on the use of natural food resources present on the field. In these cases highly toxic plants that grow naturally in the field can be eaten by animals causing poisoning what can mean death of animals or a significant loss in production. The onset of these adverse effects can be quite sudden or take some time to develop. Fortunately, among the thousands of plants in the environment of animals, relatively few cause acute, life-threatening illnesses when ingested.

Among the toxic plants, one of the most important group, due to their high toxicity and frequency of outbreaks, is the group of plants that have a primary toxic action on the nervous system (neurotoxic plants) that belong to different taxonomic classes.

The pathological findings are varied and can range from an absence of them (Oenanthe crocata or hemlock poisoning), nonspecific (necrosis, degeneration ...) or some more specific and localized findings like intracytoplasmic accumulations of toxic compounds (vacuolization) in neurons (Phalaris spp., Astragalus and Oxytropis spp.) or polioencephalomalacia similar to B1 avitaminosis (Pteridium and Equisetum).

The birdseeds (Phalaris spp.) are grasses that cause hyperexcitability and muscle contractions due to accumulation of the toxic principle in the cytoplasm of neurons, causing the appearance of bilateral symmetrical pigmentations throughout the CNS.

Different species of Cistaceae (Cistus spp. Xolantha spp.) cause generalized muscle contractions after stimulus, followed in chronic cases by renal impairment. Nonspecific nerve (diffuse neuronal degeneration and demyelination) and renal (bladder distention, tubulonephrosis, multifocal tubular necrosis and even pyelonephritis) changes are usually observed.

Among the legume some species of Astragalus spp. cause acute or chronic poisoning with intense vacuolization in CNS and other tissues. Lupinus produce acute poisoning being the most interesting finding for the diagnosis the observation of the seeds in the digestive content. This is important also in the case of certain Umbelliferae (Oenanthe crocata, Conium maculatum) because poisoning occurs from eating roots, and they can be in the stomach content as the death is rapid, with no specific diagnostic lesions at post-mortem examination.

Other neurotoxic plants to highlight include Datura stramonio, Nicotiana tabacum, Solanum spp. or Lathyrus and Vicia spp. that cause two syndromes: osteolathyrism and neurolathyrism.

Some plants produce toxic action on the kidney by two ways: causing deposits of oxalate crystals in the tubular lumen or in the medulla (Oxalis,

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Rumex ...) or by alteration of the cells of the nephron due to a chelating action on proteins (tannins, as contained in *Quercus* spp.). In the latter case pale swollen kidneys, perirenal edema, subcutaneous edema, ascites, and hydrothorax are common necropsy findings. Histopathologic examination of the kidney reveals coagulative necrosis of the proximal convoluted tubules and pink-staining casts of epithelial cells and protein.

Other plants produce a photosensitizing effect on unpigmented areas of skin, such as eyelids, muzzles and udders, exposed to bright sunlight. Early signs are erythema and edema, and serious signs that occur later in the course of the disease include exudation, ulceration, exfoliation of damaged epidermis develops and possibly blindness. Photosensitization is most likely to occur in sunny climates and during the spring and summer when sunlight is more intense or of longer duration each day. Primary photosensitization occurs when a plant which contains a photodynamic agent (*Hypericum* spp., *Fagopirum esculentum*) is directly ingested, and the major effects occur in the skin; other organs are usually spared. Secondary (hepatogenous) photosensitization occurs as a result of compromised liver function, which reduces the excretion of plant pigment metabolites from the body. Several toxic plants are known to cause hepatogenous photosensitizers, and in this case liver damage and involvement of other organ systems may accompany the expected skin-related signs and lesions of photosensitization.

Some plants (*Senecio* spp.) contain pyrrolizidine alkaloids that primarily affect the liver by direct injury to hepatocytes in a chronic way. These poisonings are chronic (several weeks or months) and post-mortem examination reveals cirrhosis of the liver, with characteristic histological changes (portal fibrosis, megalocytosis, hepatocellular necrosis, bile duct hyperplasia, bile stasis and nodular hyperplasia).

Other toxic plants cause a severe bleeding usually fatal syndrome due to the presence of hydroxi-coumarins, as *Ferula communis*, which occupies the leading role. Other plants (*Medicago* spp., sweet clover poisoning) are called indirect anticoagulants because the anticoagulant substance formation occurs when these plants suffer from degradation and fungal infestation. In these cases haemorrhage is the characteristic necropsy finding and large extravasations of blood are common in subcutaneous and connective tissues.

Pteridium aquilinum (bracken fern) may induce several syndromes always related to a continuous consumption of the plant: thiamine deficiency in horses and pigs; an acute hemorrhagic syndrome in cattle due to thrombocytopenia by an aplastic anemia factor; a chronic enzootic hematuria syndrome in cattle in which bladder contains small haemorrhages, dilated vessels, or tumors which can be vascular, fibrous, or epithelial; an a bright blindness syndrome in sheep which is a progressive retinal atrophy that derives its name from the hyperreflectivity of the tapetum.

Oral communications

CI - CANINE GLIOMA AS ANIMAL MODEL FOR HUMAN GLIOMA: CANCER STEM CELL STUDY FOR DETECTION OF NEW DIAGNOSTIC AND TERAPEUTIC TARGETS

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Introduction: Cancer stem cell (CSC) hypothesis implies that not all the cells in a tumor have the same capability to proliferate and maintain the growth of the tumor. CSCs have special properties being chemo- and radio resistant. Our research on canine neuroncology is based on CSC hypothesis to generate tumors in nervous tissue. We aim to demonstrate the usefulness of dog, having spontaneous gliomas, as animal model for the study of human gliomas.

Materials and Methods: Natural canine gliomas from spontaneous cases diagnosed at the HCV-UAB are obtained. We use “in vitro” and “in vivo” techniques: histochemistry, immunohistochemistry (IHC) and Western Blot (WB), to identify, classify and grade canine and murine tumors, cell cultures for neurosphere assays and neural differentiation; and reproduction of the tumor by inoculation of dissociated cells from neurospheres in immune deficient mice. We use control healthy dogs to obtain non tumor-derived neurospheres.

Results: We identified and characterized CSC in canine gliomas by IHC. Correlation between immunohistochemical and molecular studies were confirmed (p53 study). Glio-spheres were obtained from the tumor and differentiation techniques supplied several neural phenotypes. Glio-spheres were large and abundant when using tissue originating from the tumour core. In control dogs, neurospheres were obtained from the subventricular zone (SVZ), the hippocampus and IV ventricle SVZ.

New information about previously unknown cellular targets may be applicable to diagnosis and prognosis of both canine and human gliomas and useful to obtain new therapeutics tools that could be tested on dogs.

C2 - HISTOLOGICAL MALIGNANCY GRADES IN CANINE MAMMARY TUMORS: RELATIONSHIP WITH CLINICAL STAGE AND HISTOPATHOLOGICAL DIAGNOSES.

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Introduction: Histological diversity of canine mammary tumors (CMT) makes difficult their diagnosis. The histological malignancy grade, has been proposed to better accurate the tumor information provided from the pathologist to the clinician. In this study, the possible associations among the histological grade and clinical and histopathological characteristics of CMT are evaluated.

Materials and Methods: In this study 42 female dogs with malignant CMT were included. Animals were presented at Complutense University Veterinary Teaching Hospital (Madrid), clinically evaluated and surgically treated through 2008. Histological diagnosis and tumor malignancy grading were performed using a new classification system for CMT (Goldschmidt, Peña et al., 2011). In patients with more than one malignant CMT, only one was selected for statistical evaluation (grade I, n=20; grade II, n=11; grade III, n=11). Epidemiological, clinical and histological variables were considered. Statistical analyses were performed with a significant level $p < 0.05$.

Results: Histological malignancy grades were related to clinical stage ($p=0.03$), skin ulceration ($p=0.05$), histological type of diagnosis ($p=0.02$) and lymph node metastases ($p < 0.01$).

Discussion and Conclusion: Histological grading system of canine mammary tumors is a useful diagnostic tool associated to clinical and histopathological characteristics

C3 - ADHESION MOLECULES EXPRESSION IN METASTASIC AND NON METASTASIC FELINE MAMMARY CARCINOMAS

Peñañiel-Verdú C¹, Buendía, A¹, Ramirez, G², Altimira, J², Villafranca, M², Navarro, JA¹, Sánchez, J¹

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Introduction: Feline simple mammary carcinoma is a highly malignant neoplasia. It is thought that there are several mechanisms implicated in tumoral progression such as loss of epithelial adhesion molecules: E-cadherin and beta-catenin.

Materials and Methods: From a sample of 138 simple mammary carcinomas (66 non-metastatic and 72 with regional lymph node metastasis) were studied the expression of adhesion molecules and their relation to basal (K5, K14) and luminal (K18) cytokeratins expression. It is known that in human breast cancer the expression of K18 reveals a better prognosis than carcinomas which express basal cytokeratins.

Results: Our results shown that expression of E-cadherin and beta-catenin are significantly higher in carcinomas without metastasis. Metastatic carcinomas present loss of E-cadherin expression and only 14% of these neoplasias have a functional expression (beta-catenin coexpression).

Discussion and Conclusion: Functional expression of E-cadherin was significantly associated with high expression of K18 and low expression of K5

C4 - HYPER-LDL CHOLESTEROLAEMIA FAVOURS ACUTE LYMPHOBLASTIC LEUKAEMIA DISSEMINATION AND INVASION OF THE NERVOUS SYSTEM

Carvalho, T, Fragoso, R, Gomes, AL, Serpa, J, Caiado, F, Matias, I, Remedio, L, Bastos, A, Marcos, F, Martins, L, Cardoso, B, Silva, MG, Barata, J, Dias, S

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Central nervous system (CNS) relapse in acute lymphoblastic leukaemia (ALL) is a major obstacle to cure, accounting for 30–40% of initial relapses. Children with ALL show abnormal lipid metabolism at diagnosis, with altered serum lipid profiles. Furthermore, obesity, generally associated with high cholesterol levels, predicts likelihood of relapse in paediatric ALL patients. Elevated total cholesterol and low density lipoprotein (LDL) levels are also thought to facilitate the development of distant metastasis in certain solid tumors.

Using a murine B-ALL model (cell line 697), we found that leukaemic mice displayed significantly-increased LDL cholesterol levels, when compared to healthy mice, and that there was a positive correlation between plasma LDL cholesterol levels and the percentage of circulating B-ALL cells. After a fat diet feeding trial, mice on a high-cholesterol diet displayed increased leukaemia dissemination to the central/peripheral nervous system, with infiltration of the leptomeninges, perineural spread of tumour along the fifth cranial nerves and invasion of the facial muscles. Furthermore, B-ALL cells in the CNS are immunophenotypically distinct from those of bone marrow; while the latter are typically TdT/Pax5 positive, B-ALL cells in the leptomeninges undergo some kind of cellular reprogramming, with loss of TdT and Pax5 expression. The molecular mechanisms involved in CNS and perineural invasion include, among others, chemotactic cytokines and their receptors. We found that CX3CL1 (a transmembrane chemokine also known as fractalkine or neurotactin) is up-regulated in the bone marrow and nervous tissue of mice on a high-cholesterol diet; CX3CL1 possesses intrinsic cell-adhesive properties in endothelial cells and neurons. We also found that B-ALL cells collected from the cisterna magna expressed higher levels of CX3CR1, the chemokine receptor that exclusively binds to CX3CL1. On the basis of this preliminary data, we hypothesize that the host microenvironment greatly conditions the metastatic signature of the tumours, and that altered lipid metabolism favours tumor spread to the CNS and perineural invasion.

C5 - MORPHOLOGICAL ASPECTS OF FELINE ENDOMETRIAL ADENOCARCINOMAS: A PRELIMINARY STUDY

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Introduction: Endometrial adenocarcinomas are considered rare in domestic animals, partly because they are probably underdiagnosed. This study reports on the varying morphology of feline endometrial adenocarcinomas.

Materials and Methods: Forty feline endometrial adenocarcinomas were identified by a minimum of three pathologists, on conventional haematoxylin and eosin-stained sections. The material was mostly obtained from the archives of four different laboratories, but 13 neoplasms were obtained specifically for this study, from a total of 88 ovariectomy surgical specimens.

Results: Tumours were classified as papillary serous carcinoma (with or without clear cells), clear-cell carcinoma and in situ carcinoma according to cytological criteria: malignancy, cell morphology and invasion of adjacent tissues.

Conclusion: Feline endometrial adenocarcinomas may be more common than previously assumed, and may display various morphologies. Further research is required in order to enable complete characterisation of these lesions.

C6 - INTRAOCULAR TUMOURS DIAGNOSED AT THE PATHOLOGY LABORATORY OF THE FACULTY OF VETERINARY MEDICINE, TECHNICAL UNIVERSITY OF LISBON, OVER A TEN-YEAR PERIOD

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Introduction - Tumours of the eye and its supporting tissues are rare in domestic animals, although these may be the site of development of various primary tumours, as well as metastasis. Primary intraocular tumours may arise from any of the cells present in the eye tissue layers; however the neoplastic proliferation of uveal melanocytes is the most common in all species. The metastatic intraocular neoplasm most frequently reported is lymphoma.

Materials and Methods - Intraocular neoplasms were selected from the archives of the Pathology Laboratory of Faculty of Veterinary Medicine of the Technical University of Lisbon, from 2001 to 2011. The selection was made excluding all tumours of the eye region that did not involve the eye itself.

Results - Of a total of 29 tumours, 17 were in cats, 11 in dogs and one in a hamster. The mean age in cats and dogs was 8.4 years. Most feline tumours were diffuse uveal melanomas (41.1%) and lymphomas (29.4%). In the dog, melanocytic tumors were diagnosed in 72% of cases.

Discussion - The major challenge in the diagnosis of eye melanocytic tumours has to do with the need to evaluate the prognosis in situations where the origin of the neoplasm is impossible to determine due to tissue destruction. In most cases, the parameters for malignancy grading should be the same as those used for skin melanocytic tumours. The possibility of using cell markers as a predictive tool is worth studying.

C7 - SIX CASES OF CANINE AND FELINE LYMPHOMA WITH ATYPICAL LOCATION

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Lymphomas, a term covering B-, T- and NK-cell lymphoid neoplasms, are a group of malignant tumours common in humans and all animals species.

The aetiology of canine and feline lymphoma is multifactorial in nature and may include strictly environmental as well as infectious (viral) causes in cats. The most common descriptive terms for such tumours are digestive, cutaneous, multicentric, thymic or mediastinal, and extranodal lymphomas. Multicentric lymphomas are most commonly seen in animals. With some important species differences, the tissues/organs most frequently affected are the peripheral lymph nodes (often in a symmetrical manner), liver, spleen, kidneys, heart, gastrointestinal tract, and bone. Although an anatomical site is often used to classify a lymphoma in domestic animals, seldom is the tumour confined to that site. Solitary lymphomas are rare in the dog, being more frequently identified in the cat, especially in the kidneys, usually with bilateral involvement. Symptoms associated with solitary lymphomas depend entirely on the organ distribution and consequent degree of dysfunction.

This study reports on six cases of primary canine and feline solitary lymphomas with atypical locations: one feline pericardial lymphoma, one feline juxtamammary extranodal lymphoma, two feline ocular lymphomas, one canine central nervous system lymphoma and one canine nasopharyngeal lymphoma. Animal ranged from 3 to 13 years of age. All neoplasms were diagnosed by histopathological analysis and were immunohistochemically phenotyped using anti-CD3, Pax 5 and CD79 α antibodies. CD3-positive tumours appeared to predominate.

C8 - SUDDEN DEATH IN DOGS AND CATS – A RETROSPECTIVE STUDY OF 213 CASES (2000-2009)

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Introduction: In veterinary medicine, sudden death is a syndrome affecting animals seen as healthy both by their owners and by their veterinarians. This study aimed to determine the causes of sudden death in dogs and cats, and to establish the epidemiology of the syndrome.

Materials and Methods: An analysis was made of necropsy records at the FMV/UTL Laboratory of Pathological Anatomy from January 2000 to December 2009. The data collected included clinical signs immediately before death, and post mortem histopathological reports.

Results and Discussion: Sudden death was recorded in 6.2% (n=60) of necropsies performed in cats and 6.3% (n=153) conducted in dogs. Analysis showed that most cats involved were male European Shorthairs, of between 1 and 6 years of age. Death was mainly due to exudative pneumonia, hypertrophic cardiomyopathy, acute pulmonary oedema, feline panleukopenia virus, cardiac lipomatosis, organophosphate poisoning and acute renal failure. Most sudden deaths in dogs affected male purebreds, especially the German Shepherd breed. The main cause of death was gastric dilation-volvulus syndrome, followed by pesticide poisoning, sub-aortic stenosis and dilated cardiomyopathy. In most cats and dogs, no symptoms were witnessed and in 13% and 7%, respectively, the cause of death was inconclusive

C9 - VETERINARY FORENSIC MEDICINE IN PORTUGAL: IS IT A NECESSITY?

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A concern with animal rights and welfare has increased lately, leading to a demand of a veterinary active role in forensic work.

The fields where the Veterinarian may act as an expert are plenty and diversified.

The present work aims to identify and characterize the multiple areas of Forensic Veterinary Medicine in Portugal, which are relevant to the Community.

The authors point out, not due to their importance but because they have more impact in the society, situations involving animal violence - either as victim or as perpetrator of a crime against humans or other animals. Moreover, importance was given to the necropsy, not only for being often requested to the veterinary medical class but also for being one of the specialization areas, through which Veterinarians Pathologists may contribute to medico-legal issues.

This study was performed through two questionnaires. The first one targets the general Veterinarian practitioners of Portugal. The second questionnaire specifically aimed the Veterinarians Pathologists, since they, somehow, already have a role in situations involving medical-legal issues.

We also present, in the form of diagrams, a couple of protocols for action in situations that may be worthy of the Law's attention

C10 - RECURRENT ANNUAL OUTBREAKS OF SEASONAL BOVINE CONGENITAL DEFECTS IN NORTH OF SPAIN

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Introduction: Congenital anomalies in calves have been related to genetic factors, physical agents, vitamin A and copper deficiencies and infectious or toxic causes. In this study, an outbreak of congenital anomalies of the central nervous system in newborn cattle occurred annually during February-March in a particular valley of the north of Spain is described.

Material and methods: Necropsies were performed on four animals from four different grazing herds, and tissue samples were processed using routinely histological and immunohistochemical techniques. Serum samples from these calves, their dams and other adult animals were collected for laboratory analysis.

Results: The affected animals appeared annually at the same time of the year but these outbreaks of disease only occurred in herds which grazed in a particular valley. Clinical signs were anemia weakness and ataxia, and other neurologic signs as blindness and recumbency could be occasionally observed. Myelodysplasia with the presence of aberrant central canals and the absence of septa were the main histopathological findings found in all the newborns.

Conclusions: A viral etiology or toxic plants are discussed as possible origin of these outbreaks of disease. Nutritional deficiencies have been ruled out.

C11 - SEVERE AND UNUSUAL HEPATIC LESIONS ASSOCIATED WITH ALVEOLAR EQUINOCOCCOSIS (*Echinococcus multilocularis*) IN A Gorilla *g. gorilla*.

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Introduction: A case of unusual presentation of alveolar equinococcosis in a female 11-year-old Lowland Gorilla (*Gorilla g. gorilla*) is described.

Material and methods: The necropsy and the histopathological study of a gorilla with recurrent phases of apathy, loss of weight and progressive abdominal enlargement were performed.

Results: At necropsy, the abdominal cavity contained abundant ascitic fluid. The liver had multiple, white and firm nodules, some of them with central cavities filled with purulent material (0.5-20 cm in diameter) that replaced about 70% of the parenchyma. Histologically, nodules consisted of central necrosis infiltrated by macrophages, lymphocytes, multinucleated giant cells and in a less number by eosinophils and neutrophils and surrounded by fibrous connective tissue. The necrotic areas contained remnants of a laminated membrane, calcareous corpuscles and in the periphery, alveolar cysts with rests of the germinal epithelium. A few hidatid cysts with scolices were also observed, and the remaining liver parenchyma presented atrophy and fibrosis.

Conclusions: These pathological findings are related to hydatid cyst typical of *Echinococcus multilocularis* but a diferencial diagnosis must be done with other parasitic infections such as *E. granulosus*, *E. vogeli* and *Cysticercus spp*, abscesses and tumors. An intense and unusual inflammatory response against the parasite could result in this atypical presentation of a fatal granulomatous and necrotizing hepatitis

C12 - LUNG INFECTION BY *Crenosoma striatum* AND PERFORATED GASTRIC ULCER IN A HEDGEHOG (*Erinaceus europaeus*)

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Introduction: Though wildlife pathology studies are steadily growing worldwide, they remain relatively fragmentary in Portugal. This paper reports on a case of *Crenosoma striatum* lung infection and a perforated gastric ulcer in a hedgehog.

Materials and Methods: In April 2009, a sexually mature male hedgehog was rescued from a trap. It exhibited no remarkable clinical signs. Six days later, it was found dead. It was suspected that the hedgehog had either been attacked by a dog or run over by a car, so necropsy was requested. Samples of lung, myocardium, stomach, liver, pancreas, adrenal glands, kidneys, testicles and prostate were collected, fixed in 10% formalin and embedded in paraffin. Histological sections were stained with haematoxylin and eosin.

Results: Necropsy revealed slight anemia, presence of ectoparasites (ticks), recent fracture calluses in five consecutive ribs on the right side and a perforated ulcer in 2 mm in diameter in the pylorus. Histopathological examination showed: lungs – focal atelectasia and emphysema, presence of Nematoda in bronchiolar lumen and hypertrophy of bronchiolar muscles; kidneys – nephrosis; testes –mild focal degeneration of the seminal epithelium; prostate – cystic dilatation of a significant number of tubuloalveoli.

Discussion and Conclusion: The cause of death was perforation of an ulcer, which microscopic features indicated was chronic. Death by traumatic injury was ruled out. According to biological and epidemiological data and morphologic characteristics, the Nematoda lodged in the bronchioles were Metastrongylidae, probably of the species *Crenosoma striatum*. This appears to be the first report of lung infection by Nematoda in hedgehogs in Portugal.

C13 - UNREPORTED NEOPLASIA IN PRAIRIE DOGS (*Cynomis ludovicianus*)

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To date, limited information is available regarding tumors in prairie dogs, as they have only recently become popular as pets. The most common neoplasia is odontoma of the incisors. Hepatocellular carcinoma is also a commonly diagnosed disease, with a hypothesized link to hepadnaviral hepatitis as seen in woodchucks and humans. Single case reports of epiglottal fibrosarcoma, mediastinal lipoma, multicentric linfoma, maxillary osteosarcoma and two cases of salivary gland adenoma have been published in the prairie dog.

Here, we present four new tumors that have not yet been described in this species. These are a squamous cell carcinoma in the eyelid, a multilobular apocrine gland adenoma in the dorsal neck, an apocrine gland adenocarcinoma with carcinomatous lymphangiosis below the right ear and a retrobulbar tumor of epithelial origin.

It is likely that geriatric conditions such as neoplasia will continue to develop in the prairie dog, considering that the improvement of management by owners will increase life expectancy in this species.

C14 - AVIAN TUBERCULOSIS IN AN EAGLE OWL

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Introduction: Avian tuberculosis is a worldwide disease which affects companion, captive exotic, wild and domestic birds. It is a slowly spreading, chronic bacterial infection most commonly caused by *Mycobacterium avium sp. avium*. The disease is more common in captive than in wild birds although mycobacterial infection has been reported in different wild species. This work describes a clinical case of avian tuberculosis in an eagle owl that was remitted to the Veterinary Pathology Diagnostic Service of the University of León.

Material and methods: Necropsy was performed on the animal, and tissue samples were collected for light microscopy. Samples were stained with H&E and Zielh-Neelsen, and tested by PCR from paraffin embedded samples against the specified sequence IS901 of *Mycobacterium avium sp. avium*.

Results: At necropsy a subcutaneous yellowish, caseous, big mass, involving the neck which surrounded the cervical vertebrae and trachea and compressed the esophagus was observed. Histopathological examination of lesions in samples of the cervical region revealed a severe granulomatous inflammation in the subcutaneous tissue characterised by a well-defined area of central necrosis surrounded mainly by macrophages and giant cells, with high amount of acid-fast bacilli within the lesions. By PCR *Mycobacterium avium sp. avium* was detected. No significant lesions were observed in others organs and tissues.

Conclusions: Atypical form of avian tuberculosis is described affecting only the subcutaneous tissue of neck area.

C15 - MORPHOLOGICAL EXAMINATION OF PROTEIN INCLUSIONS IN STRANDED CETACEANS

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Introduction: This paper reports on the presence, morphology and nature of intracytoplasmic eosinophilic globules in the hepatocytes of cetaceans stranded in the Canary Islands.

Materials and Methods: Liver samples from 115 cetaceans of 17 different species were formalin fixed and paraffin embedded. In samples presenting globules, histochemical and immunohistochemical techniques were used to detect glycoproteins using Periodic acid-Schiff with diastase treatment (PASd), and specific proteins: alpha-1-antitrypsine (A1AT) and fibrinogen (FB). An ultrastructural study of the globules was also performed.

