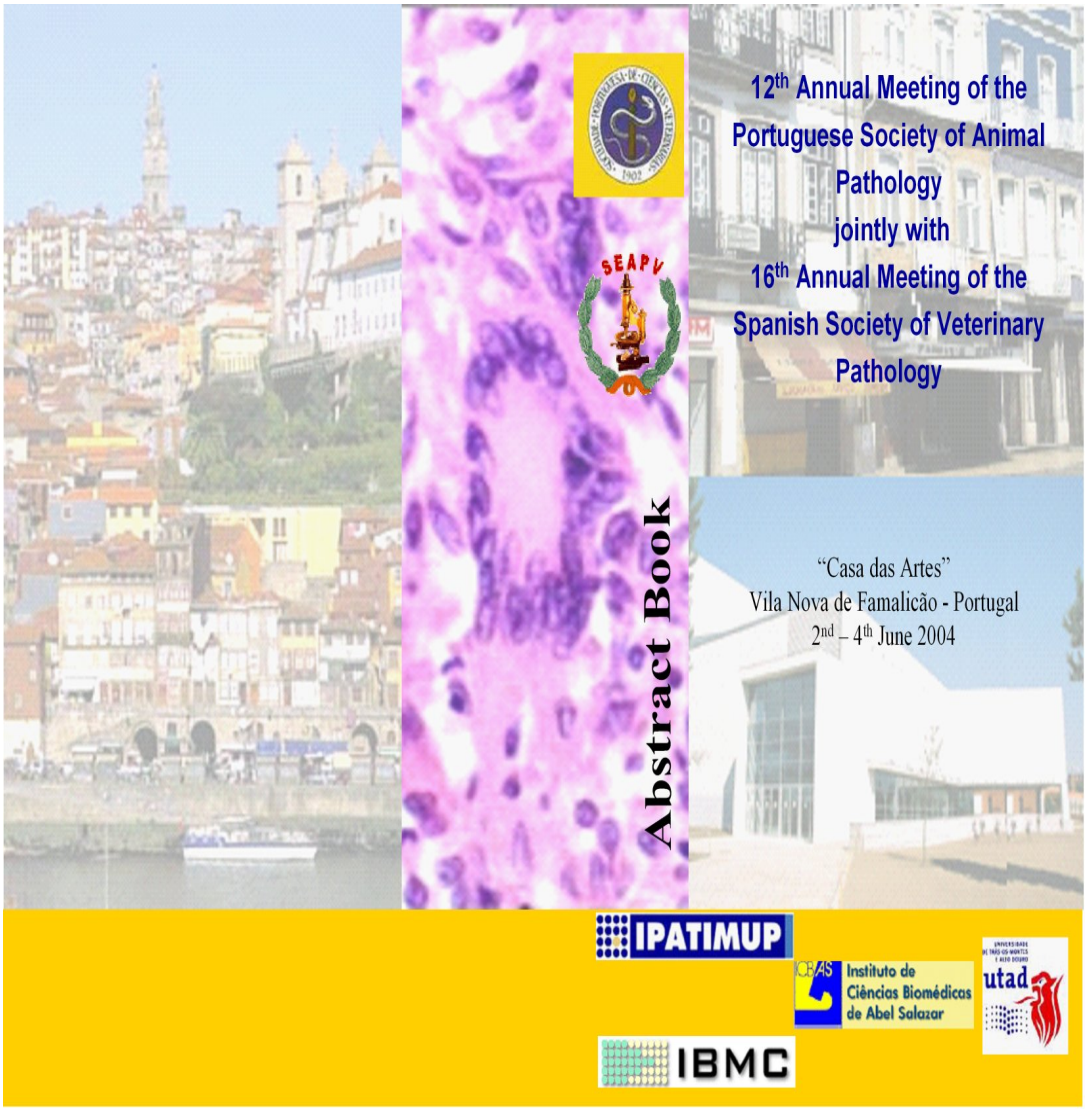
12th Annual Meeting of the
Portuguese Society of Animal
Pathology
jointly with
16th Annual Meeting of the
Spanish Society of Veterinary
Pathology
SEAPV
Abstract Book
"Casa das Artes"
Vila Nova de Famalicão - Portugal
2nd – 4th June 2004
IPATIMUP
Instituto de
Ciências Biomédicas
de Abel Salazar
utad
IBMC

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Sociedade Espanhola de Anatomia Patológica Veterinária (SEAPV)

Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP)

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Propecuária

7 Coffee

Vieira de Castro

The Oral Communications and Posters can be done in Portuguese, Spanish or English