Results: In 95 out of 115 (82.6%) liver specimens, intracytoplasmic hyaline eosinophilic globules were observed in hepatocytes; different results were obtained for PASd, A1AT and FB staining, and ultrastructural morphology also differed.

Conclusion: Intracytoplasmic protein inclusions (hyaline globules) are present in a range of inflammatory and cardiovascular disorders in stranded cetaceans. Further research is required in terrestrial mammals to compare morphological findings with those reported here for stranded cetaceans.

C16 - THYMIC SQUAMOUS CELL CARCINOMA? DO YOU CONFIRM THIS DIAGNOSIS?

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Introduction: In dogs thymic carcinoma is considered rare and distinguishes itself from thymoma by its cytologically malignant features, extensive local invasion, and a substantial potential for metastasis. Surgical excision is the treatment of choice for most thymic tumours, with the exception of lymphoma.

Medical History: A 9 years old cross-breed male dog was presented due to cough and respiratory distress with duration of 4 weeks. The animal presented with inspiratory dyspnea and cyanosis. A mass, dorsal to the pharynx, was diagnosed after clinical and radiographical examination, suspected of thyroid neoplasia. Cytology was performed and revealed cells consistent with malignancy, apparently carcinoma with high parameters of aggressiveness. As a form of treatment and to obtain a definitive diagnosis, was decided to surgically removed the mass which measured 7.8x3.7x3.5cm.

Material and Methods: The sample was fixed in 10 % buffered formalin solution for histological evaluation and sent to the Anatomic Pathology Laboratory of the Agrarian Superior School of Viseu, in Portugal, for histological evaluation.

Results: Microscopic examination revealed that we were in presence of a lymphoid organ, with peculiar features, namely a small to intermediate-sized lymphoid cells, "starry-sky" pattern and rounded eosinophilic perivascular arrangements resembling Hassall's corpuscles. Concomitantly, typical features of squamous cell carcinoma were evident.

Discussion and Conclusion: In conclusion, based on its histological features, the authors suggested the diagnosis of Thymic Squamous Cell Carcinoma with an unusual location. Thymic tumours in dogs are predominantly located in the anterior mediastinum but they may extend from the neck to the posterior mediastinum.

C17 - ABDOMINAL MASS IN AN ASSUMED NEUTERED DOG

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Clinical case: A Labrador dog was present for necropsy following euthanasia after 15 days of weight loss, anorexia, prostration and reluctance in movement. The anamnesis indicated that the dog had been neutered before adoption. At clinical examination an abdominal swelling, anaemia and thrombocytopenia were found. Abdominal ultrasonography showed a heteroechoic mass in medial abdomen, and the prostate increased in size and heteroechoic in texture. Ascites was also present. Echo-guided cytology of the mass was found poor at fusiform cells, and considered inconclusive.

At *necropsy*, mild ginecomasty and increased skin pigmentation was observed, in parallel with absence of scrotal content. The incision of the abdomen showed haemoperitoneum and a large mass with 10x12x9cm caudal to the left kidney. The mass was composed of soft white tissues, with gelatine-like texture. It presented anatomical continuity with a cord-like structure running near the bladder; a contra-lateral similar structure was found extending into an atrophic gonad. No other lesions were perceived in abdominal organs other than the spleen showing multiple haemorrhagic nodules, and the increase-sized prostate. Histopathology demonstrated the mass as being a testis, with neoplastic proliferation of Sertoli cells (Sertolinoma) and increased amount of fibrous and myxoid tissues. The atrophic, contralateral testis showed dysgenesis and marked hialinization of the tubules, and foci of intraductal seminoma. Fibrous hyperplasia and squamous metaplasia of the prostate was found.

The misleading information provided to the owners at adoption allowed the animal to develop testicular neoplasia in a situation of bilateral cryptorchidism, which could be prevented by orchietomy at young age. In association to gonadal retention, Sertolinoma developed undiagnosed until paraneoplastic clinical signs were present.

C18 - CYSTIC LESION IN THE PHARYNX OF A YOUNG BULLDOG

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Introduction: Structures occupying space in the pharynx of small dogs or in dogs from breeds with brachiocephalic skull are difficult problems to deal with, in most cases arising the suspicion of neoplasia. Rx and fine needle aspiration cytology are good diagnostic tools, although not always successful in achieving a conclusion.

Clinical case: “Spoon” is a five years old French Bulldog with a clinical history of epilepsy controlled with phenobarbital. In December 2010, Spoon showed abnormal sneezing and cough. In early April, a space occupying structure was detected in the pharynx, close to the right tonsil, with three centimetres in diameter. Cytology revealed macrophages and non degenerated neutrophils, surrounding bluish amorphous material. The pharyngeal mass was surgically removed in late April and the material processed for routine histopathological analysis.

Histopathology showed that a thick wall of dense connective tissue limited a central cavity, with multiple small rounded digitations projecting into the lumen free of content. In areas where the wall is thicker, three small islets of well differentiated bone tissue can be seen. No microbial agents could be identified.

Two weeks after the surgery, Spoon had fully recovered.

Discussion: A non controversial final diagnosis was issued of a cystic chronic pharyngitis. The challenge in this case is the etiology of this peculiar inflammatory lesion.

C19 - CAN ESTROGEN ADMINISTRATION BE A CAUSE OF ACUTE HEMORRHAGES IN DOGS? – A CASE REPORT

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Clinical case: A 3 year-old female Epagneul Breton was submitted to the Faculty of Veterinary Medicine (Córdoba University) to perform the necropsy due to the owner suspects that the animal death came after a treatment. No other clinical signs were included in the clinical history. At necropsy, samples from different organs were collected and fixed in 10% buffered formalin for histopathologic examination.

Results: Macroscopic findings consisted of pale examined mucosa. There were blood clots in the abdominal cavity. The mesentery, pancreas, urinary bladder and uterus presented extensive hemorrhages. Microscopic findings included mild alveolar edema and spleen contraction. Severe diffuse hemorrhages were present in pancreas and urinary bladder. Also, a mild glomerulonephritis was seen in the kidney. The morphologic diagnosis was acute extensive internal hemorrhage and the differential diagnosis comprised traumatism and intoxication with dicumarol, strychnine or estrogens.

Conclusion: The normal dose of estradiol is 0.5 – 1 mg/kg and in this case, the animal received 10 mg/kg. There is evidence that an overdose of estrogens can cause severe hemorrhages because of bone marrow toxicity. Accordingly, the doses of estradiol together with the gross and histopathologic lesions suggest that the dog suffered estradiol intoxication

C20 - HYDRONEPHROSIS DUE TO SUPERNUMERARY OVARY AND UTERUS

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Introduction: Hydronephrosis is a distention and dilation of the renal pelvis, usually caused by obstruction of the free flow of urine from the kidney, leading to progressive kidney atrophy. The obstruction of urine outflow can happen at any site from the renal pelvis to the urethral orifice. Unilateral hydronephrosis may occur without any symptoms.

Material and Methods: A seven-month-old European cat was brought to the clinic for vaccination. Routine physical examination revealed a palpable mass in the cranial abdomen. Severe left hydronephrosis was diagnosed after clinical, radiographical and ultrasonographic examination. There was no evidence of azotemia. Surgery was required in order to reach a final aetiological diagnosis and decide on treatment. Left ureteronephrectomy and ovariohysterectomy were performed. The cat retained kidney function, with no increase in serum urea or creatinine, and no postoperative complications.

Results: At gross examination, longitudinal sectioning of the sample revealed underdeveloped female genital organs near the renal hilum. Histopathological analysis revealed a kidney with a very thin wall and clear atrophy of the cortical region, fibrosis of the ureter and a supernumerary ovary and uterus (adjacent to the renal hilum).

Discussion/Conclusion: Supernumerary ovaries reported in cats are most frequently located on the broad ligament within 1 to 4 cm from the ovary, but not in the location reported here. To the authors' knowledge, this is the first case of double uterus and supernumerary ovary to cause hydronephrosis. Treatment by ureteronephrectomy was successful.

**C21- PATHOLOGIC AND IMMUNOHISTOCHEMICAL FINDINGS
IN RED-LEGGED PARTRIDGES (*Alectoris rufa*)
EXPERIMENTALLY INFECTED WITH TWO MEDITERRANEAN
STRAINS OF WEST NILE VIRUS (WNV)**

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Introduction: West Nile virus (WNV) is an arthropod-borne *Flavivirus* that cycles naturally and silently between birds and mosquitoes, with horses and humans as accidental hosts. In Europe, it has demonstrated pathogenicity in wild raptors. The red-legged partridge is a Mediterranean species of ecologic and economic value that is frequently raised in outdoor operations. An experimental study was set up in order to evaluate the susceptibility of the species to WNV infection and disease, and its role as a reservoir. Here we evaluate differences in tissue distribution, and presence and severity of lesions caused by two different Mediterranean isolates.

Materials and methods: Two groups of fourteen partridges were inoculated, subcutaneously, with 10⁴ UFP/0.1ml DMEM of either of two strains of WNV isolated in Spain (Sp07) and Morocco (Mo03). Tissue samples were collected during necropsies, fixed in 10% neutral-buffered formalin and processed for histopathology and immunohistochemical detection of WNV antigen.

Results: Both groups had gliosis in the brain, pulmonary congestion and inflammation, myocardial necrosis and myocarditis, renal necrosis and nephritis and lymphoid depletion in the spleen and bursa. Necrotic foci related to mixed inflammatory infiltrates in the liver, spleen, intestine, pancreas and kidney were more severe in partridges infected with Mo03. WNV antigen was detected mostly in the heart, kidney and spleen.

Conclusions: The red-legged partridge proved to be susceptible to WNV infection and developed lesions similar to those described in other birds. Higher pathogenicity of Mo03 was reflected by more severe lesions and involvement of the gastrointestinal tract.

C22 - PROGRESSIVE DEMYELINATION, NEURONAL LOSS, AND ASTROGLIOSIS IN CEREBELLUM AND HIPPOCAMPUS OF EXPERIMENTALLY POISONED CATTLE BY INGESTION OF *Solanum bonariense* L.

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Introduction: Progressive demyelination, neuronal loss, and astrocyte reaction were evaluated in tissues from cerebellum and hippocampus of experimentally poisoned cattle by ingestion of *Solanum bonariense*.

Material and methods: Briefly, tissues for the present study included archived formalin-fixed, paraffin-embedded samples from 2 experimentally poisoned Holstein steers with mild or severe cerebellar symptoms, and 1 normal steer as a control. Firstly, samples were sectioned and different techniques were carried out:

Kluver-Barrera and Bielschowsky staining methods were used to evaluate demyelination in cerebellar white matter.

Cerebellar and Hippocampal neuronal loss were evaluated using immunohistochemistry (IHC) against a neurofilament marker (NF-200KDa primary antibody), and reactive astrogliosis of the same regions were performed using an antibody for glial fibrillar acid protein (GFAP), associated with a peroxidase-labelled polymer system and Diaminobenzidine as a chromogen.

Results: Cerebellar white matter have a progressive staining loss to Kluver-Barrera and Bielschowsky from control to most affected steers, suggesting an increasing that confirm axonal degeneration previously reported. These findings fit properly with a decreasing number of NF-200KDa positive cerebellar Purkinje cells and hippocampal neurons observed in our case. IHC using an antibody for GFAP showed reactive astrogliosis, which was most extensive and pronounced at the later time points, showing in the case of hippocampus a most intense immunostaining around blood vessels.

Discussion: According to previous results, we confirm Purkinje cell loss, demyelination and axonal degeneration, and reactive astrogliosis and a decreasing number of NF-200KDa positive cerebellar and hippocampal neurons suggesting that hippocampus could be affected in *S. bonariense* toxicosis in cattle.

C23 - CYTOSKELETAL DERANGEMENT IN CEREBELLAR PURKINJE CELLS OF *Solanum bonariense* L. INTOXICATED BOVINES. AN IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY

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Introduction: *Solanum bonariense* L. intoxication in cattle leads to cerebellar degeneration characterized histologically by Purkinje cell perikaryal vacuolation, axonal swelling and progressive cell death, and ultrastructurally by intravesicular accumulation of electron-dense bodies in the perikarya of affected cells, and axonal accumulation of similar vesicles and mitochondria. Cytoskeletal derangement and subsequent altered cell-specific axonal transport could play a role in the pathogenesis of the disease.

Material and methods: Immunohistochemistry and transmission electron microscopy (TEM) were used to characterize Purkinje cell cytoskeletal alterations. Formalin-fixed cerebellums from 7 natural and experimental cases and 2 control bovines were sectioned and immunostained with a monoclonal antibody to phosphorylated neurofilament protein (SMI-31), a monoclonal anti- β -tubulin antibody, and phalloidin, a high affinity actin marker (Alexa Fluor 647 phalloidin). Epon-embedded glutaraldehyde-fixed samples from the same animals were used to assess the Purkinje cell cytoskeleton by TEM.

Results: Immunoreactivity for SMI-31, β -tubulin and affinity reaction against phalloidin revealed an altered distribution of the three interconnected components of neuronal cytoskeleton in intoxicated cattle. TEM confirmed an abnormal distribution of microtubules and neurofilaments.

Discussion: We demonstrate that there is a deranged distribution of cytoskeletal components (particularly accumulation of phosphorylated neurofilaments and microtubules) in the perikaryon of Purkinje cells of *Solanum bonariense*-intoxicated cattle and postulate that this cytoskeletal alteration is somehow related to the miss-accumulation of membrane-bound cytoplasmic/axonal vesicles seen in these neurons in affected animals. Further investigation is needed to understand where in the pathogenic cascade these cytoskeletal alterations occur or whether they are the primary event leading to the vesicular miss-accumulations or a secondary/downstream event.

C24 - PANCREATIC LESIONS AND METABOLIC AGGRAVATION ARE PREVENTED BY LOW DOSES OF SITAGLIPTIN IN A RAT MODEL OF TYPE 2 DIABETES

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Introduction: Management of type 2 diabetes is aimed at reducing disease-related complications and improving long-term outcomes. Inhibition of dipeptidyl peptidase-4 (DPP-4) activity by sitagliptin has been shown to improve glycaemic control in patients with type 2 diabetes Mellitus (T2DM) by prolonging the actions of incretin hormones, but the real impact of low-dose sitagliptin treatment on cardiometabolic risk factors and pancreatic lesions is almost unknown. This study aimed to evaluate the effects of low doses of sitagliptin on cardiovascular risk factors and histological pancreas parameters in Zucker Diabetic Fatty rats (ZDF (fa/fa), an animal model of T2DM.

Materials and Methods: Twenty-week-old diabetic obese (fa/fa) ZDF male rats were treated with vehicle or sitagliptin (10 mg/kg BW/day) for 6 weeks (n=8 each). The following parameters were assessed: glycaemia, HbA1c, insulin, lipid profile; blood pressure. Pancreas specimens for histopathological examination were stained with haematoxylin-eosin and periodic-acid-Shiff and examined under light microscopy. Endocrine and exocrine pancreas were evaluated semiquantitatively for inflammatory infiltrate, fibrosis, vacuolisation and congestion, and scored from 0 (absent) to 3 (severe and extensive damage).

Results: Sitagliptin in diabetic obese ZDF rats exerted a positive effect on dysglycaemia and dyslipidaemia, and prevented increases in blood pressure. Endocrine and exocrine pancreas displayed a reduction/improvement in fibrosis severity, inflammatory infiltrate, intra-islet vacuolation, and congestion with respect to vehicle-treated diabetic rats.

Conclusion: Simultaneous and sustainable improvement in the glycaemic profile and in pancreatic histopathological lesions supports the favorable cardiovascular risk profile and may prove beneficial in reducing the long-term complications of T2DM.

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C25 - INHIBITION OF AFRICAN SWINE FEVER VIRUS REPLICATION BY QUINOLONES: A POTENTIAL WINDOW FOR ANTIVIRAL CHEMOTHERAPY

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Introduction: African swine fever (ASF) is a major life-threatening disease of pigs, for which no vaccine or treatment is currently available. ASF virus (ASFV), the only known virus infecting mammalian cells, codes for a type II topoisomerase (ASFV-TOPOII) that displays extended sequence homology with bacterial topoisomerase IV and gyrase A. This prompted us to evaluate the potential blockage of viral DNA replication through quinolone treatment.

Materials and Methods: Screening was performed in VERO cells infected with ASFV strain Ba71V (MOI=0,1) and treatment was started with 30 quinolones. The inhibitory effect of these drugs, after 10 days' treatment, was confirmed by PCR analysis (ASFV gene A238L). Pulsed-field gel electrophoresis was carried out to confirm viral DNA fragmentation and the possible viricidal action of quinolones. To determine the best therapeutic window throughout infection, ASFV-TOPOII mRNA levels were measured by qRT-PCR. Treatment cytotoxicity was analyzed through cell viability assays (MTT/TUNEL) and using Western Blot (caspase3/8/9 and PARP-1).

Results: Results showed that combinations of several fluoroquinolones (duplets and triplets of a selected group of six) abrogated ASFV infection in a dose-dependent manner. Moreover, the cytopathic effect induced by ASFV was detected, though less commonly, in experimental groups when treatment was instituted prior to infection.

Conclusion: The promising results for the prophylactic and therapeutic action of quinolones in in vitro ASFV infection open up new perspectives for the use of these drugs as complementary measures in ASF control.

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C26 - DEVELOPMENT OF A VITREOUS FLUOROMETRY PROTOCOL WITH A MODIFIED LASER CONFOCAL SCANNING LASER OPHTHALMOSCOPE IN RABBIT EYES TO BE APPLIED IN DIABETIC RETINOPATHY STUDIES

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Introduction: The increasing incidence of diabetes makes prevention and control of diabetic retinopathy (DR), a microvascular complication of the disease and the most frequent cause of blindness in the western world, a mandatory field of research. Its role as the key factor in the breakdown of the Blood-Retinal Barrier (BRB), which is the baseline for the evolution of DR and the histological vascular changes it promotes, is already established. Vitreous fluorometry quantifies this early breakdown by fluorescein leakage, indicating endothelial lesion. This study aimed to validate a modified ophthalmoscope and establish a protocol for further studies using this animal retinopathy model.

Materials and Methods: New Zealand White rabbits were intravenously (n=12) or intravitreously (n=2) injected with a 10% solution of sodium fluorescein. Fluorescein levels in retinal vessels, retinal avascular areas and vitreous areas, were evaluated with a Modified Confocal Scanning Laser Ophthalmoscope (Zeiss). Axial graphics were analysed and a method was developed calculate the inward (*Pin*) and outward permeability (*Pout*) of the BRB. *Pout* was determined by the half-life of intravitreous fluorescein.

Results: The *Pin* values obtained in vascular and avascular areas of the retina were, 14.30×10^{-5} and 1.03×10^{-5} cm/hour, respectively. *Pout* attained a half-life of 5 hours.

Conclusion: The modified cSLO may be used in further trials, since the results obtained for retinal measurements were comparable to those of previous studies, reflecting a workable baseline for this experimental animal model. Protocol alterations are required for vitreous evaluation.

C27 - COX-2 IMMUNOLocalIZATION IN CANINE ENDOMETRIUM DURING THE OESTROUS CYCLE

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Introduction: Cyclooxygenase (COX) is an enzyme with several isoforms. COX-2 is the inducible form that catalyzes the rate-limiting step of the conversion of arachidonic acid into prostaglandins and other prostanoids. This enzyme has been associated with a number of reproductive events, especially during the implantation period. . This study sought to obtain new information on COX-2 expression throughout the canine oestrous cycle and to report on its localization in particular structures of the endometrium.

Material and methods: Formalin-fixed canine endometrium samples (n=25) were examined immunohistochemically using a streptavidin-biotin-peroxidase technique. The primary antibody (clone SP21, Neomarkers®/LabVision Corporation, Fremont, CA, USA) was used at 1:75. Scoring intensity (weak, moderate or strong) was recorded for each epithelial structure.

Results: Positive staining for COX-2 was observed throughout all stages of the canine oestrous cycle in the endometrial epithelia but not in stroma. Regardless of the cycle stage, surface epithelium (SE) and superficial glandular epithelium (SGE) recorded higher intensity scores than deep glandular epithelium (DGE). Intensity scores increased in early dioestrus, particularly in SGE, with respect to other stages in the cycle; scores decreased slightly in dioestrus. The lowest intensity scores, in all epithelial structures, were recorded from anoestrus to oestrus.

Discussion/Conclusion: COX-2 expression in canine endometrium is mainly due to epithelial production, since positive stromal staining was not detected here; this has already been reported in humans but not hitherto in domestic mammal species.

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C28 - FADD AND DAXX MARKERS TO STUDY APOPTOSIS PATHWAYS IN PORCINE PARAFFIN-EMBEDDED TISSUES

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Introduction: Fas-associated protein with death domain (FADD) is the key adaptor protein transmitting apoptotic signals mediated by the main death receptors (1). Several complex cellular functions have been revealed for death associated protein 6 (DAXX) in diverse pathways ranging from apoptosis to transcriptional regulation (2). The main aim of this study was to determine the ideal fixative and antigen retrieval method in porcine paraffin embedded tissues for the immunohistochemical detection of FADD and DAXX.

Materials and methods: To carry out this study we used 5 week old pigs from a previous experiment (3). At the necropsy samples from tonsil and lymph node were collected and fixed in different fixatives: 10% neutral buffered formalin, Bouin solution and Zinc salts solution. Two primary antibodies were used for the immunohistochemical study: anti-human FADD rabbit polyclonal antibody and anti-mouse DAXX goat polyclonal antibody both from SANTA CRUZ Biotechnology. Different antigen retrieval methods were carried out: no pretreatment, Tween 20 and Citrate microwave. All the samples were evaluated determining the intensity of the positive reaction as well as the background.

Results: The best fixatives and antigen retrieval methods were Zinc salt fixative and no pretreatment for the antibody against FADD, and Bouin solution and antigen retrieval with Tween 20 to determine the expression of DAXX.

The immunolabelled was observed in the cytoplasm (FADD, DAXX) and nuclei (DAXX) from macrophages and lymphocytes from paracortical areas of lymphoid organs. Few fibroblasts were immunolabelled against DAXX.

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C30 - ALPHA AND BETA TUBULIN AS MARKERS FOR ENTERIC NERVOUS SYSTEM IN SIX FARMED TELEOST SPECIES: AN IMMUNOHISTOCHEMICAL STUDY

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Introduction: Alpha and β -tubulin are the major constituent of microtubules, which are assembled from heterodimers of these two globular proteins. Microtubules are an important element in the functional cytoskeleton of neurons and play a prominent role in the maintenance and growth of nerve fibers. The enteric nervous system (ENS) is the only part of the peripheral nervous system that contains extensive neural circuits that are capable of local, autonomous function. In higher vertebrates the immunohistochemical staining with anti α - and β -tubulin antibodies has been shown to be a valuable tool for studying congenital and acquired diseases of ENS, as well as, for ontogenetic studies.

Materials and Methods: We performed and optimized immunohistochemical assays with polyclonal antibodies against α - and β -tubulin in sections from the alimentary canal of six farmed teleost species: *Onchoryncus mykiss* (Walbaum), *Solea senegalensis* (Kaup), *Psetta maxima* (L.), *Sparus aurata* (L.), *Cyprinus carpio* (L.) and *Pollachius pollachius* (L.).

Results: The immunoreaction of both tubulins was observed in the myenteric and submucous ganglionated plexuses in all gut regions as a fine fibrillary pattern in the cytoplasm, not only in neuronal soma, but also in neurites. Moreover, immunoreactive nerve fibres were seen running in smooth muscle, submucosal and mucosal layers, and surrounding the endothelium of blood vessels. In some species it was possible to identify positivity for enteroendocrine cells, stomach epithelium and isolated cells in intestinal epithelium.

Conclusion: Results showed that α - and β -tubulin immunoassays could be useful to investigate the anatomy, ontogeny and pathology of ENS in farmed fishes.

C31 - QUANTITATIVE AND QUALITATIVE EVALUATION OF THE INDUCIBLE ISOFORM OF NOS EXPRESSION IN TURBOT (*Psetta maxima*) INFECTED WITH *Enteromyxum scophthalmi*

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Introduction: *Enteromyxum scophthalmi* is the causative agent of turbot enteromyxosis, an intestinal parasitosis that produces severe desquamative enteritis that leads to a cachectic syndrome and eventually death. It is well known the importance of the innate immune response against parasites in fish, with the production of antimicrobial substances such as reactive oxygen and nitrogen species, which one of its sources is the inducible nitric oxide synthase (iNOS). This enzyme is mainly found in phagocytes, but also in the intestinal mucosa. The aim of this study was to characterize iNOS in intestine and lymphohaematopoietic organs (anterior kidney and spleen) of turbot by means of immunohistochemistry. The presence of the enzyme was evaluated in control and *E. scophthalmi*-infected turbot.