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PROGRAMME

Wednesday, 2th June, 2004

16h00 Registration "Casa das Artes" de V. N. de Famalicão

18h00 **Moderators:** Maria Castaño e Fátima Gärtner

Sessão Plenária – "***Cloning: A Pathologists Perspective***" – Professora Susan Rhind, Royal (Dick) School of Veterinary Studies, University of Edinburgh. **(Plenary Lectures)**

19h00 Wellcome Reception "Casa das Artes" V. N Famalicão

Thursday, 3rd June, 2004

8h30 **Moderators:** Mª Isabel Quiroga e António Afonso

"***Gill Diseases in Salmonids***" – Professor Hugh Ferguson, University of Stirling/Institute of Aquacultura, Scotland. **(Plenary Lectures)**

9h30 **Oral Communications**–AQUATIC PATHOLOGY

Moderators: Eduardo Rocha e Mª Isabel Quiroga

Evolución de los Parámetros Hematológicos de Rodaballos (*Scophthalmus maximus*) infectados experimentalment por *Enteromyxum scophthalmi* – R Bermúdez. MJ Redondo, P Álvarez. MI Quiroga y JM Nieto.

The Leucocyte Response of Sea Bass (*Dicentrarchus labrax L.*) to Intraperitoneal Vaccination against Vibriosis and Pasteurelosis. – Sónia Gomes and António Afonso.

Pathobiology Associated with Bucephalus sp. (Trematoda digenia) Metacercariae in the Grey Mullet: Histological Aspects and Parasite Molecular Identification. – Pedro Rodrigues, Cristina Moreira, Filipe Pereira, Susana Pina and Teresa Barandela.

10h00 **Coffee break / Poster view"**

10h450 **Comunicações livres** – TUMOUR PATHOLOGY I

Moderators: Conceição Peleteiro e Augusto Faustino

Comparison of Canine Mammary Tumours Routine Histopathological Diagnosis Given by Different Grups of Pathologists and Applicability of the 1999 WHO's Classification. – Juana Martín de las Mulas and Laura Peña

11h30 **Evaluation of Interfase AgNOR Quantification Technique in Canine Diagnosis of High and Low Grade Malignant Lymphoma. A Study of 24 Cases.** – Jorge Correia, José Cabeçadas, Victor Alves, José Silva and Maria Peleteiro.

A Comparative study of Proliferation in 3 Canine T-Cell "Centroblastic" Malignant Lymphomas, Using Ki-67 Immunohistochemical Detection. – Jorge Correia, José Cabeçadas, Victor Alves, José Silva and Maria Peleteiro.

Enteropathy associated T cell Lymphoma with Epitheliotropism in a Horse. – A Rodriguez-Bertos, A Benito, B Sánchez, A Sainz-Terreros, J I Vargas, M Castaño and J M Flores.

Lymphoma-Like Lesions in Clafes Infected by Theileria annulata. – Sandra Branco, João Orvalho, Alexandre Leitão, Isodora Pereira, Manuel Malta, Isabel Mariano, Tânia Carvalho, Rui Baptista and Conceição Peleteiro.

Immunohistochemical Evaluation of the Mitogen Activited Protein Kinase ERK ½ Signalling Pathway in Contagious Respiratory Tumours of Sheep and Goats. – M de las Heras, A Ortín, M Pérez de Villareal and A Benito.

Human Lung Adenocarcinomas Contain Proteins Related with the *gag* Gene but not with the *env* Gene products of the JAAGSIEKTE Sheep Retrovirus. – M de las Heras, M Palmarini, L Borderias, R Alvarez, J J Rivas, A Artal, W Wallace, D Salvatore, A Ortín, A Benito and J M Sharp.

Haemangiomas in Young Horses: Na Histopathological and Immunohistochemical Study. – Rafael Zafra, Elena Mozos, José Carlos Estepa, Antonio Espinosa de los Monteros, Aniceto Méndez and José Pérez.

Tumores Primitivos en el Sistema Nervioso Central del Perro: Caracterización Histopatologica e Imunohistoquímica de los Casos. – Enric Vidal, Carme Costa, Anna Domènech, Raül Tortosa y Martín Pumarola.

The Value of Cell Proliferation and Oestrogen Receptors in the Biological Behaviour Assessment of Bovine Ovarian Granulosa Cell Tumours. – A J Durán, C Pérez-Martínez, R A Gracía-Fernández, J Espinosa, A Escudero and M J Gracía-Iglesias.

13h00 **Lunch –** Restaurante D. Antonieta

14h30 **Moderadores:** Aniceto Méndez e Juana de las Mulas

"***Skin Tumours as it Pertains to the Dog and Cat***" – Professor Michael Goldschmidt, University of Pennsylvania. **(Plenary Lectures)**

15h30 **Oral Communications** – TUMOR PATHOLOGY II

Moderators: Juana de las Mulas e Laura Peña

Squamous Cell Carcinoma in a *Gorilla Gorilla*: Immunopathological Analysis. – Elena Mozos, Hugo Fernández-Bellon, Rafael Zafra, José Pérez and Jesús Fernández.

Immunoexpression of MHC Class II Antigen in Canine Cutaneous Histocytoma. An Histological and Ultrastructural Study. – Isabel Pires, Anabela Alves, Paula Rodrigues, Rui Mestre and Carlos Lopes.

Canine Ocular Melanomas: Folow-up of 15 Cases. – Sónia Miranda, Carolina Naranjo, Teresa Peña and Toni Ramis.

Normal and Hiperplastic Canine Prostate – DNA Ploidy Cytophotometric Data. – J F Silva, V D Alves and J J Correia.

Three Cases of Canine Prostatic Tumours with Peculiar Features. – J F Silva, F Seixas, P Rodrigues, J Prada, R Rayan-Carreira, L Pinto and M A Pires.

A Case of Prostatic Micrometastases of Hemangiosarcoma in a Dog (*Canis familiares*). – J F Silva.

Analysis of p53 Mutations and Polymorphisms in Dog and Cat Squamous Cell Carcinoma. - Salomé Pinto, Célia Lopes, Luis Cirnes, Rui Maio, Júlio Carvalheira and Fátima Gärtner

16h30 **Coffee break / Poster view**

17h00 **Oral Communications–** MISCELLANEOUS

Moderators: Maria dos Anjos Pires e M. Azevedo Ramos

Clinical and Pathological Study of an Outbreak of Ovine Facial Eczema in Asturias. – M Fuertes, A Martínez, J Benevides, J Menéndez, J F García Marín, V Pérez and M C Ferreras.

Immunohistochemistry and Electron Microscopy Correlation in Canine Mekel Cells. – Gustavo A Ramírez, Tomás García-Caballero, Francisco Rodríguez, José R Jaber, Antonio Fernández and Antonio Espinosa de los Monteros.

Forensic pathology in Livestock Insurance Reports. Experience in Cattle in Southwest of Asturias. – J González Fernández.

Preliminary Results on na Outbreack of Mortality in Grower Iberian Pigs Associated with Miocardial and Encephalic Lesions. – Bernadino Moreno, Gorka Aduriz and Pedro Villar.

Characterization of Circulating T Cell Polpulations in Canine Leishmaniosis. – S Martorell, M Costa, H Fernández, T Ramis and L I Ferrer.

Fat Embolism Causing Hemothorax in a Cat. – Eva Sierra, Pedro Herráez, Francisco Rodríguez, Gustavo Ramírez and Antonio Espinosa de los Monteros.

Main Causes of Condemnation in Wasting Pigs at Slaughter. A Study for one Year.- J Martínez, P J Jaro, B Peris, P Segura, J Porcel, V J Cayo, G Adúriz, J C Ibabe, I Ziluaga and J M Corpa.

18h15 **Moderators:** Maria Castaño e Aniceto Méndez

"La Docencia de la Anatomia Patológica Veterinaria en España". – Manuel Pizarro Diaz, Prof. Titular de Anatomía Patológica y Marta Gonzalez Huecas, Prof.Titular de Anatomía Patológica y Vicedecana de alunos de la Facultad de

Veterinaria de Madrid (Universidad Complutense de Madrid) **(Plenary Lecture)**

"Futuro de la Docencia de la Anatomia Patologica Veterinaria en España en el Marco de la Convergencia Européa." – Librado Carrasco Otero. Prof. Titular deAnatomía Patológica y Vicedecano de Ordenación académica de la Facultad de Veterinaria de Cordoba (Universidad de Cordoba). **(Plenary Lecture)**

18h45 **SEAPV** (Annual General Meeting)

SPPA (Annual General Meeting)

20h30 **Social Dinner – "**QUINTA DO COVELO"

Friday, 4th June, 2004

8h30 **Moderators:** J Ferreira da Silva e Lluis Luján

"***Advantages and Handicaps of quantitatibe Morphology in Histology and Histopathology – The Past the Present and the Future of Stereological and other Image Analysis Methods***". – Professor Eduardo Rocha, Instituto de Ciências Biomédicas Abel Salazar (ICBAS) da Universidade do Porto. **(Plenary Lecture)**

9h30 **Oral Communications**– PATHOLOGY OF EXOTIC ANIMALS

Moderators: Valentin Peréz e Lluis Luján

High Prevalence of Glomerulonephristis in Iberian Lyn (Lynx pardinis): Histopathological and Immunohistochemical Study.- M Ángela Jiménez, Belén Sanchez, Pilar García, Margarida Galka, Pilar Fernández, Alberto Benito, Luis Galego, M Eugenia Palencia, Clara Calvo, Célia Sánchez and Laura Peña.