Results: The results showed immunoreactivity in the apical border of enterocytes and mild staining of goblet cells. This staining was more evident and extended in infected turbot compared to control. Moderate numbers of iNOS+ cells were present in the lamina propria-submucosa of turbot with moderate and severe inflammatory infiltrates. In anterior kidney and spleen, iNOS+ cells were scattered through the parenchyma and, in cases of moderate and severe enteromyxosis, tended to be allocated near the vascular structures and melanomacrophage centres. The number of positive cells at the lymphohaematopoietic organs was significantly higher in infected turbot and increased along the infection.

Conclusion: The increase in the expression of iNOS in the tissues of *E. scophthalmi*-infected turbot is more evident in individuals with severer lesions.

This work was funded with the Projects AGL2006-13158-C03 and AGL2009-13282-C02-02 from the Spanish “Ministerio de Ciencia e Innovación”.

C32 - LOCALIZATION OF VACCINE ANTIGEN AND HUMORAL IMMUNE RESPONSE IN TURBOT (*Psetta maxima*) VACCINATED AGAINST FURUNCULOSIS

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Introduction: Furunculosis is one of the most important bacterial diseases affecting cultured turbot. In this study, we have examined the distribution of vaccine antigen (AsaV) and immune response of immunized fish with *Aeromonas salmonicida subsp. salmonicida* bacterins.

Materials and Methods: One group was immunized by intracoelomic injection with non-adjuvanted vaccine, and a second group was injected with oil-adjuvanted vaccine. Fish from each group were euthanized and necropsy was performed. The organs from coelomic cavity were collected, fixed in Bouin's liquid and processed by routine methods. For immunohistochemical methods, polyclonal rabbit antibodies anti-Asa and anti-turbot IgM were used.

Results: Pathologic evaluation showed intracoelomic fibrosis and granulomatous coelomitis only in the oil-adjuvanted group. On the one hand, in the non-adjuvanted group, AsaV was detected in macrophage aggregates in the coelomic cavity and into macrophages of the spleen and kidney. Also, in spleen, the antigen was found within the melanomacrophage centres (MMCs). However, AsaV was only found near the centre of the granulomas in the oil-adjuvant vaccinated group. On the other hand, quantification of IgM+ cells, showed significantly increased number of IgM+ cells in spleen and kidney in fish from non-adjuvanted group respecting oil-adjuvanted group.

Conclusion: Our results suggest that antigens were transported from the injection site to spleen and kidney in the non-adjuvanted group, whereas in the oil-adjuvanted group AsaV was retained at coelomic granulomas. Besides, the MMCs in spleen were the main place for the uptake and retention of AsaV in turbot. Finally, our results suggest that non-adjuvanted vaccine induced a humoral immune response with increase of IgM+ cells in haematopoietic organs.

This work was funded by the Xunta de Galicia project 08MMA011200PR.

C33 - SKELETAL MALFORMATIONS IN EARLY STAGES OF DEVELOPMENT OF SENEGALESE SOLE (*Solea senegalensis*, KAUP 1858) REARED AT DIFFERENT DENSITIES

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Introduction: In the last years, Spanish and Portuguese aquaculture placed a great expectation on Senegalese sole farming. One of the most important constraints in its culture is the incidence of vertebral malformations. This is not only a health problem but it also affects the industry with higher rejection and sacrifice rates, decreasing market value of produced fish. Despite the interest in this species, few studies have been reported about rearing conditions. The objective of this investigation is to study vertebral deformities in larval stages of hatchery-reared Senegalese sole cultured in different production densities.

Material and Methods: Samples were caught and evaluated at 16, 24, 30 and 37 days after hatching (DAH). Double staining for cartilage and bone with Alcian Blue and Alizarin Red was used to detect malformations.

Results: In this investigation, we observed an incidence of 55.6% of individuals that presented at least one vertebral anomaly. Caudal fin complex was the anatomical area with more malformations. In addition, fusions were the higher number of anomalies within type of deformities.

Discussion: Total number of malformations showed no significant differences between production densities although at 16 DAH there were some considerable variations in deformed specimens. However, the high incidence of malformations observed in our study suggests that other problems due to rearing and/or feeding conditions may affect deformity development. Therefore more extended investigation is needed to achieve novel results that can help interpretate this preliminary data.

This work was supported by “Consellería de Economía e Industria” of Xunta de Galicia (10MMA020E), Spain.

C34 - IMMUNOHISTOCHEMICAL ASSAYS WITH COMMERCIAL ANTIGEN-PRESENTING CELL ANTIBODIES IN TURBOT (*Psetta maxima* L.)

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Introduction: Antigen-presenting cells (APCs) process antigens and present them to lymphocytes. Some professional APCs are dendritic cells, found disperse throughout the organism of mammals, and M cells in the specialized epithelium covering the Peyer's patches of the intestine. Although APCs have been well-documented in mammals, the lack of appropriate cell markers in fish turns it difficult to characterize them.

The aim of this study is to standardize immunohistochemical techniques in turbot (*Psetta maxima* L.) tissues to detect antigen-presenting cells employing anti-human CXCL13, annexin V, clusterin α , CD207 and CD21 antibodies.

Material and Methods: Samples of the digestive tract, pancreas, liver, thymus, kidney, gills and skin were fixed in Bouin's fluid for 24 hours and embedded in paraffin.

Results: CXCL13 positivity was seen in the cytoplasm of macrophage-like cells disperse in the parenchyma of various organs. Annexin V showed positive spotted labelling in epithelial cell membranes, in the cytoplasm of mononucleated large cells of skin, gills and rodlet cells in several digestive sections. Clusterin α immunostaining was in goblet and enteric nerve plexus cells. As to CD 207, cytoplasmic reactivity was found in elongated cells of some digestive sections, mononucleated large cells of kidney and mainly of spleen, and stellated cells in epidermis. Presently, no immunoreactivity was achieved with the CD21 antibody in turbot tissues.

Discussion: These results suggest that some turbot cells are analogous to the mammalian APCs in antigenicity, morphology and distribution but further studies will be necessary to establish this functionality.

C35 - PRELIMINARY STUDY OF INJURIES ASSOCIATED WITH MYCOBACTERIOSIS IN HORSE MACKEREL (*Trachurus trachurus*)

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Introduction: The mycobacteriosis is a critical disease that concerns numerous species of fishes, even at sweet or salty water. The principal agents involved are *Mycobacterium marinum*, *Mycobacterium fortuitum* and *Mycobacterium chelonae* that produce a chronic, systemic disease with granulomas in internal organs and tissues; it also deals with immunosuppression quite often, and it can even be fatal in a few cases. The most frequent microscopic injury is the formation of granulomas, which consist of concentric strata of epithelioid cells, necrosis and calcification of the central region, surrounded by a fibrotic and/or leucocytic capsule. In collaboration with the Oceanogràfic of Valencia, it has been developed a study about the prevalence of compatible lesions with mycobacteriosis in 100 horse mackerels (*Trachurus trachurus*). Hence, the aim of this study was to describe the distribution of lesions caused both in the skin and in internal organs and the histopathologic characterization of the granulomas.

Material and Methods: All the fishes included in this study showed lesions consistent with mycobacteriosis in several internal organs, with presence of acid-fast bacilli in the center of granulomas.

Results: Firstly, the most affected organs were the spleen and kidney (97% of cases), followed by the heart (70%), digestive system (66%), liver (63%) and gills (27%). Granulomas were classified into four types: cellular granulomas, cellular granulomas with necrosis, granulomas with melanomacrophages and laminar granulomas. Every single fish studied had granulomas of all types.

Discussion: In less affected specimens predominated cellular granulomas; however, in the more affected fishes the spleen and kidneys were very injured, with more than 30-40 granulomas per field (100x), with a predominance of laminar granulomas type.

C36 - DESCRIPTION OF A LYMPHOMA OUTBREAK IN CULTURED TENCH (*Tinca tinca*)

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Samples of fresh and fixed tissues of adult tench *Tinca tinca* (L.) from several culture facilities in Spain showing large whitish nodules in the skin were submitted for evaluation. These lesions were reported since 2001, generally associated to low or negligible mortalities. The incidence of these skin lesions was higher during Spring-Summer, when temperatures increased and also when tench acquired sexual maturity. Spontaneous regression of these lesions was also observed when temperature decreased. Nodules showed different sizes and shapes could be found in different localizations in the skin and also in fins.

At necropsy, smaller nodules could also be observed in different organs such as liver, kidney, spleen, heart and gonads. On the histological examination of these nodules, large numbers of mononuclear cells that resembled lymphoblasts occurred in the both epidermis and dermis in skin, in the liver surrounding portal spaces, spleen, kidney, gonad, heart and also in the perivisceral fat. Lymphoblast aggregates were usually densely packed or can be found infiltrating the structures of the different organs but without causing apparent damage. Mitotic figures were scattered throughout infiltrative masses although they were not abundant. By means of TEM these cells showed rounded or irregular, sometimes deeply clefted nucleus with inconspicuous nucleoli. The thin rim of cytoplasm contained a large amount of ribosomes, few large mitochondria and dense-core granules.

Morphological and ultrastructural characteristics of the lesions suggest a lymphoblastic lymphoma diagnostic. Taking into account the observed epidemiological evidences it is reasonably to suspect a possible viral aetiology, although no viral particles have been identified by means of TEM.

C37 - IMPLEMENTATION OF THE TEACHING-LEARNING SYSTEM BASED ON PROBLEM SOLVING AND ENGLISH USE IN THE CORE SUBJECT ANATOMÍA PATOLÓGICA GENERAL IN VETERINARY

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Introduction: The aim of this educational innovation project was to progressively introduce the method of teaching and learning based on evidence and problem solving (PBL) in the core subject *Anatomía Patológica General*.

Training Method: The experience was carried out during the academic years 2009-10 and 2010-11. The experience has focused on developing a “Images-case-problem work” in groups of 2-3 students. These students received a powerpoint presentation with 5-6 macroscopic and microscopic images from unpublished lesions through the Virtual Platform of the subject; at the same time, the literature to be consulted for the resolution of the work was also provided. Specific questions related to the following items: precise identification of normal histological structures, description of lesions (application of recently acquired concepts together with the terminology and vocabulary previously provided in theoretical and practical lessons), morphological diagnosis, differential diagnosis and, finally, the elaboration of the pathogenesis for each case. Finally, works were presented by the student and discussed in practical joint sessions (3 hours were employed for 7 working groups). Teachers-tutors monitored working groups during one month, the span of time devoted to the experience. The work was written and presented in English.

Results and Conclusions: The results of this experience have shown that the project promotes teamwork, is perceived by students as a challenge and improves student’s bibliographical management for the solution of real problems that they will find in their near future as graduates as well as their command of English language in a professional context.

Posters

P1 - FELINE'S NEOPLASIA DISTRIBUTION STUDY IN DNATECH (2008-2010)

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Introduction: A veterinary cancer registry is lacking in the Portuguese veterinary community, in spite of its recognizable value in the evaluation of cancer prevalence in both animals and humans, assessment of changes in cancer occurrence over time and in the identification of risk factors in carcinogenesis.

Material and Methods: The authors would like to contribute to that goal by presenting a study of feline's neoplastic lesions distribution during a three year period (2008-2010) from material received in the Laboratory of Histopathology of Dnatech.

Results: Out of a total of 992 cases received from feline patients: 707 (71%) were neoplastic lesions. From these, 286 (40,5%) were mammary gland tumors; 224 (31,7%) mesenchymal tumors of skin and soft tissue tumors; 13,2% (n=93) epithelial and melanocytic tumors of the skin; 7,4 (n= 52) tumors of the alimentary system; 5,7% (n=40) hematopoietic tumors; 2,3% (n=16) ocular and optic tumors; 0,7% (n=5) urinary system; 0,6% (n=4) respiratory system and finally, 1,7% were classified to other systems (bone and joint, endocrine and nervous system).

Within mammary gland tumors, 69 % were malignant, followed by 20% classified as mammary hyperplasias/dysplasias and 11% classified as benign. In terms of mesenchymal tumors, 79 % were malignant and 67% were addressed as tumors of fibrous tissue. Within epithelial tumors, 60% were malignant and the most common tumor was the squamous cell carcinoma with 48% of the cases.

Discussion: Neoplasia was most prominent in females (66%). and the age group from 9 to 11 years old was the most affected with 34% of the tumors observed (tumors were registered between the age of 1 until 20 years old).

P2 - FELINE INFLAMMATORY MAMMARY CARCINOMA: A CASE REPORT

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Introduction: Inflammatory mammary cancer (IMC) is a rare and aggressive type of mammary cancer associated with aggressive behaviour in women, and in the dog. The diagnosis of this disease depends on a combination of pathological confirmation of invasive carcinoma and a set of clinical criteria including diffuse erythema, oedema, tenderness, and rapid enlargement of the of the mammary gland, often without an underlying mass. It is considered the most malignant type of breast carcinoma with a fulminant clinical course and extremely poor survival rate. The dog was considered the only natural model in which to study inflammatory breast cancer until 2004 when Pena *et al* described the first three cases in the queen.

Case report: we report the clinicopathological findings of a case of feline inflammatory mammary carcinoma in a 10 year old domestic shorthair queen. It was present to the clinician with rapidly growing lesions affecting inguinal and abdominal mammary glands; the overlying skin was thickening, edematous and presented multiple small ulcerated nodules.

Results and discussion: The animal was euthanized. The necropsy examination revealed extensive invasion of subcutaneous tissue and muscles and lung metastasis. Histologically it was an invasive tubulopapillary carcinoma with extensive necrosis and lymphovascular invasion.

Conclusions: Based on the clinical history, macro and microscopic findings the diagnosis of inflammatory carcinoma was established.

P3 - CANINE MESOTHELIOMA WITH CHONDRO-OSSEOUS METAPLASIA

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Introduction: Mesotheliomas are rare tumours originating in the mesothelial lining of coelomic cavities. Most canine mesotheliomas develop in old animals and arise from the pleura or pericardium, disseminating and causing persistent pleural effusion. Invasive and metastatic behaviour are considered to be two hallmarks of malignant mesotheliomas. Depending on cellular morphology and arrangement, these tumours may be classified as predominantly epithelioid, fibrous (spindle cell) or biphasic (mixed). Mitotic index is usually low even in metastatic tumours. Areas of necrosis may suffer dystrophic mineralisation. The present paper reports on a case of malignant mesothelioma with chondro-osseous metaplasia of the supporting stroma.

Clinical Case, Results and Discussion: “Bora” a 12-year-old male Golden Retriever, was presented for clinical examination due to respiratory distress secondary to persistent pleural effusion. Hematology confirmed anaemia and leukocytosis. Radiology revealed an intrathoracic mass; cytological findings were consistent with mesothelioma. Medical treatment resulted in temporary recovery, but euthanasia was eventually decided upon, due to recurrence of clinical symptoms and evidence of multiple organ failure. The cadaver was sent for necropsy, which revealed the presence of plaques of mesothelial neoplastic tissue in pleura and lung metastases. Histologically, neoplastic tissue was associated with extensive areas of chondro-osseous metaplasia of the supporting stroma. A diagnosis of malignant mesothelioma triggering stromal chondro-osseous metaplasia was made. The authors have found no other report of malignant canine mesothelioma associated with metaplastic bone and cartilage formation, suggesting that this may be the first reported case.

P4 - PROGNOSTIC VALUE OF MACROPHAGES INFILTRATION IN CANINE MAMMARY TUMOURS

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Introduction: Tumour-Associated Macrophages (TAMs) have already been associated in human breast cancer (HBC) to a poor prognosis, due to their involvement in angiogenesis as well as in other cancer hallmarks, through the production of several chemokines. As a part of a tumoral microenvironment, TAMs have an important contribution influencing neoplastic progression. To our knowledge, so far, in canine mammary tumors (CMT) the prognostic value of TAMs has not been reported.

Materials and Methods: In the present study MAC387 immunohistochemical expression was evaluated in 59 CMT (20 benign and 39 malignant). Statistical analysis of variance was performed to confirm TAMs differences between benign and malignant CMT. Among malignant CMT, Pearson Chi-Square test was used to verify associations with clinicopathological variables and Kaplan-Meier survival analysis performed to explore TAMs prognostic value.

Results: There are significant differences in the number of TAMs between benign and malignant CMT ($p=0,011$). In malignant CMT, TAMs were associated with cutaneous ulceration ($p=0,022$), histological type ($p=0,027$), lymph node metastasis ($p=0,029$), nuclear grade ($p=0,031$) and clinical stage ($p=0,043$). A Kaplan-Meier analysis revealed a very significant association between tumors with higher levels of TAMs and the decrease in overall survival ($p=0,03$).

Conclusion: In this study, TAMs have proven to have a prognostic value, as they are significantly increased in animals with reduced survival times and related to clinicopathological features of tumoral aggressiveness. These findings are in agreement with results of similar studies performed in HBC and suggest the possibility of using TAMs as a novel therapeutic target in CMT.

P5 - INTRACRANIAL NEOPLASMS IN DOGS: A COLLECTION OF 183 CASES

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Introduction: Nervous tumours in domestic animals are infrequent. Most have been diagnosed in dogs (60-80%) and cats (10-20%). Primary intracranial tumours in dogs have a higher incidence (14.5 per 100,000 dogs) than in humans (4-5 tumours per 100,000 humans). The incidence in brachiocephalic breeds is 23 times higher.

Material and methods: We performed a retrospective study of brain tumours in dogs, diagnosed by the Department of Diagnostic Veterinary Pathology of the Universitat Autònoma de Barcelona, between 1999 and 2010.

Results: In total, 183 cases were collected. 138 of the cases reported (76%) corresponded to primary neoplasms and 45 cases (24%) corresponded to secondary neoplasms. As for primary neoplasms, 64 glial tumours, 2 primitive neuroectodermal tumours, 65 meningiomas and 3 meningoangiomatosis were diagnosed. The primary non-neural diagnosed tumours were 1 granular cell tumour, 1 germ cell tumour, 1 primary lymphoma and 1 chromophobe pituitary adenoma. As for secondary neoplasms, 23 carcinomas, 9 sarcomas, 7 lymphomas, 3 hemangiosarcomas, 1 infiltrative leukemia, 1 melanoma and 1 mastocytoma were diagnosed. Boxers (49 cases), cross-breeds (37 cases) and German shepherds (21 cases) were the most represented breeds. The age of the affected animals varied between 1.5 and 15 years; the sex distribution was 110 cases in males (60%) and 73 cases in females (40%).

P6 - STUDY OF THE PRESENCE OF INTRATUMORAL NEUTROPHILS IN FELINE MAMMARY CARCINOMAS

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Several studies have demonstrated the presence of neutrophils associated to breast, colon, kidney and lung neoplasias. These inflammatory cells release diverse substances such as proteinases with the capacity to modify the tumoral stroma and to favour the growth and invasiveness of neoplasia.

During the study of several feline simple mammary carcinomas, we observed the presence of numerous neutrophils associated mainly to the epithelial neoplastic cells with a high grade of anaplasia in a higher number of metastatic carcinomas than in carcinomas that did not have metastasis. This finding reinforces the theory of the recruitment of neutrophils by some carcinomas to enhance their invasion. The neutrophils were localized inside the ducts, in the tumoral stroma and within epithelial neoplastic cells, in such way that it resembles a neutrophil phagocytic process by these cells.

P7 - FELINE MAMMARY GLAND COMPLEX CARCINOMAS

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Introduction: Complex carcinomas are rare in cats and the presence of myoepithelial cells is apparently associated to better prognosis. It has been reported that in feline mammary carcinomas the presence of myoepithelial cells was not always clearly detected in neoplastic lesions, with most carcinomas being incorrectly classified as solid or tubular. The two cell components, epithelial and myoepithelial, were not always clearly observed in all neoplastic areas with haematoxylin and eosin (H&E) stain. However, the biphasic epithelial-myoepithelial pattern was highlighted by immunohistochemistry, with a epithelial:myoepithelial cell ratio of 1:1.

Materials and Methods: This study of P-cadherin immunoexpression in feline mammary gland included samples of normal ($n=4$), hyperplastic ($n=12$), benign ($n=6$) and malignant ($n=39$) feline mammary tissues. The p63 protein antibody was used as a marker for the myoepithelial component of the mammary gland.

Results: From the 39 feline malignant mammary tumours studied 18 were classified as tubulopapillary carcinomas, 20 as solid carcinomas and 1 as cribriform carcinoma with H&E stain. However when immunostaining for p63 protein antibody was performed it was observed that 3 tumours classified as solid carcinomas showed a cell population positive to p63. This cell component was not clearly detected in H&E routine staining.

Discussion and Conclusion: The immunohistochemical detection of myoepithelial cells appears to be an important tool for accurate classification of feline carcinomas, and therefore a correct prognosis. In conclusion, we can assume that our findings corroborate the result of other authors in respect to complex carcinomas erroneously classified as simple-type carcinoma.

P8 - IMMUNOHISTOCHEMICAL EXPRESSION OF COX-2, Ki-67 AND p53 IN A SQUAMOUS CELL CARCINOMA OF A HORSE

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Introduction: A 12-year-old Hispano-Luso bullfighting male horse was referred to our Hospital with left facial swelling, unilateral nasal discharge and a soft tissue mass (15 x 15 cm) protruding below the left cantus of the left eye.

Results: Sinus radiographs revealed a soft tissue opacity in the left frontomaxillary sinuses and nasal meatus deviation. The mass was excised through a routine left frontal bone flap performed under general anaesthesia. Histopathological examination revealed islands and cords of neoplastic cells showing a variable degree of squamous differentiation with keratin pearls formation consistent with a squamous cell carcinoma (SCC). Due to this type of neoplasm, a non steroidal anti-inflammatory drug treatment were given. At 10 months, the horse began to show mild signs of respiratory dyspnoea, unilateral nasal discharge and slight facial deformity over the left frontal bone. The endoscopy revealed infected necrotic tissue and a cauliflower-like soft tissue mass, protruding through the opening of the left frontal sinus. Histopathological evaluation of the mass was consistent with a recurrence of the SCC.

COX-2, Ki-67 and p53 indexes are used as prognostic factors of squamous cell carcinoma tumours. Immunohistochemical evaluation of COX-2, Ki-67 and p53 were performed in both biopsies.

Conclusion: When comparing the immunohistochemical index of COX-2, Ki-67 and p53 before and after treatment, we observed that the expressions of these proteins within the tissue were reduced after treatment.

P9 - UNILATERAL NEPHROBLASTOMA WITH PULMONARY METASTASES IN AN ADULT CAT

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Introduction: Nephroblastoma is a tumor which arises from the metanephric blastema during nephrogenesis or postnatally from nephrogenic rests. It consists of glomerular buds, tubules and mesenchymal tissue. It usually affects fetuses or young animals of all species, being more frequent in males.

Results: We report a case of a 17-years-old male Angora cat. Necropsy findings included a lobulated, firm, 3x2 cm, poorly demarcated lesion in the cranial pole of the left kidney. A 2 cm pearly, well defined and not encapsulated nodule was found in lungs. Histopathologically, there was a neoplastic proliferation of embryonic tissues with a mixture of epithelial and mesenchymal origin located in the renal cortex. The epithelial cells formed tubules and organized acini of several sizes. These cells yielded an intense immunohistochemical positivity for cytokeratin. Mesenchymal stromal cells showed a disorganized and loose sheet proliferation. Blastema mesenchymal cells layed in dense aggregates of elongated or polygonal undifferentiated cells. Both mesenchymal cell types were strongly positive for vimentin. The pulmonary nodular lesion displayed the same characteristics of the renal neoplasm.

Conclusion: Nephroblastoma is a tumor that usually occurs in fetuses or young animals derived from the malignant transformation of embryonic tissue during nephrogenesis. To the author's knowledge this is the first report of a nephroblastoma in an adult cat (17 years old).

P 10 - EPITHELIOID VARIANT OF HEMANGIO-SARCOMA IN A DOG: HISTOLOGIC, AND IMMUNO-HISTOCHEMICAL CORRELATIONS

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Introduction: This study aims to report a case of hemangiosarcoma, with presentation in heart, lung, liver and spleen.

Materiales and Methods: A female Cocker Spaniel 14 years old was referred to the Veterinary Hospital of the University of Extremadura to be clinically evaluated because it suffers a rapid deterioration with chronic cough, anorexia and cachexia. The animal underwent a physical examination and abdominal ultrasonographic examination. Cytology aspirate samples were obtained by ultrasound guidance from the heart, lung, liver and spleen mass. Blood and biochemistry analyzes were also carried out. The animal died a week later and it necropsies. Samples of, liver, lung and spleen were collected for histopathological examination.

Results: Cytologic evaluation of biopsy of liver and spleen of anaplastic cells clustered revealed that lack of convincing differentiation tissue. Necropsy examination revealed splenomegaly and multiple, beige to dark red nodules that ranged from 0.5 to 3 cm in diameter in the heart, lung, liver and spleen. In histopathological examination, multiple nest of anaplastic epithelioid cells were found in sections of all affected organs. Immunohistochemical revealed widespread expression of CD31 and Factor VIII-related antigen.

Conclusions: The diagnosis was epithelioid hemangiosarcoma located in the miocardio, lung, liver and spleen.