Pathological Findings in Wild and Zoo Animals: A Retrospective and Comparative Analysis. – M P Martín, M A Quevedo, J M Aguilar and E Mozos.

Estudio Neuropatológico del Encéfalo de un Gorila Albino. – M Márquez, E Vidal, C Costa, R Tortosa, A Domenech, I Ferrer y Martí Pumarola.

Pathological Findings in Sauria Pets. – Elena Mozos, Mª Josefa Ruiz, Rafael Zafra, Rafael Guerra and José Peréz.

H-DAF Transgenic Pig Liver Prevents Hyperacute Rejection after Orthotopic Xenotransplantation in Baboons: Immunopathologic Study. – A Sánchez, P Ramírez, G Pino, R Chavez, M Majado, V Minitiz, A Muñoz, C G-Palenciano, J Yelamos, M Rodríguez-Gago and P Parrilla.

Acute gastric Dilatation in Laboratory-Housed Baboons. – N García, A Sánchez, L Martínez, A Muñoz and P Ramírez.

10h30 **Coffe break / Poster view**

11h00 **Moderators:** Juana Flores e Luis Gómez Gordo

"***Vasculitis in Dermatopathology***" Doctor Marianne Heimann, Institut de Pathologie et Génétique, Loverval, Belgium. **(Plenary Lecture)**

12h00 **Oral Communication**– INFECTIOUS DISEASES I

Moderators: Rogério Monteiro e Luis Gómez Gordo

Clinico-Lesional Study of Experimental Mastitis caused by *Mycoplasma agalactiae* in Goats. – Ayoze Castro-Alonso, Christian de la Fé, Antonio Fernández, Francisco Rodríguez, Bismarck Poveda and Pedro Herráez.

Pathology of Bovine Tuberculosis in the Red Deer and the Wild Boar in Central Spain. Evidence for Transmission Routes and Epidemiology of the Disease. – Mª Paz Martín, Ursula Höfle, Joaquin Vicente, Marta Barral, Joseba Garrido, Ramón Juste and Christian Gortazar.

Experimental Infection with *Mycobacterium avium* subespecies *paratuberculosis* of Calves Previously Immunized. – M Muñoz, A Verna, O Moreno, C García-Parente, L E Reyes, J Benavides, M Fuertes, M C Ferreras, J F García-Marín and V Pérez.

Abscess Disease in Sheep: Pathology, Diagnostic and Use of Autovaccines. – L Lujan, E Blasco, E Biescas, M Pérez, F Vargas, I Cuartielles, R Baselga, E Fantova and J J Badiola.

Different Roles of CD4 and CD8 T Cells *Chlamydophila abortus* in a Mouse Model of Primary Infection. – C M Martínez, A J Buendia, N Ortega, J Sanchez, M R Caro, J A Navarro, M A Gallego, F Cuello and J Salinas.

Diagnosis of Tuberculosis in the Lymph Nodes of Wild Boards Hunted in la Rioja Community. – L E Reyes Avila. O Moreno Buitrago, A Verna, S Jiménez Palacios, J Medrano Gómez, V Pérez and J F García Marín.

13h00 **Lunch –** Restaurant D. Antonieta

14h30 **Moderators:** Anabela Alves e Fátima Gärtner

"***Feline Mammary carcinomas as an Animal Model in Comparative Oncology***" – Professor Massimo Castagnaro, Universitá delgi Studi di Padova. **(Plenary Lecture)**

15h30 **Oral Commnunications**– INFECTIOUS DISEASES II

Moderators: Dolors Fondevila e Madalena Monteiro

Evaluation of the Pathogenicity of Several Strains of *Mycobacterium avium* subspecies *paratuberculosis* in an Experimental Infection of Lambs. – A Verna, M Muñoz, O Moreno, C García Parente, L E Reyes, M Geijo, M I Romano, F Paolicchi, J F García Marín and V Pérez.