P11 - CLINICAL AND ANATOMOPATHOLOGICAL FEATURES OF LARYNGEAL AND TRACHEAL CARTILAGE NEOPLASIA IN THE DOG (1995-2010)

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Introduction: Cartilage neoplasia of larynx and trachea is very rare in domestic animals. The present study describes the clinical and anatomopathologic features of neoplasia arise from the laryngeal and the tracheal cartilaginous component diagnosed at our institution between 1995 and 2010.

Results: Ten cases were included in the study: 3/10 were laryngeal tumors and 7/10 were tracheal tumors. All laryngeal neoplasia (3/3) showed benign characteristics (chondromas). Tracheal tumors presented an extraluminal growth towards cervical soft tissues (3/7) or intraluminal development (4/7). The former included benign forms (osteochondroma, 1/3) and malignant forms (chondrosarcomas, 2/3). Similarly, intraluminal tumors were benign (osteochondroma, 3/4) or malignant (chondrosarcoma, 1/4).

Affected breeds were numerous and no age predilection was noted. Eight/10 were male dogs. Most common clinical signs were dyspnea, respiratory distress, sporadic or persistent cough, cyanosis and/or syncope. Two/10 animals were asymptomatic.

Benign forms (7/10) consisted of solid, nodular o lobulated, unencapsulated, masses composed of lobules of isomorphic and isochromic hyaline cartilage with variable amounts of osseous tissue. Malignant variants (3/10) were formed by multiple, irregularly-shaped nodules of soft-viscous to moderately firm tissue composed of hyaline cartilage and basophilic myxoid or mucinous matrix.

Surgical resection was made in 9/10 cases. One animal was euthanized. All benign tumors showed a favorable follow-up. Only 1/3 malignant tumors showed local recurrence after a three-month follow-up period. Metastases were not reported.

Conclusion: Cartilage neoplasia arising in larynx and trachea in the dog appears as low-aggressive and good-prognosis following complete surgical resection.

P12 - DISSEMINATED WELL-DIFFERENTIATED LIPOSARCOMA IN A DOG

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We describe a well-differentiated liposarcoma in a 10 years old, male, crossbred dog. Clinical exploration revealed caquexia and a wide, hard mass that extended to the entire ventral abdominal wall. The dog presented ascitis and pleural effusion. A thoracoscopy and laparotomy exploration showed white nodules on parietal pleura and a subcutaneous mass of 20 x 10 x 5 cm that included both rectus abdominal muscles. Moreover, several white nodules were random distributed within the abdomen. The dog was euthanized and the postmortem examination confirmed that the white mass was replacing the rectus abdominal muscles and extended cranially into the ventral thoracic wall. Caudally, the mass went on the bladder wall and prostate. Disseminated white nodules or spots of different sizes were distributed by parietal pleura and pericardium, diaphragm, external layer of stomach and intestine, and epiploon.

A well-differentiated liposarcoma in soft tissue with metastasis in mesenteric lymph node was diagnosed. On histopathological examination the characteristics of masses were variable. The neoplasm of the thoracic and abdominal wall corresponded to a mature fibrotic and adipocytic tissue. Others small masses were composed of atypical stromal cells, in a slightly fibrillary collagenous stroma background, admixed with lipocytes of a significant size variation and mature lipocytes.

In conclusion, we present a well-differentiated liposarcoma in ventral abdominal wall, disseminated by three different ways: direct invasion of abdominal muscles and ventral thoracic wall; implantation into body cavities, as the nodules in pleura, epiploon, stomach, etc; and lymphatic dissemination as in the mesenteric lymph node.

This study was funded by F.E.D.E.R. and JUNTA DE EXTREMADURA

P13 - EVALUATION OF HER2 PROTEIN EXPRESSION IN FELINE MAMMARY CARCINOMAS (FMCS) - COMPARISON AND OPTIMIZATION OF TWO DIFFERENT ANTIBODIES

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Introduction: In mammals, HER2/neu proto-oncogene encodes a transmembrane glycoprotein that plays an important role in cell cycle regulation and differentiation. HER2 overexpression is present in 25% to 40% of all women breast cancer cases and its evaluation is considered to be an important prognostic marker and an essential tool for therapeutic decisions. Despite this, no indication concerning the best method to evaluate fHER2 protein status in FMCs, is clearly defined for Veterinary Medicine.

Materials and Methods: In order to evaluate the impact of different immunohistochemical protocols in the assessment of HER2 expression two different antigen retrieval methods were used for each anti-human HER2 primary antibodies tested: CB11 monoclonal antibody, from Zytomed, and TAB250 monoclonal antibody, from Zymed. Since fixation time and tissue processing method can influence the immunodetection of HER2, the American Society of Clinical Oncology guidelines were used as standard procedures for 30 FMC samples.

Results: Best results were obtained conjugating longer antigen retrieval time with CB11 antibody: 60% were scored as 0 (negative), 33,3% showed HER2 weak or incomplete membrane labeling (+) and 6,6% FMCs were classified as HER2 weakly positive (++) . Positive samples corresponded to highly malignant tumors. Mild membrane labeling was frequently observed in dermal adjoined cells. Positive and negative human controls labeled as expected, in all evaluations.

Conclusion: The present study supports similar incidence of HER2 overexpression in FMCs to a recent publication (Rasotto et al., 2010), but markedly lower than in many reports demanding for a continuity of exploratory work.

P14 - PRIMARY CARDIAC LYMPHOMA IN A GERMAN SHEPHERD DOG

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Primary cardiac neoplasms in domestic animals are rare and primary cardiac lymphoma is an infrequent type.

We report a case of a 5 year-old male German shepherd dog that suffered subaortic stenosis, third degree atrioventricular block and renal failure.

Complete necropsy was performed and at gross examination there was subcutaneous oedema in the hind limbs. In the heart, at opening the left ventricle, there were mural thrombi in the subaortic region. These thrombi were associated with a whitish firm mass in the inter-ventricular septum, immediately ventral to the non-coronary cusp of the aortic valve. The mass slightly protruded into the lumen of the left ventricle and at cutting it was a solid well-circumscribed lesion. At opening the abdominal aorta, a thrombus was found cranial to the iliac artery bifurcation occluding the vascular lumen. In both kidneys, multiple nodules were noted on the surface that deepened to the parenchyma. At cutting haemorrhagic pyramidal lesions corresponding to infarction were observed.

At microscopic examination, the myocardium was severely infiltrated by lymphoblasts. In the kidneys, the nodules also corresponded with neoplastic lymphocyte infiltration.

Immunohistochemical assays with the antibodies anti-CD3, CD79, MAC387 and lysozyme were performed. Neoplastic cells showed immunoreactivity for CD3, in heart as well as in kidney, and they were negative for the rest of antibodies. The single mass located in the heart was considered the primary tumour and the renal nodules were metastases.

P15 - PREFERENTIAL EXPRESSION OF RECEPTOR ACTIVATOR OF NFκB (RANK) IN MYOEPIHELIAL CELLS OF COMPLEX AND MIXED TUMORS OF THE MAMMARY GLAND OF THE DOG

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Introduction: Receptor activator of NFκB (RANK) is a key regulator of bone metabolism expressed in bone cells and tumours. However, RANK expression is not restricted to these locations and has been also observed in several normal tissues, tumour cell lines and solid tumours.

Material and methods: Formalin-fixed, paraffin embedded tissue samples of 6 complex adenomas, 3 mixed benign tumours and 1 fibroadenoma were stained with the ABC immunohistochemical method using a commercially available anti-RANK polyclonal antibody diluted 1:50. Osteoclasts and tissue macrophages served as internal positive controls and non-immune rabbit serum diluted as the primary as negative control. RANK expression was evaluated semiquantitatively and expressed as percentage of the corresponding cell type.

Results: Immunoreactive products to RANK antibody were observed in the cytoplasm of osteoclasts and tissue macrophages as well as of different cell types in all tumours analysed. Five to 10% of luminal-type epithelial cells expressed RANK in complex and mixed tumours. By contrast, 30% to 90% of myoepithelial (ME) cells expressed RANK in 11 out of 12 tumours studied. RANK expression was found in fusiform, hypertrophied and star-shaped ME cells. In addition, ME cells-extracellular matrix was stained in more than half of the tumours. Isolated stromal fusiform cells and chondrocytes expressed RANK as well.

Conclusion: RANK was preferentially expressed in ME cells of complex and mixed benign tumours of the mammary gland of the dog. Its suggested role in tumour progression should be investigated further on their malignant counterparts.

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**P16 - MAST CELLS IN CANINE MAMMARY TUMORS:
DIAGNOSTIC ISSUES**

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Introduction: The presence of abundant mast cells in the stroma of some canine mammary tumors and dysplasias was studied to investigate their possible relation with mast cell tumors and its use as a diagnostic tool.

Materials and Methods: Five female dogs with mammary tumors or dysplasias and numerous mast cells in the stroma of mammary lesions were selected. The animals were presented at the University of Zaragoza (cases 1, 2) and at the Complutense Veterinary Hospital (Madrid, cases 3-5). All tumors were pathologically revised and clinical files and follow-up, recorded.

Results: Case 1: Mammary adenoma that showed abundant mast cells mixed with other inflammatory cells in the stroma. Cases 2 and 3: Prominent mastocytosis affecting benign and malignant mammary tumors. Case 4: Low grade mastocytoma in a mammary complex adenoma and hyperplasia. Case 5: Massive embolization of mast cells in the lymphatics of all mammary nodules, including an adenosquamous metastatic mammary carcinoma.

Discussion: The presence of abundant stromal mast cells, mastocytosis and mastocytomas in the mammary samples is an unusual finding that was related to the presence of skin mast cell tumors in 3 out of 5 cases. The possibility of primary mast cell tumors in the mammary tumors analyzed will be discussed. In some cases, mastocytosis of the mammary gland samples could be indicative of an undetected mast cell tumor.

P17 - MATRIX METALLOPROTEINASE-2 AND 9 EXPRESSION IN CANINE MELANOCYTIC TUMOURS

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Introduction: The gelatinases matrix metalloproteinase (MMP) -2 and -9 are reported to play a major role in human melanoma invasion and progression. No research has addressed this issue in dogs. This study sought to investigate the expression of MMP-2 and MMP-9 in canine melanocytic tumours in order to clarify their contribution to this highly-aggressive disease.

Material and Methods: Seventy-two tissue samples from canine melanocytic tumours (12 melanocytomas and 60 melanomas) were stained immunohistochemically for MMP-2 and MMP-9, using polyclonal antibodies (Neomarkers®). Positivity was assessed semi-quantitatively, considering only tumour cells. A tumour was defined as overexpressing MMP-2 and MMP-9 if over 20% of malignant cells were positive. Statistical analysis was performed to determine differences in expression between benign and malignant tumours.

Results: Positivity for both MMP-2 and MMP-9 was observed in almost all tumours. Overexpression of MMP-2 (>20% of neoplastic cells positive) was found in 100% of melanocytomas and 66.7% of melanomas. Overexpression of MMP-9 was observed in 16.7% of melanocytomas and 50% of melanomas. MMP-2 expression was significantly higher in benign tumours, while MMP-9 was significantly overexpressed mainly in malignant tumours.

Conclusion: Most melanocytic tumours expressed MMP-2 and MMP-9. The results suggest that MMP-2 and MMP-9 may be implicated in different ways in canine melanoma progression. Further research is required to identify the pathways involved.

P18 - ENZOOTIC NASAL ADENOCARCINOMA IN GOATS: A CASE REPORT

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Introduction: Enzootic nasal adenocarcinoma (ENA) is a contagious nasal neoplasm of sheep and goats caused by the Enzootic Nasal Tumor Virus (ENTV), a retrovirus closely related to the Jaagsiekte Sheep Retrovirus Virus (JSRV). This disease can cause serious economic losses mainly due to respiratory distress. As the tumor grows in the nasal cavities a wide variety of symptoms may appear, ranging from a serousmucous nasal discharge to skull-bone deformation.

Material and Methods: This paper reports on two cases of ENA from the same flock of goats, involving moderate to severe deformation of the nasal bone.

Results: Histopathological examination of the tumor mass revealed a carcinoma with a papillary pattern, invading and destroying surrounding bone tissue.

Conclusion: The diagnosis of ENA was confirmed by PCR, which identified ENTV 2 as the causal agent

P19 - THREE PRIMARY TUMOURS IN A CAT: A CASE REPORT

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Introduction: Multiple primary tumours are occasionally reported in veterinary medicine, involving deficient tumour-suppression mechanisms.

Materials and Methods: A 12-year-old female cat with a 5-year history of iris pigmentation and recent-onset unilateral ocular discharge was brought for ophthalmic evaluation. Clinical examination revealed diffuse iris pigmentation with unilateral secondary glaucoma, suggestive of diffuse iris melanoma as well as the presence of a nasal mass responsible for the ocular discharge due to tear-duct compression. Computed tomography (CT) scan revealed a hypodense nasal mass and also a cerebellar mass showing strong homogeneous contrast enhancement. Cytological examination of a fine needle aspiration of the nasal mass enabled diagnosis of nasal sarcomatous neoplasia. No other abnormal signs were detected in blood tests, chest x-rays or abdominal ultrasound scan. Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) serologies were negative. Support treatment was initiated. The cat was euthanized 2 months later for humanitarian reasons due to severe worsening of clinical signs.

Results: Gross and microscopic examination revealed three different tumours occurring simultaneously: a strongly invasive leiomyosarcoma in the nasal region, a fourth ventricle choroid plexus papilloma and a diffuse iris melanoma.

Conclusion: The simultaneous finding of multiple tumours with different anatomical origins may be a clear indication of the ability of the immune system to deal with the cellular and molecular changes leading to carcinogenesis. Examples such as the present one should be studied in greater depth in order to evaluate the mechanisms that may have failed.

P20 - FELINE CUTANEOUS LYMPHANGIOMA: A CASE REPORT

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Introduction: An 8-year-old male Persian cat was referred to us in January 2011 with a cutaneous mass close to the eyelid, presenting grossly as a cyst measuring 6 mm in diameter, with a thickness of 4 mm.

Materials and Methods: Histological examination with hematoxylin-eosin (H&E) staining revealed skin-tissue neoplasm with a clearly-defined increase in the number and size of vessels; there was no epidermis involvement or invasion of associated structures. There was a relative absence of erythrocytes in vascular spaces, with evidence of stromal edema, mucin, and lymphoplasmacytic infiltration. Macrophages with intracytoplasmic foamy vacuoles were also observed.

Immunohistochemistry revealed endothelial neoplastic cells positive for the lymphatic vascular marker DAKO clone D240 (ref. M3619), equivalent to Lymphatic Vessel Endothelial receptor – 1 (LYVE-1) antiserum, at a concentration of 1:10.

Diagnosis: Cutaneous lymphangioma.

Discussion: Lymphangiomas in animals are rare, and the small number of reported cases suggests a predilection for intertriginous areas. The histological features here were suggestive of lymphangioma, but were also consistent with a bloodless cavernous hemangioma or lymphangiomatosis. The diagnosis was based on the immunohistochemistry result and on histological features including the absence of erythrocytes in vessels and the observation of lymphoplasmacytic infiltration, these features being rare in hemangiomas. Macrophages with intracytoplasmic foamy vacuoles suggested lymph phagocytosis processes consistent with lymphangioma. Lymphangiomatosis occurs more frequently in young animals and masses are bigger (up to 18 cm in diameter) and commonly ulcerated.

P21 - LOMBAR TRANVERSE-PROCESS OSTEO-SARCOMA

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Introduction: Osteosarcoma (OSA) is the most common primary bone tumour in dogs. Between 20 and 25% of canine OSAs arise from bones of the axial skeleton, and these tumours are also the most common extradural spinal neoplasm (46%). The radiographic appearance is nonspecific and inconsistent. Pain without neurological signs is the predominant initial finding with vertebral OSA.

Materials and Methods: An 8-year-old cross-bred male dog was brought to the surgery with progressive weight loss, apathy and nonspecific pain. A paralumbar mass on the left side was diagnosed after clinical and radiographical examination. Cytology of the mass was performed and the animal was hospitalized for pain control and nutritional support until definitive diagnosis. During hospitalization, the clinical status of the animal deteriorated, and neurological abnormalities emerged. Cytology examination revealed the presence of cells characteristic of a neoplastic process of mesenchymal origin, apparently sarcoma. A myelogram revealed spinal cord compression at L4, caused by the mass. Due to the deterioration of the dog's clinical state and in view of the diagnosis, the owners opted for euthanasia.

Results: At necropsy, thickening of the transverse process of vertebra L4 was observed, due to a mass measuring 6.3 x 7.0 x 4.5 cm. Histopathological examination revealed an OSA with distinct histological characteristics.

Discussion and Conclusion: An unsuccessful-outcome OSA was diagnosed. Adequate treatment of local disease in vertebral OSA is very difficult. Surgery is aimed at decompression in dogs with neurologic deficits or intractable pain. Present recommendations are to perform surgery for decompression and institute radiation and chemotherapy.

P22 - EVALUATION OF C-KIT EXPRESSION IN CANINE MELANOCYTIC TUMORS

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Introduction: The tyrosine kinase c-Kit, a transmembrane receptor protein activated by stem cell factor, plays a crucial role in the development of many cell types, including melanocytes. c-Kit mutations may lead to several types of cancer. Since the dog is considered a good model for human malignant melanoma, this study sought to evaluate c-Kit expression in canine melanocytic tumours, in order to explore its potential in comparative pathology.

Material and methods: c-Kit expression was evaluated immunohistochemically in 56 tumors (12 melanocytomas and 44 malignant melanomas) and scored in terms of the percentage of positive cells and staining intensity.

Results: Staining was negative in 23.2% of tumours, while in 30.4% of tumours over 50% of cells were positive. Staining intensity was generally weak/moderate, strong staining being observed in only 19.6% of tumours. Melanocytomas exhibited a higher percentage of positive cells than malignant melanomas ($p < 0.001$). However, differences in staining intensity were not statistically significant.

Discussion and Conclusion: c-Kit expression was observed in normal melanocytes and in most melanocytic tumors. Staining differences between melanocytomas and malignant melanomas suggest that loss of c-Kit expression may be an indicator of clinical aggressiveness. The results also showed that the standard pattern of c-kit expression in dog melanocytic tumours is similar to that observed in humans, which is an additional indication that canine melanoma can be considered a good model for studying the disease in humans. This marker may also be useful in the diagnosis of these tumors

P23 - DIFFERENTIAL DIAGNOSIS BETWEEN LYMPHOMA AND IBD IN CANINE DUODENAL ENDOSCOPIC BIOPSY SAMPLES.

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Introduction: Inflammatory bowel disease (IBD) and intestinal lymphoma induce similar chronic digestive symptoms. To achieve an accurate diagnosis of these diseases, histopathological analysis of biopsy specimens is needed. There are circumstances that make this diagnosis very difficult, especially when only endoscopic biopsy specimens are available. The aim of this investigation is to evaluate the histomorphologic parameters of both diseases, looking for their possible differences. Also, considering the limitations of routine techniques, we want to establish the diagnostic difference between microscopic examination of HE-stained sections alone, and HE-stained sections plus immunohistochemical labeled (ICH) sections for CD3 and CD79.

Materials and Methods: Twenty-two dogs were included in this retrospective study, all with a clinical history of chronic diarrhea, chronic vomiting, or weight loss. All dogs underwent endoscopy and duodenal biopsy samples were obtained. Histopathological evaluation of H-E sections was performed. Immunoreactivity of CD3 and CD79 was investigated by immunohistochemistry, using the streptavidin-biotin-peroxidase complex method.

Results and Conclusion: Initially 13 dogs were diagnosed as IBDs and 9 were diagnosed as lymphomas. After combining the H-E evaluation with CD3, CD79 and immunoexpression, the diagnosis was changed from IBD to lymphoma in 5 cases, and from lymphoma to IBD in one case. Differential diagnosis between IBD and intestinal lymphoma resulted extremely difficult in H-E stained sections alone, especially when infiltrates of small lymphocytes were present, which is the most common. Thus, immunohistochemical study of these proteins could represent an appropriate technique to enhance the diagnosis of lymphoproliferative disorders, so as to establish the appropriate treatment.

P24 - CANINE CARCINOSARCOMA OF URINARY BLADDER

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Introduction: Neoplasms of the urinary bladder are relatively common in dogs, accounting for 0,5-1,0% of all canine neoplasms. About 90% are of epithelial origin, 85-98% of these are classified histologically as malignant, and 50-90% of these will have metastases. Approximately 75-90% are transitional cell carcinomas and only 10% have mesenchymal origin. Carcinosarcomas can occur in cattle, mostly associated with enzootic hematuria.

This report describes an unprecedented case of a carcinosarcoma of the urinary bladder in a 10-years-old Saint Bernard male dog, presented for examination with a one-year history of hematuria. Radiographically, lesions of pulmonary metastases were detectable.

Material and Methods: Tissues were routinely processed, sections were stained with haematoxylin and eosin and submitted to microscopic evaluation.

Results: The histological examination revealed an irregular and highly infiltrative multinodular neoplastic proliferation, composed of cells morphologically resembling epithelial components (globular or polyhedral epithelial cells, predominantly organized in a solid pattern) and malignant mesenchymal components (multiple foci of osseous differentiation). Both cell populations showed marked atypia and some mitotic figures.

Conclusion: In human medicine carcinosarcoma of the urinary bladder is a well defined entity, already described and recognized by WHO. In our case, the morphological findings clearly showed the intimately admixed epithelial and mesenchymal malignant elements of the neoplasia. Thus, a diagnose of canine carcinosarcoma of the urinary bladder was done.

P25 - PROTOZOAL MENINGOENCEPHALITIS IN A FOSSA (*Cryptoprocta ferox*).

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An adult female fossa (*Cryptoprocta ferox*), an endangered species from Madagascar, was submitted to the Clinical Veterinary Hospital of the Universidad CEU Cardenal Herrera for necropsy from the Bioparc (Valencia).

The animal died after several months of illness characterized by weakness, recumbency, and hind leg ataxia. The most important pathological finding was a moderate multifocal non-suppurative meningoencephalitis. Multifocally, small numbers of protozoal cysts were observed surrounded by a severe lymphoplasmocytic and histiocytic infiltrate. The morphology and size of the cysts were consistent with protozoal infection, including *Toxoplasma gondii*, *Sarcocystis neurona* and *Neospora canis*.

Immunohistochemistry was faintly positive for *T. gondii* and negative for *Sarcocystis neurona* and *Neospora canis*. To the best of our knowledge, this is the first case of protozoal meningoencephalitis described in a fossa.

P26 - CITROBACTER FREUNDII SEPTICEMIA IN A NEWBORN STRANDED WHALE (*Ziphius cavirostris*)

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Citrobacter freundii, a gram-negative bacterium of the Enterobacteriaceae family, is considered a ubiquitous and opportunistic pathogen. In humans, *Citrobacter spp.* have been associated with urinary tract infections, respiratory infections, bacteremia, septicemia and meningitis (Doran, 1999; Drelichman and Band, 1985).

From a group of human cases diagnosed of *Citrobacter freundii* septicemia, 48% died (Drelichman and Band, 1985). In humans, person to person contact appears to be the usual mode of transmission; in young children, maternal transmission is possible (Doran, 1999) and *Citrobacter koseri* and *C. freundii* have been reported in vertically acquired infection (Doran, 1999; Malpas et al., 2003).

In veterinary sciences, *Citrobacter freundii* is a potential cause of bacteremia-septicemia in puppies or immunocompromized adult dogs (Galarnau et al., 2003).

C. freundii has infrequently been identified in cetaceans (Higgins, 2000), and although not reported as a cause of death, a carrier state and its likely importance in relation to dolphin health have been hypothesized (Buck et al., 2006).

Here we report a case of a stranded neonatal beaked whale showing a bacteremia-septicemia caused by a systemic infection of *Citrobacter freundii*.

P27 - A WASTING SYNDROME IN MHORR GAZELLA (*Gazella dama mhorrr*). CLINICAL AND HISTO-PATHOLOGICAL FEATURES

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Introduction: The Mhorr gazelle is an endangered species from the southwestern Morocco (former Spanish Sahara). Currently a captive stock (about 170 individuals descendant from only 19 animals) are living in captivity around the world. This work describes the clinic-pathological findings of a wasting syndrome observed in captive Mhorr gazelles of the Zoobotanic Jerez in the last ten years.

Material and methods: Twenty one (out of 53 born and living in Jerez zoo), male/females, ranging 3 month-9 year-old gazelles, suffer chronic (2-24 month), progressive weight loss, lethargy, variable degree of oral mucosal necrotizing lesions, diarrhoea or respiratory disorders and finally died. Absence or partial response to treatments was observed. Blood, serological, urine, microbiological and anatomopathological analysis were performed.

Results and conclusion: Haematology and blood biochemistry were within normal reference ranges for the species with a slight increase in blood urea nitrogen.

Bacteriology: in some cases a bacterial agent such as *Fusobacterium necrophorum*, *Pasteurella sp* or *Clostridium perfringens* were isolated. Virological analysis, both serological assay and PCR, produced negative results to Bovine viral diarrhoea (BVD), Infectious bovine rhinotracheitis (IBR), Malignant catarrhal fever, Pesti des petits ruminants virus, Rinderpest virus (RPV) and Bluetongue virus (BTV).

The main and constant pathological findings were chronic necrotizing-ulcerative oral, pharyngeal and rumenal inflammation as well as lung, splenic and hepatic abscesses consisted with *Fusobacterium necrophorum* or/and fungi proliferation.

Similar findings were reported by Worley et al., (1995) but the etiology of this syndrome still been unknown and further studies are necessary because the impact of any disease is critic in these limited stocks of gazelles.

P28 - CASE REPORT OF A SUDDEN DEATH IN A CAPTIVE CHIMPANZEE (*Pan troglodytes*)

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The present work describes the case report of an adult male chimpanzee that was submitted to the Histology and Anatomic Pathology Department of the Clinical Veterinary Hospital (Universidad CEU Cardenal Herrera), from a primate lifetime care center. The animal was taken to the sanctuary after being rescued from a small zoo in Germany with no means of support, where he lived in a cage without any kind of shelter from weather adversities. The animal died within one or two minutes, after a short period of loud screaming and convulsions. Immediately after this, the dominant male chimpanzee took, hit and bite him several times.

The necropsy of the chimpanzee was performed on the following day. The most significant pathological findings were a severe, locally extensive, myocardial necrosis, with extensive fibrosis in the heart and a moderate, diffuse, interstitial pneumonia with presence of syncytia and histiocytic infiltration. Moreover, the animal showed several hemorrhages and congestion in different body locations and organs, probably related with bites and perimortem traumas.

Based on the above-mentioned pathological findings, the cause of death was likely a cardiac failure. The fibrosis observed in the heart is indicative of a chronic process. The etiology of the myocardial fibrosis remains unknown but it might have been caused by different infectious agents, toxins or nutritional imbalances. The lesions observed in lungs might be compatible with a viral process.

P29 - OCULAR LESIONS ASSOCIATED TO *Chlamydia suis* IN A WILD BOAR PIGLET

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Introduction: The role of the wild boar as a reservoir for a large number of pathogens that can affect both domestic animals and humans has been widely studied in the last few years. However, the impact of some of these pathogens on the health of these animals is still being determined. This paper presents a clinical case involving a two months old piglet that came from a fenced hunting preserve that developed a severe bilateral keratoconjunctivitis.

Material and methods: Histopathological and microbiological studies were carried out. Samples obtained for the histopathological study were processed according to the habitual methodology for optical microscopy and were stained with hematoxylin-eosin. Samples used for the microbiological study were cultured in Blood Agar and McConkey. In addition, a specific PCR was used to determine the presence of *Chlamydia spp.*

Results: We found complete loss of the surface cells layer of the cornea on almost the entire cornea. In addition, the presence of inflammatory cells was observed, mainly lymphocytes and, to a lesser degree plasma cells, some macrophages and neutrophils among the different layers of the cornea, the choroid membrane and the retina. In the latter structure a degeneration of the layer of nerve fibers, with alterations similar to those observed in the optical nerve was also detected. There were signs of demyelization accompanied by a proliferation of glial cells. The presence of *Chlamydia suis* was detected in both eyes.

Discussion: This finding is the first description of this microorganism causing ocular disease in a wild boar. The visual handicap resulting from this type of lesion makes very difficult the survival of the piglet in its semi-captive environment.

P30 - IMMUNOHISTOCHEMICAL CHARACTERISATION OF INDUCED GRANULOMAS IN WILD BOAR (*Sus scrofa*) NATURALLY INFECTED BY *Mycobacterium bovis*.

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Introduction: *Mycobacterium bovis*, the etiological agent of bovine tuberculosis (bTB), affect a wide range of domestic and wild animal species. It is known the role of some wild species in maintaining bTB and acting as a reservoir of infection for livestock. Specifically the European wild boar (*Sus scrofa*) is a major reservoir in some regions of central and south Spain. There are many scientific studies about the management and the role of the wild boar as disease reservoir of bTB and some about the lesion patterns and histopathology but the immunopathogenesis of the infection is unknown in wild boar.

Material and methods: In this study we describe the immunohistochemical characterisation of 202 granulomas in different tissues of wild boar with natural bTB infection. The granulomas has been classified into 4 stages (I-IV) based on the pathological characterisation of bTB granulomas in cattle by Wangoo et al (2005). Immunohistochemistry has been used to characterise the cell populations and soluble markers in granulomas of different stages: CD3, CD79, MAC387, IFN-gamma and iNOS.

Results: Stage I/II granulomas were composed mainly by macrophages (MAC387) and marked expression of IFN-gamma expressing cells, both decreasing in stage III/IV granulomas. iNOS was predominantly expressed in scattered macrophages in stage I/II lesions and forming a rim of positive macrophages in stage III/IV. The number of T cells showed a mild increase from stage I to stage IV granulomas, having this fact not observed in bTB lesions in other species. The number of B cells also suffered an increase in stage III/IV when compared to stage I/II. Further analysis will be necessary to elucidate the role of the different cell populations composing the bTB induced lesions in this species.

P31 - IMMUNOHISTOCHEMICAL CHARACTERISATION OF LUNG GRANULOMAS FROM FALLOW DEER (*Dama dama*) NATURALLY INFECTED BY *Mycobacterium bovis*.

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Introduction: Bovine tuberculosis (bTB) is an important zoonotic disease caused by infection with *Mycobacterium bovis* and may affect a wide range of domestic and wild animal species. *Mycobacterium bovis* has been reported from fallow deer by several authors and this species seem to be highly susceptible to bTB. There is little knowledge about the pathogenesis of bTB in fallow deer and only some scarce publications refer to the histological characterization of the granuloma in this species.

Material and methods: In this study we show the immunohistochemical characterization of the granuloma induced in the lung of four fallow deer with natural bTB infection. The granulomas have been classified into 4 stages (I-IV) based on the pathological characterization of bTB granulomas in cattle by Wangoo et al (2005). Immunohistochemistry has been used to characterise the cell populations and soluble markers in granulomas of different stages: CD3, CD79, MAC387, IFN-gamma, TNF-alpha and iNOS. **Results:** Stage I/II granulomas showed a marked presence of macrophages (MAC387) expressing high level of iNOS while stage III/IV granulomas showed a decrease in the number of these cells, that were forming a rim surrounding the necrotic foci. This was correlated with the presence of IFN-gamma expressing cell counts, much higher in stage I/II than in stage III/IV. Interestingly the expression of TNF-alpha was very low in all stages. The number of T cells did not show any significant change among the granuloma stages while B cells were more predominant in stage III/IV granulomas. This characterization of the local immune response may be helpful to augment the vaccine efficacy and disease severity and the development of improved diagnostic tools.

P32 - A SUSPECTED CASE OF CALICIVIRUS DISEASE IN AN IBERIAN HARE (*Lepus granatensis*).

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Introduction: European brown hare syndrome (EBHS) is caused by a calicivirus closely related to rabbit haemorrhagic disease (RHD) virus, both accompanied by similar clinicals symptoms as well as pathological and histopathological changes. In Spain, only one case has been reported in European Hare (*Lepus europaeus*).

Material and methods: In a few days, two adult Iberian Hares (*Lepus granatensis*) died acutely with epistaxis as the only clinical sign. Both were kept isolated for the last two months in a wild animal rehabilitation centre with other three adult hares that remained healthy.

Results: At necropsy, severe hemorrhages and edemas in the lungs as well as hepatomegaly with friable consistence of the liver were the main gross lesions. Microscopically, vacuolation, karyolysis and karyorrhexis were observed widespread in individual hepatocytes, and foci of small number of necrotic hepatocytes were found associated with neutrofiles in the perilobulillar areas. Disseminated intravascular coagulation was also observed. Immunohistochemistry with an antiserum against VP-60 viral antigen was performed with positive result with the same distribution of lesions. Results from the analysis of anticoagulants rodenticides were negative.

Conclusion: A calicivirus infection has been considered as a possible origin of the disease.

P33 - SPERM WHALE MASS STRANDING IN ITALY: GAS AND FAT EMBOLI ANALYSES.

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On the 10th of December of 2009, seven sperm whales were found beached in Gargano coast, Italy. There was a fast and coordinated stranding response that enabled a multidisciplinary study with the collaboration of several scientific institutions. Many hypotheses were considered and analysed including the “gas and fat embolic syndrome”. For the first time, a standardized methodology for the study of gas embolism was applied to a mass stranding of sperm whales.

Because of field work-conditions, complete necropsies were only performed in the three sperm whales that stranded alive and survived beached for several hours. Gas bubbles were only found in the coronary veins. Bubbles were sampled with an insulin syringe and stored in a 5 ml vacutainer without additives. Analyses were done by gas chromatography.

Results showed a composition of 70% of N₂, 15% of CO₂ and 15% of O₂ in the freshest animal, in contrast with 30% of N₂, 30% of CO₂, 6% of O₂ and 33% of H₂ in the most decomposed animal. These results excluded putrefaction as the sole source of gas formation and dissection as artefact, since composition was not the same as the atmospheric air and bubbles were not found in other veins. Bubbles were not widely distributed, nor either massively, through out the rest of the body as described in the “gas and fat embolic syndrome”. Fat embolism was excluded because lung samples from all three sperm whales showed very few or none fat emboli (grade 0 or 1). These results on fat emboli confirmed that this evidence is not commonly found in stranded cetaceans, even if they spent hours stranded alive on the shores, differentiating this event with atypical mass stranding related to sonar exposure. Thus the “gas and fat embolic syndrome” was ruled out as the cause of the stranding.

P34 - MORPHOLOGICAL CHARACTERISTICS OF THE LUNGS FROM CETACEANS STRANDED IN THE CANARY ISLANDS

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Introduction: Marine mammals present numerous morphophysiological adaptations for surviving in the marine environment. Of all evolutionary adaptations, the lungs set apart from the rest. This is one of the main anatomical differences respect to land mammals. In marine mammals, pulmonary viscera size has not changed so much, but there is an increasing about the number of pulmonary alveoli which represents a highest speed in the gaseous exchange.

Materials and method: Different cetacean species beached in Canary Islands were used for this study. We took photos of the different anatomical parts of the respiratory system during this necropsy. Later, clinically relevant thoracic anatomical structures were identified using different anatomical and physiological papers and books.

Results: Macroscopically, lungs were covered by thin and stretch pleura which together with the myoelastic fibers of the parenchyma provided some elasticity characteristics to the organ. The shape of the lungs was frequently prismatic with the edges more emphasized than terrestrial mammals. No lobes were observed, only a less accentuated cardiac notch at level of left lung. It was characteristic in all cetacean species observed, a higher number of lymphatic structures surrounding all the ventral edge until converging to the basal edge. These structures were among the sheets of pulmonary pleura. Furthermore, there were big size lymph nodes on the vertebral portion of the medial face. About bronchial tree distribution, for ventilating the right cranial lobe existed a tracheal bronchus and two short main bronchi. Esophageal and cranial cava vein imprints were emphasized in the right lung medial face, as the aortic arch and thoracic aorta imprints in left lung.

Discussion: Studies of marine mammal anatomy are very limited because of the difficult to find healthy specimens and the access to this kind of animals. The scarce bibliography makes reference about the physiology of the marine mammals related to the habitat, where the morphology of these animals is barely studied. It is worth highlighting, some studies about the relation of the pulmonary size and the ribcage morphology in cetaceans, and the macroscopical anatomy of the lower respiratory tract in Ringed Seal (*Pusa hispida*).

P35 - HISTOLOGICAL CHARACTERIZATION OF MARINE TURTLE FIBROPAPILLOMATOSIS IN GREEN TURTLES (*Chelonia mydas*) OF PRÍNCIPE ISLAND IN THE GULF OF GUINEA.

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In May 2010, a sea-turtle conservation initiative run by the “Programa SADA” carried out a capture-mark-recapture campaign of green turtles (*Chelonia mydas*) on Príncipe Island, in order to study the incidence of sea turtle fibropapillomatosis in this region.

Thirteen captured turtles exhibited gross lesions consistent with fibropapillomatosis, from which 22 tumour biopsies were collected.

All tumours were diagnosed as fibropapillomas and were further classified according to the following traits: shape (verrucous or fibromatous); presence of epidermal acanthosis; evidence of hyperkeratosis; presence of basal layer degeneration; existence of pustules; presence of pigment, type of inflammatory cells observed; occurrence of trematode eggs, evidence of other ectoparasites and incidence of viral inclusions.

A total of 81.2% were classified as verrucous, characterized by papillary projections of the epidermal layer supported by a fibrovascular connective tissue, while the remaining tumors were classified as fibromatous, characterized by a smooth epithelial surface supported by a hypercellular stroma composed of well differentiated fibroblasts and collagen. All samples showed acanthosis, hyperkeratosis and basal-layer degeneration. Pustules were observed in 18.2% of tumours and pigment was present in 54.6% of samples. With regard to the presence of inflammatory cells, 50% of tumours presented granulocytes, 90.9% presented lymphocytes and the same number presented foreign-body giant cells. Evidence of trematode eggs (Digenea: Spirorchidae) was observed in 86.4% of tumours, while other non-classified ectoparasites were also identified on the epidermal surface of two. Viral inclusions were not observed in any of the tumours.

This is the first histological characterization of green turtle fibropapillomas on Príncipe Island in the Gulf of Guinea.

P36 - HISTOPATHOLOGIC CHARACTERIZATION OF CARDIOMYOPATHY IN LIVE - STRANDED CETACEANS.

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Introduction and aims: The causes and consequences of live cetacean strandings are poorly understood and characterized. Live-stranded cetaceans usually are debilitated immediately upon rescue, and their condition continues to deteriorate over time. Live-stranded cetaceans are certainly stressed. Although there are benefits of physiologic stress, an extreme response with the release of catecholamines is potentially damaging the cardiac muscle. The goal of the present work was to describe the cardiac lesions found in cetaceans stranded alive.

Material and Methods: Heart tissue samples from 64 animals of 16 different species, that stranded alive along the coast of Canary Islands from 1992 to 2010, were collected, fixed in 10% neutral buffer formalin and embedded in paraffin wax. Sections were stained with Hematoxylin-Eosin, Mallory's Phosphotungstic Acid-Hematoxylin (PTAH), Masson's Trichrome and Periodic Acid-Shiff (PAS). In addition, tissue samples were also immunostained using anti-myoglobin and anti-fibrinogen antibodies.

Results and discussion: Myocardial histological lesions were detected in all animals, and consisted of congestion, hemorrhages, hypercontracted and wavy fibres with sarcoplasmic and perinuclear vacuolization and contraction band necrosis (CBN). CBN develops after transient ischemia, and it is the characteristic myocardial lesion associated with elevated endogenous catecholamines. Degenerated cardiomyocytes showed depletion of myoglobin and deposition of fibrinogen. Acute ischemia produces early myocardial cell membrane rupture, causing depletion of cytoplasmic myoglobin and deposition of plasma proteins, such as fibrinogen, in myocytes. The cardiac lesions found were interpreted as physiological stress related to stranding and transportation. These observations may explain why these animals die suddenly from handling and transportation when stranded alive and why the mortality of rescued cetaceans is very high.

P37 - RENAL NEOPLASIA IN A ROE DEER (*Capreolus capreolus*): A CASE REPORT

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Introduction: The roe deer (*Capreolus capreolus*), also known as Western roe deer, is a fairly common species in Europe. In the wild, roe deer are widespread in Western Europe, from the Mediterranean to Scandinavia, and from the British Isles to the Caucasus. Due to their growing popularity, they have also become common in zoos. Spontaneous renal neoplasia is rare, both in domestic mammals and in zoo and wild mammal species. We report on a papillary renal cell carcinoma in a young male roe deer.

Materials and Methods: The animal was found dead, trapped in the wire fence of a national park in northern Portugal. Necropsy revealed a solitary subcapsular spherical mass in the left kidney, measuring approximately 5 cm in diameter. Morphological and immunohistochemical examinations were performed.

Results: Histological examination revealed a tumour mass comprising polyhedral to cuboidal cells with abundant eosinophilic cytoplasm lining long papillae. Neoplastic cell nuclei were vesicular and varied in size, showing some pleomorphism. Mitoses were infrequent. Sparse neoplastic stroma comprised connective tissue. There was also evidence of necrosis and haemorrhage. Ultrastructurally, tumour cells displayed abundant cytoplasmic mitochondria and distinct microvilli on the apical surface, resembling proximal convoluted tubular epithelium. For immunohistochemical examination, sections were stained for vimentin and cytokeratin AE1/AE3.

Conclusion: Based on its morphological and immunophenotypic features, the tumour was classified as a papillary renal cell carcinoma.

P38- INVERTED PAPILLOMA IN A RUSSIAN HAMSTER

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Introduction: Cutaneous papilloma is a benign epithelial neoplasm arising in epidermal keratinocytes. Papillomaviruses belong to the papovavirus family, causing single or multiple verrucous projections, variable in size, well circumscribed and keratinized. This type of tumour is common in horses, cattle and humans; it is less frequent in dogs and goats and rare in sheep, pigs, cats, guinea pigs and hamsters.

Case history: An 18-month-old intact Russian hamster presented with a right lateral axillary subcutaneous ulcerated nodule. No other abnormalities were detected on clinical examination. The approach taken was total surgical excision.

Material and Methods: Specimens were fixed in 10% buffered formalin solution for histological evaluation and referred to the Anatomical Pathology Laboratory, Agrarian Superior School of Viseu, in Portugal.

Results: At gross examination, the cut section revealed a radial arrangement of whitish tissues converging on the ulcerated area. Microscopic examination revealed a cystic cavity, lined by apparently-inverted hyperplastic squamous epithelium, together with ortho and parakeratotic hyperkeratosis forming a feather-like pattern. Epithelium displayed acanthosis, hypergranulosis and large keratohyalin granules. Koilocytosis was also observed, as well as sparse eosinophilic intracytoplasmic bodies, in cells exhibiting ballooning degeneration.

Discussion and Conclusion: While the histopathological features observed were consistent with papilloma, the uncharacteristic inverted image of the epithelium, producing keratin convergent to the cystic cavity, which assumed a feather-like appearance, fits with reported descriptions of inverted papilloma, although this type of tumor, especially of such large dimensions, has apparently never been reported hitherto in hamsters.

P39 - SIMULTANEOUS INFESTATION BY *Aspergillus spp.* AND HIGHLY-RESISTANT ENTEROBACTERIA IN A PEREGRINE FALCON (*Falco peregrinus*).

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Introduction: Aspergillosis is among the leading and most common diseases in birds in captivity. Hawks inhale the airborne and soil-borne spores of *Aspergillus* spp. (usually *Aspergillus fumigatus*) the lungs and air sacs; the resulting infection can even invade other anatomical sites, giving rise to different clinical (e.g. digestive, nervous). Affected hawks are more susceptible to other infections due to weakening of the immune system. **History:** A 7-month-old male peregrine falcon (*Falco peregrinus*) from a flock raised in captivity and used to control airport species, showed clinical symptoms of depression, lack of appetite, vomiting, anorexia, and laboured breathing. The body was referred to the pathology department within 48 hours of death for necropsy; samples were taken for histological examination.

Gross lesions: The bird was thin, and displayed evidence of respiratory and digestive disorders. The crop was greenish-brown in colour, the serosa appeared dry and the fungus-coated mucosa exhibited wall thickening. :

Microscopic lesions: Extensive necrosis and tissue destruction were observed. *Aspergillus* hyphae and sporangia were growing in tissues, and numerous granulomas were visible, comprising gram-negative coccidial colonies surrounded by necrotic granulocytes.

Laboratory techniques isolated *Aspergillus spp.* and highly-resistant Enterobacteriaceae.

Conclusion: Aspergillosis in this falcon may have been due to stress, excessive humidity, poor ventilation, presence of irritant (disinfectant) gases in the atmosphere, immunosuppression and inappropriate use of antibiotics, all of which are predisposing factors for the onset of illness.

P40 - FOLLICULAR DISPLASIA IN SPANISH WATER DOGS: CLINICAL AND HISTOPATHOLOGICAL FEATURES

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Introduction: Canine follicular dysplasia (CFD) includes a group of skin diseases with unknown etiology but marked breed predilection (Siberian husky, Irish water spaniel, Portuguese water dog or Curly coated retriever), that suggests a genetic basis. Clinically CFD is characterized by poor, frizzy hair coat quality, color change and variable degrees of progressive alopecia. Diagnosis is based on clinical features, endocrinology evaluation and histopathology.

Aim: to describe the clinical, histological and immunohistochemical features of follicular dysplasia in Spanish water dogs and contribute to better know this dermatopathy.

Material y methods: Multiples skin biopsies of five Spanish water dogs (3 females and 2 males, ranging 3-6 year-old), formalin-fixed and paraffin-embedded, were analysed. Antibodies against a panel of cytokeratins and vimentin were evaluated using avidin-biotin complex immunohistochemical method.

Results and conclusion: The five dogs showed non inflammatory symmetrical truncal alopecia and histopathological results were consistent with CFD. Affected hair follicles were atrophic, variably distorted; their infundibula were plugged with orthokeratotic keratin and had irregular contours. Hair shafts were absent or dysplastic and, in many cases, affected follicles showed scattered intrafollicular clumps of melanin. Discrete perifollicular fibrosis was randomly present as well as a variably altered pattern of keratin expression in dysplastic follicles.

This study is the first to describe the clinical, histological and immunohistochemical characteristics of CFD in Spanish water dogs. Our findings were similar to those reported in the follicular dysplasia of Portuguese water dogs.

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P41 - TISSUE ALTERATIONS ASSOCIATED WITH ACQUIRED HYPERFLEXION OF THE DEEP DIGITAL FLEXOR TENDON IN YOUNG LUSITANIAN HORSES

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Introduction: Since 2006, a high incidence of flexural limb deformities with hyperflexion of the deep digital flexor tendon, leading to the characteristic “club foot”, has been observed on a Lusitanian stud farm. The pathology appears in the early stages of the foals’ growth. This study sought to determine the possible cause.

Materials and methods: Flexural deformities were evaluated in ten horses by X-ray examination and measurement of the hoof-ground angle. To eliminate genetic causes, one foal was acquired from another Lusitanian horse breeder at a very early age; approximately six months after arrival, this foal exhibited the same symptoms. For humanitarian reasons, one foal was put down due to the severity of its condition. Tissue samples were studied under light microscopy.

Results: All affected foals displayed major deviation of the phalangeal axis. Microscopic examination revealed structural alterations of smooth muscle tissue, with marked myofibrillar disarray, especially in the small intestine, and changes in blood vessels, including some located in tendons.

Discussion: Acquired flexural limb deformities are usually due to rapid bone development with no corresponding growth of adjacent tissues. The major alterations observed here may have prevented normal muscle-skeletal tissue growth.

Conclusions: Further research is required to chart the correlation between microscopic findings and the development of flexural deformity. However, the alterations observed strongly suggest an as-yet-unidentified cause that triggered the onset of symptoms

P42 - ATHEROSCLEROSIS IN A DOG: A CASE REPORT

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Atherosclerosis, a rare pathological finding in dogs, is usually linked to hypothyroidism, diabetes and hypercholesterolaemia. This paper reports the case of a six-year-old male dog that died suddenly after clinical examination for dysuria.

At necropsy, the epicardial coronary arteries were markedly thickened, firm, yellow-white, and cord-like; similar lesions were also seen in renal arteries, especially in the cortical-medullary region. Multifocal haemorrhages in the myocardium and atrophy of the left thyroid lobe were also observed.

Histological examination revealed, in the tunica media of arteries, massive cholesterol deposition (cholesterol clefts), multifocal calcification and foamy macrophage infiltration; in the myocardium, there was focal fibrosis admixed with areas of recent necrosis.

**P43 - A CASE OF CARDIOMYOPATHY SUGGESTIVE OF
ARRYTHMOGENIC RIGHT VENTRICULAR
CARDIOMYOPATHY IN A HORSE**

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Introduction: Pre-existing cardiac disease, not been previously identified, could be the cause of unexpected cardiac death in horses. Arrhythmogenic right ventricular cardiomyopathy (ARVC) is characterized pathologically by fibro-fatty replacement primarily of the right ventricle in apparently healthy young people. ARVC has been described in boxer dogs, cats and horses¹.

Material and Methods: A 22-year-old crossbred gelding was submitted for necropsy. Clinical history indicated sudden onset of expiratory dyspnea, shallow breathing, and tachycardia. Symptomatic treatment was performed, but euthanized due to bad prognosis was performed. Samples were routinely processed for histopathology and on representatives areas Masson's trichrome, PAS, and immunohistochemistry (desmin, sarcomeric actine, caspase-3 and cadherin) were done.