Macroscopic and Microscopic Lesions of Paratuberculosis in Sheep and Goats in the Area of Lisbon. – Augusto Afonso, Teresa Albuquerque, Susana Mendes, Fernando Boinas and Alice Amado.

Valoración de la Prueba de la Intradermoreacción para el Diagnóstico de la Tuberculosis en Animales Infectados por *Mycobacterium avium* subspecies *Paratuberculosis*. – Ana Balseiro, José Miguel Prieto, Alberto Espí and Juan Francisco García Marín.

***Myoporum laetum* Poisoning in Cattle**. – L E Reyes Avila, O Moreno Buitrago, L Quevedo Neyra, J M San Miguel and J F García Marín.

Study of Porcine Respiratory Disease complex associated to Circovirus type 2 in Postweaning Pigs ina apig farm in Gran Canaria.- M Andrada, M J Rodríguez, A Sanz, I O Quesada, N García, G López, E Polo, L Dominguez and A Fernández.

Etiopathogenic Study in Naturally Infected Pigs with Porcine Circovirus. – A Andrada, M J Rodríguez, I O Quesada, E Sierra, E Rodondo, A Afonso and A Fernández.

16h30 **Coffe break / Poster view**

17h00 **ORAL COMMUNICATIONS–** INFECTIOUS DISEASES III

Moderators: Mª dos Anjos Pires e Augusto Faustino

Susceptibility Patterns of Inbred Mice to Experimental Vírus Infection Suggest a Significant Influence of their Genetic Background. – Pedro Faísca, Dao Bui Tran Anh and Daniel Desmecht.

Preliminary Results in the Epidemiological Studies of the JAAGSIEKTE Retrovirus Infection in a Naturally Infected Flock. – M de las Heras, A Ortín, M Pérez de Villareal, A Benito, L M Ferrer, J A García de Jalón and J Fanlo.

Relationship between Acute Phase Protein Levels and Lesion Scores in PMWS Clinically Affected Pigs. – F J Pallarés, S Gómez, J Seva, J J Cerón, J S Martínez, F Tecles, S Martínez, M A Gómez, A Bernabé and A Muñoz.

Immunohistochemical Characterization of Humoral immune Response on Paraffin-Embedded Tissues of Pigs Inoculated with the Classical Swine Fever Virus. – J L Romero-Trevejo, P J Sánchez-Cordón, A I Raya, M Pedrera, A Núñez and J C Gómez-Villamandos.

Pathological Characterization of the Nervous Form of Ovine Maedi-Visna in Natural Cases. – J Benavides, N Gómez, M C Ferreras, M Fuertes, J F García Marín and V Pérez.

Pathological Characterization of a Porcine-Adapted BSE Prion. – F Diaz-San Segundo, F J Salguero, J Castilla, A Gutiérrez-Adán, B Pintado, B Parra, M A Ramírez, M J Cano, A Brun and J M Torres.

19h30 **Bus to Porto**

20h30 **Trip with Dinner on "Douro" River**

PLENARY LECTURES

CLONING: A PATHOLOGISTS PERSPECTIVE

Susan M. Rhind BVMS, PhD, MRCPath, MRCVS

Royal (Dick) School of Veterinary Studies, University of Edinburgh.

The presentation will focus on nuclear transfer ('cloning') from the point of view of the pathologist and will use examples both from personal experience and from the literature to explore the evidence and consider the implications for the future of this technology both for reproductive and non-reproductive strategies.

The present nuclear transfer (NT) process is recognised as being inherently inefficient with losses occurring from the earliest developmental stages and throughout pregnancy. Further, in those animals which survive to term, post natal viability is often poor and can be associated with a range of pathological phenotypes. However, until relatively recently, the focus on cloning has, perhaps understandably, been very much on the development of the technology and the vigorous debate surrounding the ethics of human cloning.

Nevertheless, it is now well recognised that abnormalities in a range of organ systems are features of failed clones. Several recent studies have identified placental abnormalities such as rudimentary development, placentomegaly and enlarged cotyledons in ruminants. In addition, defects affecting a range of other organs have also been reported including those affecting kidneys, lungs, heart, liver, CNS and muskuloskeletal systems.

Whilst some of these phenotypes appear to be conserved across species (e.g. cardiovascular and placental defects), others have only been reported in single species (e.g. obesity in mice; body wall defects in sheep). It also appears that the severity of phenotypes reported is variable – in general mice, goats and pigs present with more subtle phenotypes than those seen in failed sheep and cattle clones. Notably however, there are many reports where 'abnormalities' are reported in particular organs with no further pathological descriptions given thus precluding any consideration of possible underlying pathogenesis in these cases. In ruminants, some of the features seen in clones are similar to those described in the 'large offspring syndrome' (LOS). LOS is a syndrome associated with a variety of embryo manipulations and is certainly not unique to cloning. This

illustrates the key point that in order to define the aetiological factors driving the abnormalities specific to cloning, careful experimental planning is required to ensure that the exact contribution of the NT technique to the induction of the abnormalities is clear and is not being masked by those induced by more general embryo manipulation techniques.