Results: Epicardium and endocardium of both ventricles showed multifocal grassy cream areas extended into the myocardium. H/E sections showed patchy to diffuse loss of myocardial fibres with replacement by fibrous and adipose tissues, where groups of cardiomyocytes were trapped. Purkinje fibres appeared disrupted. Sarcomeric-actin and desmin showed decreased immunostain in trapped cardiomyocytes. Cadherin and caspase-3 were not detected.

Discussion: The case that we report has similarities with previous descriptions in horse. The pathogenesis of the disease is thought to be due to defective cell adhesion proteins in the desmosomes, detachment of cardiomyocytes at intercalated discs and accelerated apoptosis of myocardial cells. We could not demonstrate alterations at intercalated discs, probably because the specificity of the antibody and further investigations are necessary to confirm this condition. Others studies⁷ suggest that cases in horses previously reported as idiopathic RV dilation may be similar conditions of ARVC and investigation of these cases could be of value.

P44 - EVALUATION OF THE CAUSES OF SEIZURE IN A SLAUGHTER OF BIRDS IN THE INSPECTION BEFORE AND POSTMORTEM DURING THE PERIOD 2007-2009.

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Introduction : Chicken meat is a product of mass consumption, as it has important nutritional properties that make it necessary to include in food. The purpose of this study was to analyse the causes of condemnation in poultry slaughterhouse, antemorten and postmortem, in order to avoid economic losses, know the conditions of these animals for slaughter and to improve animal welfare.

Material and Methods: Data registered in the slaughterhouse journal entry during the years 2007, 2008 and 2009 were used. In the antemorten phase, birds were inspected after transport according to their external appearance and nutritional status. All animals showing any signs of disease or pathology were rejected.

Results: The causes of death during transport were cold, hot, crowding, rubbing against cage walls, broken limbs, fasting, and noise impacts. Other causes of death were stress, delays in reception and poor handling of the operators. Lesions found were bleeding in the breast and redness of the wingtips because stunning currents above 110 mA, plucking too hard, violent flapping during collection of cages and hung up to sacrifice. In the 3 years analysed, we had a total of 13,594,275 animals slaughtered, 67,500 out of which rejected by alterations antemorten and 32,305 postmortem.

Conclusions: 1- Antemorten comisos of slaughterhouse birds were the most frequently detected in the 3 years analysed, representations 0,49 % of birds. 2- rejections were more frequent during the very hot (July, August, September) and very cold (December) months. 3 - The most frequent causes of postmortem rejection were congestion and/or haemorrhages in the wings due to excessively high stunning augments.

P45 - INTESTINAL EMPHYSEMA OF SWINE

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Introduction: Intestinal emphysema (IE) is characterized by the existence of gas filled bubbles or cysts within the intestinal wall and adjacent lymph nodes. A similar, condition commonly referred to as *Pneumatosis cystoides intestinalis*, also occurs in man. Although its incidence from 1940 to date is decreasing, aetiology and pathogenic mechanism are still unknown. In this case report, we describe a slaughterhouse finding in a pig of 100 kg of weight, without significant lesions in other organs.

Materials and Methods: Samples of small and large intestine were taken for histopathology study. In addition, gas samples were taken from the bubbles located in different sections of intestine, following the method described by Bernaldo de Quirós (2011) to be analysed its composition by gas chromatography.

Results: Lesions were located in small and large intestine and consisted in multifocal and randomly distributed, gas containing vesicle, ranging in size from 1 to 25 mm. Histologically, lesions consisted of numerous large endothelial-lined cystic structures surrounded by connective tissue in the submucosa, muscular and serosa in the different samples of small and large intestine. A mixed inflammatory infiltrate, composed mainly of neutrophils, eosinophils, lymphocytes, monocytes, multinucleated giant cells and fibroblasts, was observed among the vesicles

Conclusion: The macroscopic and microscopic findings are similar to those described in gnotobiotic pig intestinal emphysema related to bacterial aetiology (*Escherichia coli*). Although in our case an etiologic agent was not determined, gas composition was analysed to determine its likely origin and related to the pathogenesis of the process.

P46 - PATHOGENESIS OF RIFT VALLEY FEVER IN AN EXPERIMENTAL IFNAR -/- MOUSE MODEL

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Introduction: Rift Valley fever (RFV) is an important hemorrhagic zoonosis caused by a virus (RFVv) of the *Bunyaviridae* family, genus *phebovirus*, transmitted by mosquitoes *Aedes* or *Culex* mainly.

RFVv is pathogen of livestock and humans and has been responsible for outbreak of disease throughout Africa and the Arabian Peninsula. Actually it is considered as a potential cause of disease in the south of Europe, mainly due to erratic migrations of mosquitoes caused by the climate change.

Materials and Methods: Our study describes the pathology of RFV in an IFNAR -/- mouse model, using classical pathological methods, Immunohistochemistry and Electron Microscopy. For that, we used ten 8 weeks old animals, inoculated with 2×10^4 PFU of an MP-12 attenuated strain of RFVv. Animals were sacrificed at 12, 14, 48, 72 and 96 hpi.

Results: The gross pathology findings were limited to hepatic congestion and hepatomegaly, mainly at 72 dpi and more. Histopathologic lesions were evident in the liver: 12 hpi hepatocytes showed vacuolar degeneration of the cytoplasm which evolved to multifocal necrotizing hepatitis, with presence of intranuclear eosinophilic inclusions (48 hpi). Other important lesions –such as focal meningoencephalitis and brain microhemorrhages– were usually present at the end of the infection. Lymphoid organs were affected too, lymphocytosis and lymphocytic apoptosis were evident at 48 hpi and more. By means of the Immunohistochemistry we found RFVv antigen in hepatocytes, but also in many several locations –such as brain, lung, gut and lymphoid tissues– usually in the cytoplasm of macrophage-like cells. Electron Microscopy confirms the lesions before described.

Conclusion: The mouse model used in this report mimics the main lesions observed in the natural disease and it is an excellent model for further studies in RFV pathogenesis and vaccines/ therapeutics experiments.

P47 - EQUINE HERPESVIRUS- 1 (EHV-1) GLYCOPROTEIN D EXPRESSED BY A RECOMBINANT BACULOVIRUS (GPD-BAC) PROTECT MICE OF ABORTIGENIC INFECTION BY EHV-1 PATHOGENIC STRAIN.

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Introduction: Equine herpesvirus 1 (EHV-1) is a pathogen of major significance to the horse breeding industry. A mouse model provides a valid method to investigate aspects of EHV-1 disease and to analyze the vaccine potential of various immunogens. In this work we analyzed the probable effect protective of gpD-Bac for to prevent abortigenic infection in mice.

Materials and Methods: A gpD-Bac was produced in Hi5cells and then purified by metal ions (Ni). Five groups of females were used. Three of them (A, B, C) received twice gpD-Bac intranasally. All animals were bled ten days after 2nd immunization for antibodies (Ab) detection by ELISA technique. Groups A and B were pregnant and 12 days after, the group A with groups C and D were infected with a pathogenic Argentine EHV-1 strain. Groups B and E were used as negative infection controls. Weight loss, appearance of clinical signs and premature parturition were controlled. At 48 h post infection (pi) two females from group A, two from C and two from D were sacrificed to determine the presence of virus in blood and lung by PCR and virus isolation, histological and immunohistochemical (IHC) studies.

Results: Mice immunized developed specific Ab. Signs of illness, weight loss and viral DNA was detected only in mice of group D. In addition, the typical histological lesions in lungs were observed and IHC was positive. The parturients went to full term of gestation and gave birth to litters of normal offspring.

Conclusion: These previous results suggest that gpD-Bac would have protected the mice from EHV-1 infection.

P48 - LIVER PATHOLOGY AND HOST RESPONSE IN GOATS IMMUNIZED WITH RECOMBINANT CL1 IN MONTANIDE AND INFECTED WITH *Fasciola hepatica*

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Introduction: Fasciolosis is a economically important disease of ruminants, current control is based in the use of anthelmintics, but there is an increasing interest for developing vaccines which could avoid problems of anthelmintic resistance and risk of metabolites in foods. The aim of the present work was to evaluate the hepatic changes and local host response in goats immunised with recombinant cathepsin L1 (CL1) and challenged with *F. hepatica*.

Material and Methods: 21 goats were used: group 1 (n=8) was vaccinated with CL1 in montanide ISA 70 adjuvant, group 2 (n=8) with adjuvant and group 3 (n=5) was used as uninfected control. Animals were infected with 200 metacercariae and euthanised at 15 weeks post-infection. Tissue samples from the liver were fixed in formalin and embedded in paraffin, or snap frozen for histopathological and immunohistochemical studies, respectively. T cell subsets (CD2, CD4, CD8, $\gamma\delta$, IgG, IL4 and IFN- γ) were analysed in snap frozen sections.

Results: No significant fluke burden reduction was found in the CL1 group respect to the infected control. Hepatic lesions (chronic cholangitis, portal fibrosis, bile duct hyperplasia and granulomas with necrotic centre) were similar in the vaccinated and infected control group. The hepatic infiltrate of T cell subsets was severe and similar in the two infected groups.

Conclusion: The results of the present work revealed a strong local non protective immune response in the vaccinated and infected control group, suggesting that montanide may be not appropriate adjuvant for this vaccine.

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P49 - HEPATIC CHANGES DURING EARLY AND LATE INFECTION STAGES IN GOATS VACCINATED WITH RECOMBINANT GST - SIGMA CLASS AND CHALLENGED WITH *Fasciola hepatica*

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Introduction: Vaccine development for fasciolosis control in ruminants has been relatively slow. To date vaccine trials in ruminants have been focused in evaluation of protection, however, the mechanisms of the host response are crucial to improve vaccine formulation and efficacy. The aim of the present work was to evaluate hepatic changes during early and late infection stages in goats vaccinated with recombinant glutathione S transferase sigma class (GST) and challenged with *Fasciola hepatica*.

Material and Methods: Group 1 (n=7) was unimmunised and uninfected; group 2 (n=10) was immunised with adjuvant Quil A and infected; group 3 (n=10) was immunised with GST and infected. Three goats from each group were killed at 7, 8 and 9 days post-infection. The remaining goats from groups 2 and 3 were killed at 12 weeks post-infection (wpi). Hepatic lesions were evaluated gross and histopathologically.

Results and Conclusions: At early stages of infection two goats from the vaccinated group showed mild hepatic lesions. In these animals migrating larvae were surrounded by a heavy infiltration of eosinophils suggesting this cell type play an important role in the effective host response during early migratory stages. GST vaccination did not induced significant fluke burdens reduction, but three goats at showed low fluke burdens at 12 wpi and mild hepatic lesions, suggesting that effective host response occurs during early infection stages. This work support further studies to evaluate host response during early stages of infection in vaccine trials.

Acknowledgments: Work supported by projects 023025-DELIVER and P07-AGR-02900.

P50 - EXPRESSION OF FORKHEAD-BOX P3 (FoxP3) REGULATORY T CELLS IN EARLY AND LATE STAGES OF CAPRINE FASCIOSIS

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Introduction: Currently, there is an increasing interest about the study of regulatory T cells (Tregs) and their role in the establishment of an effective immune response to infections. In ruminants this T cell subset has not been thoroughly studied.

Material and Methods: Eighteen Malagueña breed goats divided in three groups of 6 animal each were used for this study: group 1 was immunised with Glutathione-s-transferase (GST) in Quil A adjuvant; group 2 was immunised with Cathepsin-L1 in Quil A adjuvant and group 3 was the adjuvant control (only with Quil A). The animals were immunised and infected at 10 weeks post-infection with 100 metacercariae of *F. hepatica*. Necropsy was carried out at two points of sacrifice: at 7-9 days post-infection and 12 weeks post-infection. Samples from liver and hepatic lymph nodes were taken and embedded in paraffin and sectioned at 4µm-thick for the immunohistochemical study.

Results: Immunohistochemistry showed positive Foxp3 cells in goat formalin-fixed tissue samples from the liver. The goats sacrificed at early stages showed more Foxp3 positive cells in comparison with the animals from the late stages.

Discussion and Conclusions: This is the first report about the expression of Tregs positive cells in formalin tissue from goats parasitized with *F. hepatica*. The high expression in early stages respect the late stages of the diseases could explain the lack of a protective immune response in goats against this parasite, although more studies should be carried out.

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P51 - *Fasciola hepatica* INDUCES APOPTOSIS OF DIFFERENT CELL TYPES DURING EARLY HEPATIC MIGRATORY STAGES

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Introduction: *Fasciola hepatica* has shown a variety of mechanisms to evade the host immune response. Recently has been reported that excretory secretory products from *F. hepatica* induces apoptosis of eosinophils in rats. The aim of the present work was to evaluate if *F. hepatica* is able to induce apoptosis in vivo in goats during early stages of infection.

Material and Methods: 12 goats were used for the study. They were allocated in 4 groups, the first one was immunised with Quil A, the second one with recombinant glutathione S transferase (GST) and the third one with recombinant cathepsin L1 (CL1). Group 4 was used as uninfected control. Animals were orally infected with 100 metacercariae and euthanized at 7-9 days post-infection. Liver tissue samples were examined for routine histopathology, transmission electron microscopy (TEM) and immunohistochemistry for caspase 3 and different cell markers (CD3 and lysozyme).

Results: All infected animals showed caspase 3 expression in hepatocytes, macrophages, T lymphocytes and eosinophils located in hepatic necrotic tracts and foci caused by migrating larvae. Hepatic tissue adjacent to migrating larvae did not show caspase 3 expression, but it was found in tracts behind the larvae. TEM studies confirm apoptosis in the above mentioned cell types.

Conclusion: The present study revealed that *F. hepatica* causes apoptosis in vivo during early stages of hepatic migration in different cell types, which may be an important mechanism to evade the host immune response during the migratory phase of the parasite.

Acknowledgments: Work supported by projects 023025-DELIVER and P07-AGR-02900.

P52 - EXPRESSION OF INTERLEUKIN-1B, TUMOR NECROSIS FACTOR ALPHA AND INTERLEUKIN-8, IN LUNG OF LAMBS EXPERIMENTALLY INFECTED WITH *Mannheimia haemolytica*

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Introduction: The immunohistochemical expression and the lung extract concentrations of Interleukin-1 Beta (IL-1 β), Tumor Necrosis Factor Alpha (TNF α) and Interleukin-8 (IL-8) in the lung of lambs experimentally infected with *Mannheimia haemolytica* were investigated.

Methods: The lambs were randomly assigned to 2 groups: infected and uninfected controls. The inoculum in each lambs of the infected group was 1.5×10^9 colony-forming units of the *Mannheimia haemolytica* in 5 mL sterile nutrient broth. The control lambs were inoculated with 5 mL of sterile nutrient broth. The control and infected animals were killed from 1 to 15 days post-infection (dpi).

Results: These findings demonstrate a temporal association between pulmonary expression of these cytokines and lung pathology in ovine pulmonary pasteurellosis. Given that the lung expression of IL-8 was much greater than that of TNF α and IL-1 β , the anti-cytokine agents directed at this mediator could be more useful in the prevention and treatment of this disease.

Conclusions: The results of this study suggest that IL-1 β , TNF- α and IL-8 inflammatory cytokines may play an important role in enhancing the biological response of *Mannheimia haemolytica* and contribute to the development of the lung lesions in ovine pulmonary pasteurellosis

P53 - EFFECT OF THE IMMUNE SYSTEM IN THE PATHOGENESIS OF STAPHYLOCOCCAL MASTITIS IN RABBITS

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Introduction: Staphylococcal mastitis is the main cause of culling of adult does from commercial rabbitries. However, there is practically no information available on the pathology and pathogenesis of mastitis in rabbits. The aims of this work were (1) to provide a detailed description of the spectrum of microscopic pathology observed in cases of chronic staphylococcal mastitis in adult does; (2) to determine whether there was a correlation between *Staphylococcus aureus* genotypes and pathology and (3) to characterize local and peripheral immunity for to improve the knowledge in the pathogenesis of this important mammary infection in rabbits.

Material and Methods: Forty-two adult rabbits (*Oryctolagus cuniculus*) from rabbitries with previous diagnosis of chronic mastitis were studied. Next analyses were carried out on each animal: (1) genotyping of *S. aureus* strains isolated from lesions, (2) study of lymphocyte populations in peripheral blood by flow cytometry, (3) histopathologic classification of mammary lesions, (4) analysis of local immune response by immunohistochemical studies in mammary glands. Lesions were focussed on the mammary glands or on periglandular tissue. On the basis of histopathology, pathological changes were differentiated into abscesses, suppurative mastitis with a lobular pattern, cellulitis and mixed lesions.

Results: While there were different pathological presentations, these were independent of *S. aureus* genotype. There were differences in the cells populations between lesions. The number of T and B lymphocytes was decreasing with the maturation of the abscesses. The suppurative mastitis showed a less number of T lymphocytes than abscesses and the cellulitis were the type of lesion with a lower number of macrophages, plasma cells and T and B lymphocytes. The rabbit does present a decrease in the number of total CD4⁺ and CD8⁺ lymphocytes. A broad spectrum of different pathological states could be established based on the histomorphological characteristics and the cellular composition of the lesions. These manifestations may reflect different via of infection and host-pathogen interactions.

P54 – Ki 67 EXPRESSION AS A MARKER OF CELLS PROLIFERATION IN TESTICLE OF BULL FIGHTING

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Introduction: The use of growth promoters causes increases of muscle size due to promotion of positive nitrogen balance by stimulating protein, it produces retention of body water, with a lower fat content. Depending on their chemical origin they may be involved in sperm production, markedly influence hormone levels, are evident in its behavior. Growth promoters are classified as possible carcinogens..

Materials and Methods: The study was carried out on 32 fighting bulls of 4 years old. The testes were collected after slaughtering, processing of the samples were formalin fixed for 24 hours and paraffin embedded according to routine histological procedures, stained with hematoxylin-eosin. Cell proliferation was evaluated by immunohistochemical analysis performed with monoclonal antibody Ki67 (Dako Cytomation, CA, USA).

Results: Immunohistochemical analysis showed, no cellular proliferation in 12/32 (37.50%) bulls, moderate proliferation in 15/32 (46.87%) and normal proliferation in 5/32 (18.62%) suggesting a significant restriction of spermatogonial germ cells and small amounts of spermatocytes.

Conclusion: We observe a reduction in spermatocyte proliferation. The use of growth promoters have been previously associated with decrease in spermatogenesis, causing poor development of germ cells and sperm quantity and quality. These drugs can cause behavioral changes. The mechanism of action of hormonal regulation of testosterone and its analogues play an important role in spermatogenesis, particularly in the initiation and maintenance of sperm processing and inhibition of germ cells. Further studies are necessary to confirm the cause of reduction of spermatogenesis in fighting bulls.

P55 - EFFECTS OF ESTROGEN ANALOGS ON ESTROGEN RECEPTOR ALPHA, PROGESTERONE RECEPTORS AND Ki67 ANTIGEN EXPRESSION IN THE MYOMETRIUM OF OVARIECTOMYZED RATS.

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Introduction: Leiomyomas of the reproductive tract of females grow under the influence of sex steroid hormones through estrogen receptors (ER). Selective ER modulators (SERM) may influence the growth of these tumours. The ovariectomized (OVX) rat allows exploring the role of ER analogs on steroid hormones receptors expression and cell proliferation.

Materials and methods: Two-weeks-OVX adult Wistar rats were injected over three days with 0.2 ml oil, 25 µg estradiol benzoate (EB), 1.5 mg ERα and ERβ selective agonists PPT and DPN, respectively, and SERMs tamoxifen (TX, 3.0 mg) and raloxifene (RX, 1.0 mg), and pure anti-E RU58668 (RU, 0.5 mg) and ICI182780 (ICI, 0.25 mg). Formalin fixed, paraffin embedded uterine tissue samples were sectioned and stained immunohistochemically for ERα, progesterone receptor (PR) and Ki67 antigen.

Results: PPT>BE>TX decreased ERα expression, whereas DPN and RX did not. Myometrial cells of EB-, PPT-, TX- and DPN-treated OVX rats expressed PR while Ki67 was present in EB-, PPT-, DPN- and TX-treated OVX rats. A positive correlation (Spearman correlation tests) was observed between ERα and PR (R=0.42) or Ki67 (R=0.36) expression, and between PR and Ki67(R=0.8) expression.

Conclusions: Estrogens induced ERα down-regulation, PR and Ki67 expression in the myometrium of the OVX rat through activation of ERα and, to a lesser extent, ERβ. TX mimicked ERα activation whereas the other SERM did not. RX and pure anti-E appear to be the best agents to control ERα effects on PR expression and cell proliferation.

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P56 - CYCLIC CHANGES IN MUC1 EXPRESSION IN CANINE ENDOMETRIUM

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Introduction: MUC-1 is a polymorphic glycoprotein expressed in the epithelia of various organs, including the uterus. Here, mucins are responsible for local protection, since they form a natural barrier against external threats. MUC-1 activity in the uterus is regulated mainly by progesterone. This study sought to locate MUC-1 in the canine endometrium and to investigate possible changes in staining intensity in the course of the canine estrous cycle.

Material and methods: Formalin-fixed canine endometrium samples (n=43) were examined immunohistochemically using a streptavidin-biotin-peroxidase technique. The primary antibody (clone MH1 - CT2, AbCam) was used at 1:200. Scoring intensity (weak, moderate or strong) was recorded for each epithelial structure (surface epithelium – SE, glandular epithelium –GE).

Results: As expected, positive staining for MUC-1 was observed throughout all stages of the canine estrous cycle, in both uterine epithelial elements. Regardless of the stage of the cycle, stronger MUC-1 positivity was noted in SE than in GE ($p<0.001$; Fisher=36.56), with no significant difference between superficial and deep glands. The highest overall staining intensity was recorded during anestrus, proestrus and estrus ($p<0.001$; Fisher=36.26), especially in the SE, whilst the lowest positivity rates were found in diestrus. There was no significant difference in intensity between early and full diestrus.

Discussion/Conclusion: MUC-1 expression in the canine uterus displayed certain similarities to that reported in humans during the follicular and luteal stages; the decrease in MUC-1 content in progesterone-associated stages would favour embryo implantation.

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P57 - IMMUNOHISTOCHEMICAL EVALUATION OF TNF EXPRESSION IN CANINE CYSTIC ENDOMETRIAL HYPERPLASIA

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Introduction: Tumour Necrosis Factor (TNF) has been identified in the uterus of several species, and altered TNF expression is reported in some pathological conditions. This study sought to evaluate TNF expression in canine cystic endometrial hyperplasia (CEH; n=20) and compare it with expression in postpartum samples (PP; n=5).

Materials and Methods: Canine uteri presenting CEH were collected at ovariectomy for subsequent histological classification using Dow's grading system (I, II, III, IV). For immunohistochemistry, a primary monoclonal antibody raised against the canine TNF molecule (sc-80386; Santa Cruz Biotechnology Inc.) was used at a dilution of 1:50. Staining intensity was scored (0-3) in superficial and glandular epithelia (SE and GE) and in cystic epithelium (CE).

Results: Preliminary results indicated more heterogeneous TNF staining in almost all CEH samples compared to PP specimens, possibly due to the inflammatory infiltrate observed in the uterus in CEH grades II, III and IV. Overall TNF positivity differed significantly between CEH and PP specimens (p=0.046). Stromal positivity was higher for CEH grades I and II than for grades III and IV and PP; values for PP were higher than for CEH grade III and IV. In CEH specimens, staining intensity was greater in the SE than in GE, whilst scores were lower in the CE (p=0.012). The greater staining intensity for TNF recorded particularly in the early stages of CEH suggest that this factor may be involved in the onset of this pathology.

P58 - P-CADHERIN EXPRESSION IN FELINE LACTATING MAMMARY GLAND

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Introduction: In human and canine normal non-lactating breast tissue, P-cadherin expression was restricted to myoepithelial cells. However, unexpectedly, in lactating tissues, P-cadherin was expressed in luminal epithelial cells, in a staining pattern similar to secreted proteins. Feline mammary tumours, due to their histological characteristics and clinical evolution, are considered a good animal model for the study of human mammary carcinogenesis. The study of adhesion molecules, namely P-cadherin, may be an useful model to better understand the feline and women mammary carcinogenesis.

Materials and Methods: As part of the study on P-cadherin in feline mammary gland tissues, namely normal ($n=4$), hyperplastic ($n=12$), benign ($n=6$) and malignant ($n=39$) lesions, P-cadherin expression was examined in normal lactating mammary gland ($n=2$).

Results: In normal non-lactating mammary gland P-cadherin immunolabelling was restricted to myoepithelial cells. In lactating or pseudo-lactating mammary tissue, P-cadherin was found in secretory luminal epithelial cells and milk secretion. The P-cadherin expression in luminal epithelial cells was mostly cytoplasmatic, with an expression pattern similar to apocrine epithelial cells, in the apical cell region.

Discussion and Conclusion: Our findings largely corroborate the previous observations in human and canine mammary gland tissues what concerns P-cadherin expression in luminal epithelial cells, reinforcing their results. From our point of view it will be interesting to study not only the immunohistochemical expression of P-cadherin in feline lactating mammary glands, but also the possible presence of P-cadherin in the milk.

P59 - MODIFICATIONS IN KIDNEY STRUCTURE WITH AGE IN AN ANIMAL MODEL OF TYPE 2 DIABETES: FOCUS ON HYDRONEPHROSIS

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Introduction: The Zucker Diabetic Fatty (ZDF-*falfa*) rat is one of the most widely-used models for the study of type 2 diabetes (T2DM). Characterisation of renal morphology in this model may provide useful insights into the mechanism of diabetic nephropathy progression. The purpose of the present study was to determine renal morphology, identify and characterise renal dysfunction complications such as hydronephrosis, in Zucker Diabetic fatty (ZDF) rats in the course of T2DM progression.

Material and Methods: Male diabetic obese ZDF (*falfa*) rats were sacrificed at 8, 20 and 26 weeks of age and compared with lean age-matched ZDF (+/+) counterparts. Blood biochemistry was also performed to evaluate metabolic status. Kidney slices stained with hematoxylin-eosin and periodic acid-Schiff were evaluated. A semiquantitative rating was assigned for hydronephrosis and scored from 0 (normal) to 3 (severe), based on conformational aspects of the papilla and calyx and on cortex compression.

Results: A striking progression in the severity of hydronephrosis was observed. All end-stage rats, i.e. at 26 weeks of age, showed hydronephrosis, which was more severe in the diabetic rats (score: 2, 3) than in lean controls (score 1). No significant cortical atrophy was found. These abnormalities were accompanied by aggravated diabetic-metabolism dysfunction.

Conclusions: The results indicated that ZDF rats presented nephropathy with hydronephrosis. Lesions were age-related and accompanied by aggravated diabetic metabolism dysfunction, but did not hinder morphological evaluation. Therefore the ZDF rat might provide a useful model for the preclinical study of therapeutic interventions in diabetic nephropathy.

P60 - EXPRESSION AND DISTRIBUTION OF CYTOSKELETON MARKERS IN GILTHEAD SEABREAM (*Sparus aurata* L.)

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Introduction: Protein filaments that form part of the cytoskeleton constitute an internal dynamic framework which carries out vital cell functions. However, lack of species-specific antibodies is a limiting factor in conducting a detailed fish tissue study. This implies the use of antibodies developed in other species, which possess significant cross-reactivity. The objective of our study was to standardize immunohistochemical techniques in gilthead seabream (*Sparus aurata* L.) tissues to detect several cytoskeleton molecules including some involved in the cell-to-cell adhesions.

Material and methods: We used antibodies against anti-human: E-cadherin; annexin V; occludin and claudin. Samples from digestive tract, pancreas, liver, thymus and kidney fixed in Bouin's for 24 hours and embedded in paraffin were employed. Positive and negative controls were used for each antibody.

Results: Trials showed positive spotted expression in epithelial cell membranes especially of digestive mucosa, when using E-cadherin and annexin V antibodies. The later also marked rodlet cells of specific digestive tube sections. Presently, no satisfactory immunoreactivity was achieved with the occludin or claudin antibodies.

Conclusion: This data contributes to enhance knowledge regarding fish tissue structure providing a useful tool for a more accurate physiological and histopathological studies.

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P61- CLINICAL SIGNS OF *Ipomoea carnea* - INTOXICATION IN GOATS. A CLINICO-PATHOLOGICAL CORRELATION WITH SPECIAL CONSIDERATION ON THE CENTRAL NERVOUS SYSTEM

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Introduction: *Ipomoea carnea* subsp. *fistulosa*, aguapei or mandiyura, is responsible for lysosomal storage in goats. It contains alkaloids, mainly swansonine, which inhibits lysosomal α -mannosidase and Golgi mannosidase II. Poisoning occurs by inhibition of these hydrolases. There is neuronal vacuolation, endocrine dysfunction, cardiovascular and gastrointestinal injury and immune disorders. We describe the clinical and pathology of the disease in Northeast Argentina.

Material and methods: Five goats received fresh leaves and stems of *Ipomoea* during the experiment, a detailed examination of central nervous system was carried out, including state of consciousness and sensory, facie attitudes station, walking and lying and reflections. The goats were sacrificed when became recumbent. Routinely CNS sections were processed for histology and lectin histochemistry.

Results: After 21 days we found: abnormal fascie, dilated nostrils, and abnormal postures of head, cephalic tremors and nystagmus, difficulty to stay on station. Subsequently, it was a tendency to fall, always to the left, with spastic convulsions. Purkinje and deep nuclei neurons were damaged. As a consequence, voluntary movements lack coordination. The cochlear reflex originated hyperreflexia, abnormal posture, head movements and tremors. The withdrawal reflex produces flexor muscles hypersensitivity at the four members, later depression and stupor. Abnormal responses to sounds are related to colliculus lesions. Thalamic damage alters the withdrawal reflex, showing incomplete reaction. Cervical bristling may be a nociceptive response also regulated by the thalamus.

Conclusion: Due the wide variety of SNC lesions, it would be essential to reach an early diagnosis to a proper management of an economically relevant disease for goat production.

P62 - ERGONOVINE FROM *Ipomoea carnea* RESPONSIBLE OF ADDICTIVE EFFECT IN INTOXICATED GUINEA PIGS

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Ipomoea carnea is a toxic plant, able to generate a poisoning in the goats. It is characterized by hepatic damage, nervous upheavals and death. The addiction in ruminants is commonly cited as a clinical sign of *Ipomoea* intoxication. The objective of the study was to determine, through observation, whether the animals poisoned by *Ipomoea* become addicted to it, to identify the possible alkaloid responsible for this preference and the relation between serotonin and ergot derivatives. "Small balls" were elaborated with leaves of *Ipomoea* and administered to guinea pigs, during 75 days. Besides, an extract of leaves from *Ipomoea* was elaborated to conduct different tests for the lysergic acid derivatives, such as HPLC-UV. Finally, plasma serotonin (5-HT) was quantified by methods based on HPLC- with electrochemical detection. We observed that the intoxicated animals screeched when people entered the animal's room. They stopped vocalizing when they received the "small balls" and they refused the grass. Ergonovine was identified in the extract by means of the chromatography profile compared to a standard reference. Decreased activity of 5-HT in poisoned guinea pigs suggests the presence of derivatives of lysergic acid. These may act as 5-HT₂ agonists at postsynaptic 5-HT₂ receptors. Possibly, the decreased 5-HT₂ concentration was due to self-regulation. The results demonstrate the presence of ergot derivatives in the plant and the addiction in guinea pig.

P63 - HISTOPATHOLOGICAL STUDY OF A NEUROTOXIC SYNDROM CAUSED BY *Paspalum paspalodes* INFECTED BY *Claviceps paspali*

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Introduction: An accidental intoxication in bovine and horses caused by ingestion of *Paspalum*, infected by *Claviceps paspali* is reported. Frequently described in South Africa and America, there are only few cases in Spain.

In the Doñana P.N. marshlands (Huelva, Spain), a tremorgenic syndrome developed in 26 bovines and 2 horses. Clinical sings showed by affected animals included tremor, abnormal head movements, ocular globe motor alteration, and motor incoordination which was more evident in posterior members and was aggravated under stress conditions, leading to lateral recumbency showing hyperextension of the anterior members. After approximately half an hour, the animals recovered. The post-mortem study revealed no macroscopic lesions only a certain grade of adenomegaly in the mesenteric lymph node. In the microscopic study, the most important lesions were observed in the brain, and consisted in microhaemorrhages diffused through the whole parenchyma, neuronal degeneration, satellitosis, neuronophagia, gliosis, necrosis and moderate degeneration of the neuropil from the peripheral zones of the brain. The diagnosis of this tremorgenic syndrome involved ruling out other pathologies which also affect the nervous syndrome.

Conclusion: The grass was identified as *Paspalum paspaloides* (Michx.) Scribner, and the fungus was identified based on the morphology of the sclerotia as *Claviceps paspali*.

P64 - GLIAL REACTIVITY IN THE CEREBELLUM OF *Solanum bonariense* L. INTOXICATED BOVINES

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Introduction: *Solanum bonariense*, a South American native shrub has been associated with outbreaks of cerebellar dysfunction in cattle due to cerebellar cortical degeneration. Histological lesions include Purkinje cell perikaryal vacuolation, axonal swelling, gliosis and progressive cell death. Ultrastructurally, accumulation of electron-dense residual bodies in the perikarya and axons of affected Purkinje cells was described. The goal of the present study was to immunohistochemically characterize the glial cell reaction observed in the cerebellum of *S. bonariense*-intoxicated cattle.

Material and methods: Formalin-fixed paraffin-embedded cerebella from seven natural and experimentally intoxicated bovines and of two controls were sectioned and immunostained either with a polyclonal rabbit anti-glial fibrillary acidic protein (GFAP), a polyclonal rabbit anti-S-100 protein and the monoclonal anti human-S100 β antibodies. GFAP immunohistochemistry was performed using a peroxidase-labelled polymer system and diaminobenzidine as a chromogen. Triple immunofluorescent technique was carried out for detection of GFAP and S-100 β , plus DAPI for nuclei detection; samples were observed under confocal microscopy.

Results: A remarkable increase in the number of Bergmann and other astrocytes (astrocytosis) was observed around damaged Purkinje cells and in the molecular layer in the cerebella of affected animals. Small round S100 β + cells, normally aligned along Purkinje cells in control animals, were increased in number and showed a disordered distribution pattern throughout the molecular layer. Most of these cells were negative against S-100; however, a small number of cells displayed co-localization of both S-100 and S-100 β antigens.

Conclusion: The present study contributes to understand the fine gliofilament regulation in reactive astrocytosis observed in the cerebellum of *S. bonariense*-intoxicated bovines.

P65 - TOXIC BRACKEN (*Pteridium aquilinum*) COMPONENTS IN MAINLAND PORTUGAL

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Introduction: Bracken (*Pteridium aquilinum*) causes cancer in animals and is a suspected human carcinogen. Ptaquiloside is considered bracken's main carcinogen, but toxicological studies are hampered by its instability and the difficulty in isolating it. This study sought to isolate bracken compounds for toxicological testing.

Materials and Methods: Three bracken crossier samples were collected in April 2010 in Arcos de Valdevez, Portugal. Samples A and B were collected from site 1, sample C was collected from site 2. An improved ptaquiloside isolation method was adopted, with modifications. Sample A was routinely processed, frozen rather than air-dried, and silica gel-chromatographed with CHCl₃ before ethyl acetate (EtOAc). Fractions were controlled by ¹H and ¹³C nuclear magnetic resonance (NMR, 400 MHz). Ptaquiloside-containing fractions were separated twice by reverse-phase column chromatography instead of HPLC. With samples B and C the initial batch resin adsorption step was replaced by adsorption onto a resin (Amberlite XAD-2) column. Sample B CHCl₃ fractions were separated by HPLC-DAD (C₁₈ column, 4 ml/min, CH₃OH/H₂O 50/50). Two EtOAc/CH₃OH fractions were also separated (CH₃OH/H₂O 20/80). The identity of the compounds was confirmed by ¹H and ¹³C NMR (APT, DEPT, HMBC, HSQC) and mass spectrometry data.

Results: Samples A and B yielded 10 and 100 mg ptaquiloside, respectively; only vestiges were present in sample C. Sample B CHCl₃ fractions yielded 41 mg pterisin B (RT=12min). The ethyl acetate-methanol fractions yielded 67 mg prunasin (RT=8min).

Conclusions: Two main toxic bracken components were isolated from samples collected in mainland Portugal: the carcinogen ptaquiloside and the cyanogenic glycoside prunasin, partly responsible for acute bracken toxicity. Column-adsorption ptaquiloside isolation methods are more efficient than the previous batch-based strategy.

P66 - MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF PTAQUILOSIDE-INDUCED NEOPLASTIC AND PRENEOPLASTIC LESIONS IN MICE.

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Introduction: Bracken (*Pteridium aquilinum*) is known to cause cancer in animals and is a suspected human carcinogen. Ptaquiloside is considered bracken's main carcinogen. The WNT pathway is involved in human and rodent urinary bladder carcinogenesis, with β -catenin nuclear translocation and activation of proliferation-related genes.

Materials and Methods: Ptaquiloside was isolated from bracken according to previously published methods. 12 male CD-1 mice were administered 0.5mg ptaquiloside i.p. weekly for 15 weeks, followed by a further 15 weeks' incubation period. 12 control mice received saline. Two exposed animals died during the experiment. At necropsy, blood and organ samples were collected for histological analysis. Leukograms were prepared from blood smears. Flow cytometry was used to assess blood T-(CD3⁺) and B-(CD19⁺)-lymphocytes, bone marrow granulocytic (CD11b⁺/Ly-6G⁺, CD11b⁺/Ly-6G⁺) and B-lymphocytic (CD19⁺/IgM⁻, CD19⁺/IgM⁺) populations and thymic T-lineage cell (CD4⁺, CD8⁺, CD4/CD8⁺) populations. Lymphoproliferative and urothelial lesions were analyzed immunohistochemically for antibodies against CD45 and CD3 and against Ki-67, β -catenin and E-cadherin, respectively.

Results: 10/10 surviving exposed mice developed a B-cell lymphoproliferative malignancy characterized by B-(CD45+/CD3-)-lymphocytic renal (10/10) and hepatic (2/10) invasion, splenic white pulp hyperplasia (10/10) together with circulating dysplastic lymphoid cells, neutropenia and a significant ($p < 0.05$) increase in circulating B-(CD19+)-lymphocytes. No bone marrow changes were detected. 8/10 exposed mice developed bladder urothelial dysplasia with increased ($p < 0.05$) Ki-67 labelling index and identical E-cadherin and β -catenin expression compared with control animals. No lesions were detected in controls.

Conclusions: Ptaquiloside induces a B-cell lymphoproliferative malignancy and bladder dysplasia in mice. Increased proliferation is a feature of ptaquiloside-induced urothelial dysplasia but there is no evidence of WNT activation or of reduced E-cadherin expression.

P67 - EFFECTS OF HESPERIDIN ON DIETHYLNITROSAMINE - INDUCED HEPATOCELLULAR CARCINOGENESIS IN RATS.

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Introduction: Hesperidin is a biologically active flavanone glycoside occurring abundantly in citrus fruits. This flavonoid is known to possess beneficial effects against cancer but its effects on liver tumorigenesis have not been studied. In the current study, we have carried out a macroscopic and histological evaluation of hesperidin effects in a well-characterized model of rat liver carcinogenesis with diethylnitrosamine (DEN).

Material and methods: Twenty four, seven weeks-old Sprague Dawley rats were grouped (3 rats/group) as follows: Group 1 was fed with 0.01% DEN in water. Groups 2-4 received 250, 500 or 1000 ppm hesperidin, respectively, before 0.01% DEN. Control groups included rats fed with 250, 500 or 1000 ppm hesperidin (groups 5-7) and 20 g/day rat chow (group 8).

Results: Body weight was significantly lower in group 1 rats (mean 346g) than in control rats (mean 453g) while hesperidin treatments (mean 452g) did not affect this parameter. Compared with group 1, 1000 ppm hesperidin reduced the total number of visible liver nodules (55-25). Histological evaluation revealed that rats treated with hesperidin had morphological changes lighter than rats fed with DEN exclusively.

Conclusion: Altogether these results suggest that hesperidin may modify DEN-induced rat hepatocarcinogenesis. Further studies are necessary to clarify the actions of this flavonoid.

P68 - ROLE OF POLY (ADP-RIBOSE) POLIMERASES 1 AND 2 (PARP-1 AND 2) IN ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN MICE

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Introduction: PARP 1 and 2 are enzymes involved in DNA repair. DNA fragmentation in hepatocytes occurs early after acetaminophen (AAP) overdose. High DNA fragmentation can induce excessive PARP activity, which can lead to early oncotic necrosis. The aim of this report was to study the role of PARP-1 and PARP-2 enzymes in the pathophysiology of AAP-induced liver toxicity in mice.

Material and methods: Male WT (Parp1^{+/+}Parp-2^{+/+}), Parp-1 KO (Parp1^{-/-}) and Parp-2 KO (Parp-2^{-/-}) C57BL/6J mice were injected intraperitoneally with a single, sublethal (500 mg/kg) dose of AAP. Samples of liver (for histopathology, TUNEL, Western blot analysis) and blood (for aspartate (AST) and alanine (ALT) aminotransferase analysis) were collected at 6, 24 and 48 hours post injection (p.i.). Additionally, mice were injected intraperitoneally with a single lethal (700mg/kg) dose of acetaminophen to study the survival rate for 7 days.

Results: Edema, congestion and centrilobular necrosis (not apoptosis) were the main histopathological features, mainly at 24 hours p.i. in Parp-1^{-/-} and WT mice, while in Parp-2^{-/-} mice these damages were less severe. Western Blot analysis revealed PARP (1 and/or 2) activity in all groups. Biochemical analysis of blood samples showed non-significant increasing in ALT values in PARP-1^{-/-} mice at 24 and 48 hours p.i. By last, 100% of WT and Parp-1^{-/-} mice died by 48 hours p.i., while 30% of Parp-2^{-/-} mice were still alive at the end of the survival experience.

Conclusions: Results point to a protective role for PARP-1 activity, while PARP-2 appears develop a pathogenic role in acetaminophen-induced hepatotoxicity.

P69 - GILLS HISTOLOGICAL STUDY IN ZEBRAFISH (*Danio rerio*) LIKE WATER CONTAMINATION BIOMARKER AFTER BISFENOL - A EXPOSURE

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Introduction: BPA is a widespread contaminant of the aquatic environment, being the gills the major uptake sites of water contaminants and also targets used like biomarkers of aquatic contamination. In the present study, zebrafish (*Danio rerio*) has been used like a new experimental model in environmental contamination to study the gills histopathology, to evaluate the risk of the BPA presence in the environment like water contaminant.

Methods: Thirty males' zebrafish were randomly distributed in three aquariums to establish the groups of the study: Control group (n=10), and treated groups (n=20). Treated groups were exposed in water to 10 and 1000 µg/L respectively, during 14 days. Animals were sacrificed by MS-222 dilution. Immediately after death samples were taken out and fixed for histological processed.

Results: Histological modifications were observed in zebrafish gills exposed to 10 µg/L, hyperaemia and oedema processes were evident in the lamella together to microhemorrhagic processes. At electronic scanning microscopy it can be observed the degradation of the lamella surface.

In the gills of the zebrafish exposed to 1000 µg/L could be observed the same processed but more aggravated, including inflammatory focus presence. At electronic scanning microscopy exit of inflammatory exudative cells can be observed, and disorganization of the basal filaments and several inflammatory exudative cells.

Conclusion: Our results demonstrate that structural and ultrastructural study of the zebrafish (*Danio rerio*) gills could report information like biomarker, together to other biomarker in the BPA exposition.

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P70 - EXPOSURE TO ENDOCRINE DISRUPTOR AGENT 17 α -ETHINYL-ESTRADIOL IN TENCH (*Tinca tinca*). HISTOPATHOLOGICAL CHANGES IN TESTES.

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Introduction: The synthetic estrogen 17 α -ethinyl estradiol, used as a contraceptive substance and estrogen replacement therapy, reaches the aquatic environment from effluent from Wastewater Treatment Stations (ETAP). In the literature have been described alterations in the reproductive capacity of many aquatic organisms, especially fish, as a result of exposure to endocrine disruptor agent. This study aims at evaluating testicular alterations in tench (*Tinca tinca*) after chronic exposure to this substance.

Material and methods: Five groups of 15 each were used, three with intraperitoneal administration of 50 μ g/kg, 100 μ g/kg and 500 μ g/kg of 17 α -ethinyl estradiol, respectively, and two controls with and without vehicle (corn oil+methanol). The animals were euthanized, taking the testes for histological examination. Samples were fixed, processed and stained with routine techniques in histology, were photographed and examined statistically using the statistical package SPSS (version 19.0).

Results: The histopathological examination revealed changes in testis of exposed fishes. The seminiferous tubules showed variations both in number and normal morphology, even appearing devious forms, with a size bigger than usual form and with impaired normal histology of tubular epithelium, both available for loss as anarchic disposition. Sertoli cells showed changes indicative of degeneration. It was also observed a degeneration of intertubular tissue and Leydig cells. These injuries, aggravated by the increase in the dose of exposure, can adversely affect the reproductive capacity of this species

P71 - OPTIMIZED PROTOCOL TO OBTAIN RNA FROM MICRODISSECTED FORMALIN-FIXED, PARAFFIN EMBEDDED TISSUE: IMPROVEMENT OF TURBOT (*Psetta maxima L.*) TISSUE PREPARATION FOR LCM

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In fish pathology, one of the main problems we have encountered is the lack of specific antibodies to characterize the cell populations found in either normal or pathological tissues. Nevertheless, complete genome sequencing has been achieved for several fish species, thus it would be of interest to combine genetic and molecular techniques with morphological ones, to gain insight into fish histology and pathology. Laser-capture microdissection (LCM) is a technique for isolating highly pure cell populations from a heterogeneous tissue section, cytological preparation, or live cell culture via direct visualization of the cells. Until recently, only frozen section of tissue were used for the isolation of mRNA amenable to Q-PCR, microarray studies, generation of expression libraries and related techniques. However, the employment of frozen tissue lead up to greatly reduced cellular detail, which diminishes the ability to distinguish and isolate specific cell populations from complex tissues and organs. Otherwise, limited success in extracting high-quality total RNA from formalin-fixed paraffin embedded (FFPE) samples has been attributed to the ability of formalin to crosslink RNA and proteins as well as the addition of monomethylol groups to the bases. These processes are known to interfere with reverse transcription and amplification reactions. In this study digestive tract and lymphohaematopoietic organs from five healthy turbot (*Psetta maxima L.*) were sampled under RNase-free conditions to obtain RNA from FFPE microdissected samples. Detailed tissue processing will be discussed. A step-by-step analysis of RNA quantity and quality was carried out to identify and monitor possible critical points. The results demonstrate that it is possible to obtain high-quality RNA from FFPE microdissected samples.

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P72 - ASSESSMENT OF DIFFERENT MONOCLONAL ANTIBODIES FOR THE IMMUNOHISTOCHEMICAL DETECTION OF *Mycobacterium avium* subsp *paratuberculosis* IN TISSUE SECTIONS

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Introduction: The use of immunohistochemical (IHC) techniques in the detection of *Mycobacterium avium* subsp *paratuberculosis* (Map) is hampered by the lack of specific and sensitive antibodies. The aim of this study is to test different monoclonal antibodies (MoAb) produced in the frame of an European grant, in tissue sections showing different paratuberculosis lesions.

Materials and methods: Fourteen MoAb (10 purified and 4 culture supernatants) were assessed using Envision IHC technique, in samples from experimentally infected lambs showing focal lesions negative to ZN, multifocal forms with small amounts of bacilli and diffuse multibacillary lesions. In addition, two polyclonal Ab against Map, previously tested, were employed. An avian intestinal sample infected with *Mycobacterium avium* subsp *avium* (Maa) was also used.

Results: Only the four supernatant Abs gave positive results. Two of them detected Map antigens in focal lesions and in all the tissues harbouring Map, with lack of background, no unspecific immunolabelling and a better definition of the positive signal than the polyclonal Abs. In the remaining two, immunolabelling was weaker and unspecific staining was observed. All the four Ab cross reacted with Maa.

Discussion and conclusions: Negative results obtained with the purified Ab were probably due to their inability to detect the specific epitope of Map, or to its low concentration. Two of the supernatants (55.60.1A1.11 and 56.17.2A0) have shown to be good candidates for its use in IHC techniques, regarding the staining quality, bearing in mind that they can not distinguish between the two subspecies of *Mycobacterium avium*.

P73 - LIVER PATHOLOGY IN DOGS. COMPARATIVE STUDY OF THREE DIAGNOSTIC TECHNIQUES MATCHES.

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Introduction: Diagnostic techniques as ultrasound examination, true cut needle sampling or surgical excision are commonly used in clinic to determine liver diseases in dogs. This, coupled with the tendency to use less invasive methods for the study of the disorders, shows the importance of this comparative study of techniques matches.

Materials and methods: To compare diagnostic techniques, we studied 25 dogs subsequently to natural death or euthanasia. First, we did a complete ultrasound examination, to determine ultrasound pattern. Then, true cut needle samples were taken by ultrasound guided way. Finally, formerly necropsy was done, to take a sample of liver. Samples were fixed, processed, embedded in paraffin for subsequent cut and stained with hematoxylin-eosin. Examination of each sample was carried out by optical microscopy to classify all according to the guidelines of the WSAVA Standards for clinical and histological diagnosis of canine and feline liver diseases. Results were compared to observe the diagnostic matches.

Results: Diagnosis agreed completely in 11/25 cases, being compatible diagnoses. In 13/25 cases, one of the diagnose did not match with the other two. In 1/25 case, the three diagnoses did not match at all.

Discussion: The diagnostic overlap present in the study, as shown by others, confirm the reliability of the techniques studied and the validity of these test to complement each other. In addition, similar diagnostic results suggest that both ultrasonography and True cut needle sampling give us a similar amount of information about liver diseases. Choice of test to perform, as outlined by most authors, is given by clinical experience, possible diagnosis and most important, patient condition.