A retrospective study on failed cloned lambs revealed a series of abnormalities including renal developmental defects (consistent with lower urinary tract obstruction), musculoskeletal defects (including body wall defects and angular limb deformities), overgrowth, cardiovascular and hepatic abnormalities.

Gathering together the evidence from failed clones is currently difficult as there are relatively few descriptive pathology based reports. Nevertheless, what is emerging is consistent with some of the phenotypes recorded having similar characteristics to those attributed to aberrant expression of imprinted genes i.e. allele-specific methylation of DNA sequences established during embryogenesis. For example, the phenotype of overgrowth and body wall defects in sheep has similarities with Beckwith-Wiedeman syndrome (BWS) in humans. In this syndrome, affected patients show changes including overgrowth, body wall defects, skeletal and renal defects. One cause implicated in this syndrome is loss of imprinting with resultant biallelic expression of the imprinted gene insulin-like growth factor 2 (IGF2; which has a key role in regulation of foetal growth and development, having autocrine, paracrine and endocrine effects). Recent studies on this axis in LOS lambs have shown that epigenetic change in the IGF2 receptor (IGF2R) is associated with fetal overgrowth after sheep embryo culture. Similar studies in mice have also revealed epigenetic instability with reduced H19 expression in heart, liver and kidney. Further evidence that certain phenotypes are likely to be epigenetic in origin is implied by the lack of heritability e.g. in association with the obese phenotype recognised in mice.

In summary, the current situation we face is that some apparently healthy animals can be born using nuclear transfer technology however in many cases, there are serious pathological phenotypes induced as a result of the cloning process (and/or the general embryo manipulation procedures) – significantly, there is no recognised rationale behind which reconstructed embryos will survive the ‘cloning lottery’.

A further point to consider given the likelihood of human stem cells being used in the future for non reproductive cloning is the potential for the introduction of defective manipulated cells into living human tissue. Given the knowledge of the imprinting defects and phenotypic abnormalities in clones, it is certainly possible that these cells develop in an inappropriate fashion within the treated subject. Of particular concern would be the potential for neoplastic development in such cells since it is recognised that epigenetic modifications can be associated with transcriptional changes in oncogenes and in tumour suppressor genes. There is however no current good evidence for an increased tendency to neoplasia in cloned animals however very few clones have yet reached an age where an increased propensity for cancer development may be noted.

In conclusion, in order to dissect the mechanisms involved in clone pathology, it is crucial that pathologists, developmental biologists and molecular biologists work together to investigate this issue using carefully planned and controlled studies.

GILL DISEASES IN SALMONIDS

Hugh W. Ferguson,

Professor of Diagnostic Pathology & Microbiology,

Institute of Aquaculture, University of Stirling, Scotland

Gill diseases are one of the most important categories of diagnoses in a routine diagnostic laboratory. This talk will provide a general overview of gill diseases in fish including commercially reared species, in both freshwater and the marine environment. Topics covered will include those areas of basic structure and function needed to understand some aspects of pathophysiology, and therefore what is the best way to approach controlling the disease and limiting any deleterious effects on the host. Illustrations will include gross, biopsy, routine paraffin sections, as well as scanning and transmission electron microscopy. Diseases used as examples to illustrate various processes will include bacterial infections such as bacterial gill disease (*Flavobacterium branchiophilum*), water quality parameters, viral, and parasitic disease.

CLASSIFICATION OF EPITHELIAL TUMORS OF THE SKIN: NEW INSIGHTS

Michael H Goldschmidt, Professor Veterinary Pathology

University of Pennsylvania, School of Veterinary Medicine

Classification of epithelial tumors arising from the skin is based on the predominant differentiation pattern as well as their biologic behavior. The initial part of the presentation will be an overview of the revised classification published in 1998 by the WHO. The following will be discussed briefly:

Epithelial tumors without squamous or adnexal differentiation
Basal cell tumor, basal cell carcinoma.

Tumors of the epidermis
Papilloma, inverted papilloma, squamous cell carcinoma *in situ*, squamous cell carcinoma, basosquamous carcinoma.

Tumors with adnexal differentiation
Follicular tumors,
Nailbed tumors
Sebaceous and modified sebaceous gland tumors
Apocrine and modified apocrine gland tumors

Tumors metastatic to the skin
Feline pulmonary carcinoma with digital metastases

The second part of the presentation will focus on papilloma virus associated neoplasms in the dog and the cat primarily those cases seen in immunosuppressed and immunoincompetent animals receiving bone marrow transplants and a progression of these lesions to invasive and metastatic carcinoma.

ADVANTAGES AND HANDICAPS OF QUANTITATIVE MORPHOLOGY IN HISTOLOGY AND HISTOPATHOLOGY – THE PAST, THE PRESENT AND THE FUTURE OF STEREOLOGICAL AND OTHER IMAGE ANALYSIS METHODS

Eduardo Rocha, PhD

Lab. Histology and Embryology, Dept. Microscopy, Institute of Biomedical Sciences Abel Salazar – ICBAS, University of Porto, Portugal; Lab. Cellular and Molecular Studies, Interdisciplinary Centre for Marine and Environmental Research – CIIMAR, Porto, Portugal.

Qualitative observations are essential for microscope studies, either in routine diagnosis or in research. Humans can actually be very good in discriminative analyses; so, these will continue to play a key role in both initial and later stages of solving scientific problems. However, qualitative descriptions are inherently limited, often very subjective, and not prone to statistical analyses, being in addition difficult to correlate with many sorts of analytical and functional data (usually expressed in numbers). Naturally, in the need of using quantitative methods, the hallmark of contemporary scientific research, such approaches start to be introduced in microscopy studies. Many of them were (and still are) made as quite naïve "technical" approaches, because they do not taking into due account that when observing histological sections we are essentially seeing a two-dimensional (2D) space, representing structures or phenomena, that exist or occur in three-dimensions. Key questions must be asked beforehand: What to measure in sections? How the data I will get relate with the real three-dimensional (3D) biological world? It was noticed for long (i.e., XVII Century) that simple measurements made in 2D have remarkably little to do with the 3D world. Methodologies were continuously developed to take the right measurements from sections and transform them into real meaningful (3D) data. Such methodologies, based on both mathematical and statistical principles, were coined stereology (only in 1961). This term should not be confounded with morphometry, or even with image analysis. Indeed, many still do consider

stereology as a quite different “businesses”, as image analysis is usually based in simple 2D analyses. Actually, however, stereology is plainly a way to analyse diverse kinds of images (not necessarily derived from physical sections), and so stereology can be seen as a sub-set of a broad field of image analysis. In the past, most quantitative morphology tools, including stereology, heavily relied on strict assumptions regarding the size, shape and orientation of the particles (tissues, cells, organelles, etc.) in analysis. These limitative assumptions were mere approximations, in most practical cases quite far from reality, which inevitably introduced an unknown amount of bias in the estimates. So, the classic methods were theoretically sound but most relied in assumptions that seldom apply to biological scenarios. In the early 1980s, after the publication of the cornerstone disector principle paper, a cascade of new generation of methods started to appear, free from the old assumptions, and ready to provide unbiased estimates of both particle size and number, irrespective of their 3D characteristics. These methods rely and on a new set of strategies to properly sample specimens in three dimensions. In fact, despite arbitrarily samples (including the sections) may suffice for qualitative approaches they are usually inadequate for quantitation. The recent generations of techniques are known as “design-based stereological methods” or, at times, simply by “unbiased stereology”. The idea of "never ever not measure the reference space that contain the particles" was also introduced, to avoid the "reference trap" (conclusions based on ratios). The use of (20-100 μm) thick sections for quantitative studies is now common, taking advantage of optical slicing with either high numerical aperture lens in conventional microscopy or when using confocal microscopes. Despite the theoretical background may be found cumbersome for most biologists, in practice, stereological methods are based on observations made on 2D sections, applying 0D (i.e., point), 1D (i.e., linear) or 2D (i.e., planar) test probes and simply counting, e.g., the number of test points falling into the given structure of interest or the number of intersection points of test lines with the structure surface. In current design-based stereology, there is also a new trend towards using spatial estimators evaluating small 3D samples of structure when serial optical sections from thick specimens are recorded and evaluated. One advantage of such approaches, which can merge the speed / power of image analysis software with the sound stereological principles and tools, is the opportunity to avoid generating either vertical sections or isotropic