P74 - STUDY OF LIVER PATHOLOGY IN DOGS BY TRUE CUT NEEDLE BIOPSY SAMPLING. 25 CLINICAL CASES

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Introduction: Liver diseases in dogs have been widely studied in central Europe, United Kingdom and United States of America. However, knowledge of liver pathology in the Iberian Peninsula is poorly known. This study aims to determine the most common histopathological processes present in dogs near the area of Cáceres, using a routine technique such as the ultrasound guided true cut needle sampling.

Materials and methods: Samples were taken from 25 dogs after his death by natural death or euthanasia. True cut needle samples were obtained by ultrasound-guided way. Then, they were processed, embedded in paraffin for subsequent cut and stained with hematoxylin-eosin. We conducted a microscopic examination, looking at various parameters to classify all samples according to the guidelines of the WSAVA Standards for clinical and histological diagnosis of canine and feline liver diseases. Finally, we made a classification based on the number of samples that had each pathology.

Results: The most common pathology were congestion (12/25) and non-specific reactive hepatitis (12/25); followed by steroid induced hepatopathy (6/25) and presence of reactive hepatocytes (5/25). Chronic hepatitis, edema and congestive-hemorrhagic lesions were present in 4/25 cases. The rest of described lesions were present in 3 or less cases.

Discussion: Results described as most common histopathological findings not primary liver diseases. Both congestion and non specific reactive hepatitis, as described by various authors, are not problems arising from specific diseases. Linked to this, steroid-induced hepatopathy and the presence of reactive hepatocytes, have different origins and causes, in some cases not considered by the authors, such as the presence of glycogen within hepatocytes by a prolonged congestion phenomenon.

P75 - ANTIGENICITY OF TISSUES IN PARAFFIN BLOCKS STORED FOR YEARS WITH AND WITHOUT RE-EMBEDDING

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Introduction: It is known that certain antigens are damaged during long-term storage of tissue sections giving rise to spurious negative immunohistochemical results. However, the effect on antigenicity of either long-term storage or re-embedding of tissue samples in paraffin blocks has not been published.

Materials and Methods: Freshly done tissue sections from 64 paraffin blocks of canine mammary tumours tissue samples were used. Paraffin blocks had been stored for 10 (39) and 2 (25) years. In addition, 11 of the former had to be renewed because insufficient thickness for sectioning. The monoclonal VIM 3B4 (vimentin) and CALP (calponin) antibodies were used with the avidin-biotin-peroxidase complex method and results were evaluated semiquantitatively.

Results: Strong and widespread vimentin staining was seen in 73.5% and 81.3% of cases stored for 10 and 2 years, respectively. Similarly, strong calponin immunoreactivity was observed in 80.5% and 82.3% of cases at the same time points, respectively. Some 10% of cases stored for 10 years did not react with CALP antibody, while antigenicity to vimentin and calponin was completely lost in 45.5% of re-embedded tissue samples.

Discussion and Conclusion: The degradation of vimentin and calponin antigens seems to occur in a very low level in paraffin blocks stored for years. However, a small percentage of tissue samples lost calponin antigen after 10 years of storage. The process of re-embedding tissue samples in paraffin blocks produced the complete loss of antigenicity in about half of the cases. Thus, it should be taken into account to increase reliability of immunohistochemical studies.

P76 - GIEMSA OR DIFF-QUIK? WHICH IS THE OPTIMAL STAIN FOR QUICK AND AFFORDABLE EVALUATION OF *Helicobacter* INFECTION BY GASTRIC CYTOLOGY?

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Introduction: Colonisation with *Helicobacter* spp. has been linked to gastric disease in dogs and cats, much as *H. pylori* has in humans. Since resolution of clinical signs can be achieved with antibiotic treatment, interest lies in identifying the fastest and most cost-effective method of detecting these bacteria using *in situ* cytology performed by the clinician. Two commonly-used stains, Diff-Quik and Giemsa, were evaluated to determine cost-effectiveness and veterinarians' preference.

Materials and Methods: Gastric brush cytology smears were obtained from animals referred for necropsy to the FMV/UTL Pathology Department. Three slides (for May-Grünwald-Giemsa, Diff-Quik and Giemsa staining) were obtained from each animal and 27 *Helicobacter*-positive animals were identified. Veterinarians at the University Hospital were asked to evaluate the slides based on the ease of *Helicobacter* identification. The cost of each stain per slide was calculated.

Results: Veterinarians systematically preferred Diff-Quik to Giemsa when evaluating slides, except for slides containing large quantities of mucus, when 72% of veterinarians preferred the Giemsa stain. Cost per slide was calculated as 1.68€ for Diff-Quik and 0.16€ for Giemsa.

Conclusions: Giving veterinarians the tools to identify *Helicobacter*-positive smears *in situ* may enable prompt antibiotic treatment, which could result in faster resolution of clinical signs. This study shows that, although more expensive, Diff-Quik is preferred by veterinarians for rapid identification of a wider range of *Helicobacter*-positive cytology smears.

P77 - DETECTION OF LEISHMANIA IN CANINE SKIN BIOPSIES: APPLICATION OF AN ALTERNATIVE IMMUNO-HISTOCHEMICAL METHOD

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Introduction: Canine leishmaniosis (CanL) is a chronic systemic disease of the dog caused in the Old World by protozoa of the species *Leishmania infantum*. CanL is endemic in several countries around the Mediterranean basin and Portugal. Skin lesions are among the most common manifestations of CanL, but detection of *Leishmania* in conventional Giemsa-stained samples or on histopathological sections stained with hematoxylin and eosin is not always straightforward, and non-visualisation of the parasite does not rule out its presence.

Material and Methods: The present study reports on an immunohistochemical alternative to conventional techniques for the detection of *Leishmania* amastigotes in skin biopsies obtained from dogs with suspected/confirmed leishmaniosis. A streptavidin–biotin immunohistochemical technique, with canine hyperimmune serum as the primary antibody, was used to examine 40 canine skin biopsy specimens. Histopathological (HE) and immunohistochemical (IHC) methods for detection of *Leishmania* were assessed.

Results: Diffuse or nodular granulomatous dermatitis was a consistent finding in all cases examined by routine histopathology, with visible amastigote forms in 17 (42.5%) cases. Using IHC, amastigote forms of *Leishmania* were easily observed within macrophages in 18 (45.0%) skin biopsies. Parasite load was considered mild in 5 (27.7%), moderate in 6 (33.3%) and intense in 7 (38.8%) cases.

Discussion and Conclusion: The results further confirm this technique as a useful tool for the diagnosis of CanL when parasites are not clearly detectable on the slide and when pathological signs clearly point to the disease

P78 - ADVANTAGES OF THE CYTOBLOCK PREPARATION SYSTEM

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Introduction: Over the last few years, cytoblocks have routinely been prepared using fluids for cytological examination at the Faculty of Veterinary Medicine Pathology Laboratory of the Technical University of Lisbon. This routine procedure has enabled an optimal technical procedure to be established. It has also enabled diagnosis in a number of cases where diagnosis based on observation of direct smears from the same fluids proved impossible.

Materials and method: Fluids from fine needle aspirations were obtained for over 30 cases. After centrifugation, the pellets obtained were resuspended in a freshly-prepared 4% agarose solution. Once solidified, the pellet was treated like a tissue, although carefully handled due to its fragility.

Results: All attempts to prepare cytoblocks were successful. The following cases were diagnosed: haemorrhage, inflammatory exudates (both fibrinous and purulent), cystic mammary tumours, perianal gland tumours and lymphomas. Cytoblocks from lymphomas permitted the use of immunohistochemistry for cell phenotyping using CD3, CD79 α and Pax5 antibodies.

Discussion and Conclusions: Although most cytological aspirates are sufficient to obtain a diagnosis, the fact is that fluid-rich lesions may present problems due to the scarcity of cells and deficient cell preservation. The decision to prepare a cytoblock in such cases may be taken immediately after aspiration. There are no special requirements, refrigeration of the fluid or addition of the same amount of 10% formalin being sufficient. Attention is drawn to this additional diagnostic tool, which may prove very useful for replacing incisional biopsies.

**P79 - HISTOCHEMISTRY AND IMMUNOHISTOCHEMISTRY
USING FROZEN TISSUE SECTIONS**

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Introduction: This study reports on the fine-tuning of histochemical and immunohistochemical staining techniques using frozen tissue sections, and assesses their ability to provide a rapid diagnosis.

Materials and Methods: The study used fresh material obtained immediately after biopsy (canine mammary gland tumour) or necropsy (ovine lymph node, liver and lung) procedures. The material was frozen at -20°C in a cryostat and sectioned at 5µm. To determine the feasibility of rapid diagnosis, a routine stain (hematoxylin-eosin) was used. Other histochemical and immunohistochemical stains were used to enhance these techniques with frozen sections.

The following histochemical stains were used: Periodic-Acid-Shiff to test for neutral mucins; reticulin, to test for reticular fibres; and Oil Red (the only technique specific for frozen sections) for lipid identification. The immunohistochemical antibodies used were anti-Vimentin (NCL-VIM, Novocastra, dilute 1:50) and anti-CD3 (ab898, Abcam, diluted 1:50); the streptavidin-biotin-peroxidase complex method was applied.

Results: Satisfactory results were obtained for all stains and for immunohistochemical detection of antibodies in frozen tissues, when compared with paraffin-embedded specimens.

Discussion and Conclusion: In conclusion, there is a clear advantage in using frozen sections to provide a rapid diagnosis. Histochemical staining techniques displayed greater affinity for tissue structures in frozen sections, reducing the time required and obviating some steps in the procedure. In immunohistochemical assays, antigen recovery was not generally necessary, and results were specific.

P80 - HISTOCHEMICAL STUDY OF PERIPOLAR CELLS IN SHEEP

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Introduction: Peripolar cells are granulated cells located at the vascular pole of the renal corpuscle. Peripolar cells were first described in 1979, by Ryan, Coghlan and Scoggins, in sheep. Even though these cells have already been described, some histological and physiological characteristics remain unknown. .

This paper reports on a histochemical analysis of peripolar cells in sheep, in normal and damaged kidneys.

Materials and Methods: Kidney samples were stained with Hematoxylin-Eosin (HE), Toluidine Blue, Periodic Acid Schiff, and Masson Trichrome.

Results: Peripolar cells were identified by their location in the vascular pole, and by the presence of cytoplasmic granules, which stained pale red with Hematoxylin-Eosin, blue with Toluidine Blue, purple-magenta with Periodic Acid Schiff and red with Masson Trichrome.

In damaged kidneys with dilated Bowman's space, peripolar cells were easily identified, whereas in kidneys with microthrombi, identification was more difficult.

Discussion and Conclusion: Hematoxylin-Eosin, Periodic Acid Schiff and Toluidine Blue do not enable a clear identification of peripolar cells. Masson Trichrome proved to be the best histochemical method for their identification.

In kidneys with dilated Bowman's space, peripolar cells were readily identified, suggesting, that preservation of the normal histological features of Bowman's space and the absence of intraglomerular lesions enables greater precision in the identification of peripolar cells.

P81 - ASSESSMENT OF SCRAPIE PHENOTYPE VARIATION IN CASES DETECTED BY ACTIVE SURVEILLANCE

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Scrapie affects sheep and goats and is the most common form of transmissible spongiform encephalopathy (TSE), a group of disorders which includes Creutzfeldt-Jakob Disease (CJD) in humans and Bovine Spongiform Encephalopathy (BSE).

Scrapie is an endemic disease recognised for over 250 years in a number of European countries. As no obvious clinical or epidemiological connection to human disease has been revealed to date, scrapie is considered non-pathogenic for humans, at least under natural conditions. However, it has been shown that sheep can be experimentally infected with BSE, giving rise to the possibility that BSE may have been accidentally introduced into sheep. This prompted the implementation, in 2002, of a European Union surveillance plan for scrapie in small ruminants, carried out in all member States.

Between 2003 and 2010, a total of 456,923 small ruminants were screened in Portugal; atypical scrapie was detected in 417 Portuguese sheep and 7 goats. Four outbreaks of classical scrapie were identified: two in 2008 and two in 2010, with 14 confirmed cases occurring in four different flocks.

This paper reports on the phenotypic features of these scrapie cases, identified by histopathology, immunohistochemistry and *Western Immunoblotting* as well as *Prnp* sequencing. Results confirmed that atypical scrapie in Portugal was *NOR98*, a new form first described in Norway, and that classical scrapie in Portugal may emerge against a background of enzootic atypical scrapie.

Hence, in contrast to other European countries, where classical scrapie has been enzootic for decades, these data indicate that, in Portugal, *NOR98* is the predominant form of TSE in small ruminants.

P82 - GENE EXPRESSION CHANGES IN THE MEDULLA OBLONGATA OF PRESYMPTOMATIC NATURALLY INFECTED SCRAPIE SHEEP

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Introduction: The precise molecular and cellular mechanisms that underline the pathogenesis of scrapie and other prion diseases are still poorly understood. The identification of genes showing differential expression during the preclinical stage of prion infection could help us to identify novel risk genes of prion diseases. In addition, it may allow the identification of new biomarkers other than the prion protein.

Materials and Methods: The gene expression patterns from the medulla oblongata (MO) of presymptomatic naturally scrapie infected versus non-affected animals were compared using the custom CVI high-density sheep oligo microarray. Microarray results for a selection of genes that displayed changes in their expression were confirmed using Real Time qPCR. The relationship between gene expression profiles and the appearance of the scrapie related lesions (prion deposition, gliosis and spongiosis) were also studied.

Results: Over 80 probes displayed significant expression changes greater than 2-fold, from which 44 genes were identified, many of them encode proteins that, according to gene ontology classification, were involved in cell adhesion, transcription and immune response.

Discussion and Conclusion: Using a microarray approach we have been able to identify new regulated genes/sequences as well as to confirm some earlier published ones reported in experimental scrapie infected animals. Finally, the potential impacts of linked gene expression changes in MO in the scrapie preclinical stage will be discussed.

P83 - BIOASSAY OF CATALAN ATYPICAL SCRAPIE ISOLATES IN OVINE AND BOVINE PRNP MURINE TRANSGENIC MODELS

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Introduction: Lately the transmissible spongiform encephalopathies (TSE) outbreaks diagnosed in small ruminants have increasingly been classified as Atypical Scrapie. Several aspects regarding its transmission are uncertain such as the ability to infect species other than small ruminants and the risk that it might pose to human beings.

Materials and Methods: In Catalonia all TSEs diagnosed in small ruminants have been atypical cases. Four Catalan isolates were pathologically characterized and inoculums were prepared for bioassay. Intracerebral inoculation was conducted into two transgenic mice models overexpressing ovine and bovine prnp (Tg338 and boTg110). Pathological and immunohistochemical evaluation of mice brains was performed.

Results and Discussion: In tg338 mice the attack rate was of a 100% for all 4 inocula with an incubation period of 245 days post inoculation (dpi) (70 dpi for Classical scrapie). Pathological analysis of mice brains revealed no differences between the four isolates and clearly differed from the classical ones.

None of the isolates infected boTg110 mice (>700dpi). However two isolates transmitted on second passage (attack rates: 16%-58% and 51%-354 dpi respectively). The pathology in those brains was reminiscent of the one observed in the same model when inoculated with BSE.

Conclusion: According to these results atypical Scrapie is potentially transmissible to cattle. It needs to be investigated whether the passage through cattle causes the atypical prion to change its properties and whether this should pose additional risk to human beings.

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P84 - STUDY OF MITOCHONDRIAL APOPTOSIS PATHWAY IN NATURALLY INFECTED PRE-SYMPOMATIC SCRAPIE SHEEP

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Neuronal loss is one of the characteristics of scrapie neuropathology. Previous analysis of brains from sheep naturally infected with scrapie in a terminal stage did not detect a clear induction of apoptosis although molecular changes were evidenced. As neuronal death could be occurring early in scrapie, we have developed a neuropathologic and gene expression study of sheep affected with scrapie in a preclinical stage. Histopathology, pathological prion protein (PrP^{sc}), Bax and activated caspase-3 immunolabelling was developed in four central nervous system areas: Obex, diencephalon, prefrontal cortex and cerebellum. Moreover, TUNEL and NeuN immunolabelling was performed in the Obex. Finally, we analysed the expression of 6 genes involved in the regulation of the mitochondrial pathway of apoptosis. The findings have been compared with those observed in animals with terminal scrapie. PrP^{sc} immunolabelling was evident in the four areas as well as a neuropile spongiform change in lower levels than terminal animals. Cytoplasm Bax immunostaining was observed in presymptomatic medulla oblongata but was not extended to the hypothalamus like in the terminal stage, indicating the progression of Bax induction with the curse of the disease. Although neither caspase-3 immunostaining nor the TUNEL technique detected neurons with appearance of apoptosis, NeuN immunolabelled cell counting determined that preclinical animals have already suffered neuronal loss in equal or lower degree than in terminal animals. Finally, expression profiles indicated that the mitochondrial pathway of apoptosis is activated with higher intensity than in terminal sheep and confirmed the implication of genes such as BAX in the disease.

P85 - AN OUTBREAK OF ABORTION IN GOATS ASSOCIATED WITH TYPE 1 CAPRINE HERPESVIRUS INFECTION IN THE IBERIAN PENINSULA

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An abortion storm occurred in late 2010 in a Murciano-Granadina breed intensive dairy goat flock, located in Almería (South-East of Spain). Nearly 10% of the does aborted in a 45-days time span, during the autumn lamb season. In the flock, stillbirths and neonatal mortality were observed, while genital lesions in adult goats were not. Samples from the brain, lungs, liver, kidney and placenta were obtained from 3 aborted fetuses. Upon microbiological examination, common agents of abortion in goats were not detected. Histological examination revealed multifocal coagulative necrosis with mild inflammatory infiltration in the periphery of the foci in the liver, lungs and kidneys. The size of the lesions ranged from 50 to 200 micrometers. Similar lesions, but smaller, and surrounded by a slight gliosis response, were also scattered in the brain tissues. Cariorrexis and carioliolysis were observed in the centre of the necrotic spots, with some amphophilic intranuclear inclusion bodies being clearly distinguished. Caprine herpesvirus type 1 (CpHV-1) DNA was detected in real-time PCR and CpHV-1 antigens were detected using immuno-enzymatic techniques. These findings suggest that CpHV-1 may be implicated in abortion outbreaks in goats and that the virus circulate in the Iberian Peninsula. In our knowledge this is the first description of abortions caused by natural infection of this agent in the Iberian Peninsula

P86 - TUBERCULOSIS INFECTION IN THREE SHEEP FLOCKS IN SPAIN

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Introduction: Infection of sheep by members of the *Mycobacterium tuberculosis* complex (MTC) has been occasionally reported worldwide; normally associated with individual cases or flocks. In this study, three outbreaks of tuberculosis in sheep in Galicia (Northwestern Spain) that occurred between 2009 and 2010 are described.

Material and Methods: The comparative intradermal tuberculin test and bacteriological, molecular and histopathological studies were performed.

Results: MTC infection was confirmed in the three flocks. In all cases lesions were confined to the thoracic cavity and retropharyngeal lymph nodes, varying from small granulomas consisted mainly of macrophages and Langhan's giant cells to typical tuberculous granulomas, consisting of coalescing areas of caseous necrosis with central mineralization surrounded by the cellular components of chronic inflammation. *M. bovis* and *M. caprae* were isolated and characterized from two and one flock, respectively.

Discussion: This study provides evidence of tuberculosis in sheep in Spain in three different mixed flocks with various ewes affected. The source of the infection in the present outbreaks seems to be the contact with infected cattle and goats in the same area. Our findings suggest that sheep are able to maintain tuberculosis infection and could play a role in the transmission of tuberculosis, having the potential to act as an additional domestic reservoir. *Conclusion:* The role of sheep as a host for *M. bovis* should be taken into account when regarding tuberculosis control in mixed herds of ruminants or where different domestic ruminants are kept as the same epidemiological unit.

**P87 - PORCINE RESPIRATORY DISEASE COMPLEX (PRDC):
DETECTION OF PATHOGENS ASSOCIATED AND
SEROLOGICAL STUDY**

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Introduction: PRDC is a multifactorial disease of finishing pigs. The most commonly isolated pathogens are porcine reproductive and respiratory syndrome virus (PRRSv), swine influenza virus (SIV), porcine circovirus type 2 (PCV2), *Mycoplasma hyopneumoniae* (Mh) and *Actinobacillus pleuropneumoniae* (App).

The aim of the present study was to elucidate the complexity of the pathogens involved in PRDC in a farm of Gran Canaria, correlating the histopathological findings, serological profile and production parameters.

Materials and Methods: Forty animals were randomly selected at weaning. For serological study, 22 animals were sampled at 38, 77 and 136 days after birth. ELISA was performed against Mh, PRRSv, PCV2, SIV and App. At the time of slaughter, carcasses were weighed, and the prevalence, pneumonia patterns and the percentage of pulmonary affection were determined. Pulmonary tissues samples were taken and routinely processed. The samples were evaluated according to described by Livingston et al. (1972). For immunohistochemistry, antibodies against Mh, PRRSv and PCV-2 were used.

Results: The average time of shipment to slaughter was 196 days, with 72.17 kg of carcass weight. Thirty per cent of animals showed cranioventral bronchopneumonia with 10.78% of pneumonic lesions. The histology grades were represented by 33, 25, 17 and 25% (grades I-IV, respectively). Mh antigen was detected in 67%. Serological results showed a delayed response of vaccine antibodies against Mh, coinciding in time with a seroconversion to PRRSv.

Conclusion: We have demonstrated the role of PRRSV, which could be compromising the animal's response to vaccination against Mh, which not only become infected and develop the disease, having been confirmed by immunohistochemical detection.

P88 - CONGENITAL ABNORMALITIES OF FEMALE GENITAL TRACT IN CATTLE: AN ABBATOIR SURVEY

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Introduction: Congenital abnormalities of the female genitalia, though rare, have increased importance on herd fertility, limiting the ability of a cow to conceive. Clinical or pathological surveys on the occurrence of reproductive diseases in Portugal are limited. Also, the genital tract is seldom completely evaluated during carcass inspection. The aim of this work was to determine the occurrence of congenital defects on the female genitalia detected at the slaughterhouse.

Materials and Methods: Detailed inspection of the excised female genital tract was performed during a 3 months period, in parallel to the slaughter line, in three different slaughterhouses of the north-west area of Portugal. A total of 1866 carcasses were evaluated (1146 and 720 for beef and dairy breeds, respectively). Information about age, breed, origin and presence of other lesions was also recorded.

Results: Congenital abnormalities were found in 1.3% of the genital tracts (25:1866). A higher number of cases was found in beef (15:25) than in dairy breeds (10:25) although corresponding to similar relative occurrence for productive aptitude (1.31% and 1.39%, respectively). It was more common in young animals, under 2 years-old (18:25). Still, 3 cases were present in animals older than 3 years. In beef breeds, freemartinism was identified in 40% (6:15) of the cases, while 60% (9:15) of situations showed vaginal frenulum. In dairy breeds, freemartinism was found in 90% (9:10) of the cases, while vaginal frenulum was found in only 10% (1:10). Overall, the freemartinism represented 0.52% and 1.25% of abnormalities found respectively for beef and dairy breeds.

Conclusion: This survey found an increased occurrence of congenital abnormalities in comparison to the described in other countries, particularly concerning representation of freemartinism in dairy breeds.

P89 - AN STUDY OF THE PREVALENCE OF PARAMPHISTOMOSIS IN SLAUGHTERED CALVES IN CASTILLA Y LEÓN

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Introduction: Paramphistomosis is a ruminant digestive parasitosis caused by different species of trematodes (Digenea), belonging to several genera (*Paramphistomum*, *Calicophoron* and *Cotylophoron*). The objective of this study was to assess the prevalence of paramphistomosis in bovines in the slaughterhouse, using parasitological and histopathological methods.

Materials and methods: A total of 481 slaughtered calves, 452 younger and 29 older than 30 month-old, respectively, coming from different herds in the Castilla y León region (Spain) were examined in 2010. The presence of the parasites in the different regions of the rumen was evaluated and the adult flukes counted and stained. Tissue samples were taken for histological examination.

Results: A total of 17 calves (3.53%) were parasited (only one older than 30 month-old). The total parasitic burden was 1170. The ruminal atrium was the region showing the highest number of flukes (68,01%). According to the morphology, *Calicophoron daubneyi* (Dinnik, 1962) Eduardo, 1983 is the species responsible for the infection in bovines in Castilla y León. Mature flukes were attached to the ruminal papillae. In the lamina propria there was a diffuse mononuclear infiltration, with eosinophils and globular leucocytes, always associated with the areas of parasite attachment.

Discussion and conclusions: These preliminary results show that *Calicophoron daubneyi* is the main Paramphistomum species causing bovine paramphistomosis in Castilla y León. The prevalence seems to be low (3.53%); however, it has to be considered that the majority (64.7%) of the animals examined were young and the possibility of getting the infection at this moment, would not have been too high.

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