uniform random sections, the two only options over which size estimators can be applied to grant an unbiased estimate. One of the usual disadvantages attributed to quantitative microscopy is that it is time-consuming. This idea impels people into either not go for it at all, to use semi-quantitative ratings, or to embark on the "push the button" approach much seen in now countless image analysis softwares. These provide an overabundance of parameters that can be measured, but most, when not all, stick to “flat” 2D information, biased by a number of factors and related in an unknown way with the real 3D biological world. If in the past good stereological approaches were done manually, today the new technologies do help a lot in implementing sound techniques, thus improving cost-effectiveness. Several sound techniques can be implemented manually (and fast). However, some modern stereological tools can only be implemented via software. Fortunately, there are a few programmes to that can help microscopists with no strong theoretical background to perform cutting-edge stereological approaches, which in the past were not at the reach of the vast majority of histologists or pathologists. Most parameters are now easily measured, and proved very appropriate to peculiar situations, like cancer grading by volume-weighted mean volumes. The focus now should be on proper sampling (sectioning) and handling (orientation) of the material before getting the sections. Not accounting this, jeopardizes the validity of the results, whatever morphometric measurements are made. Image analysis has its value in several situations that biological microscopists face today, and algorithms are continuously improved. Three-dimensional reconstruction software can be also a precious tool for today’s microscopists. In this lecture I will illustrate a few concepts and applications (and practical problems) with a few examples derived from our research in diverse species (from fish to man), namely in cerebellum, liver, and cumulus-oocyte complexes. Future trends will pass by new methods for automatic measurement of geometrical features of microscopical structures, based on 3D image processing or surface triangulation, like the fakir method for surfaces, developed for confocal stereology; in parallel with new interactive stereological methods. Second-order stereology will be increasingly applied for the quantification of the histological texture and 3D spatial arrangements.

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VASCULITIS IN DERMATOPATHOLOGY

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INTRODUCTION:

If some vasculites are easy to recognize, some are much more subtle and doesn't seem to enter the classical histological criteria's we have been introduced to.

The purpose of this presentation is to review all the histological criteria's we may consider in the diagnostic of vasculitis or vasculopathy.

The presentation will be constructed following those chapters.

- **Cutaneous vascular system**
- **Clinical presentation**
- **Histological criteria's at the level of the vessels**
- **Histological criteria's in the surrounding tissues**
- **Classification**

Case presentation with correlated histological lesion will then illustrate those notions.

They will be limited to the cat and dog.

CUTANEOUS VASCULAR SYSTEM:

The cutaneous vascular system is rich,

organized in three plexuses.

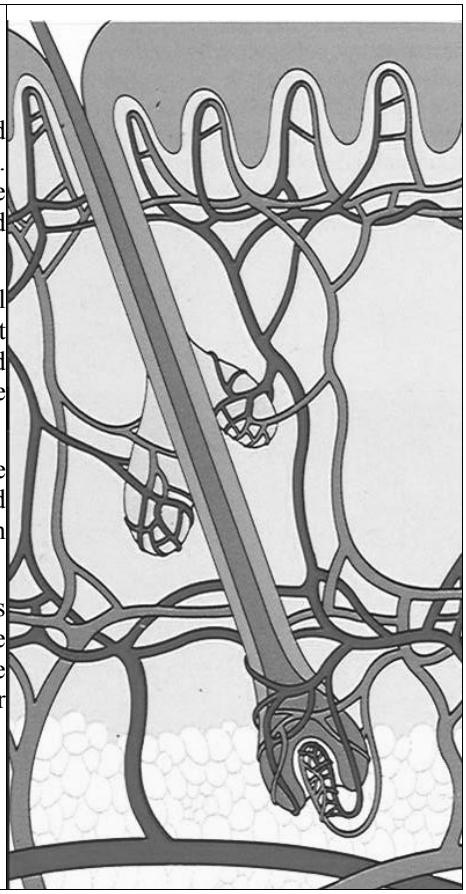
Two within the dermis: the superficial and the deep one and one within the subcutis. Those three plexi are connected but the connection varies between the species and the skin areas.

Small arterioles arise from the intradermal plexus and develop vascular meshes that surround the follicles, sebaceous and apocrine glands. The apocrine glands are also supplied by the subcutaneous plexus.

The superficial dermal plexus give also rise to a small papillary dermis plexus composed of small arterioles leading to capillaries then to post-capillaries venules.

In human being at this level, arteriovenous shunt exists which interfere with the thermoregulation. I have not found in the literature such structure described in dog or cat.

illustration from Pathology of the Skin by P McKee



CLINICAL PRESENTATION:

The systemic signs are: fever, anorexia, lethargy, myalgy, arthralgy, ptyalism, and adenopathy when the lesions are enough systemic to induce such symptom

The only **specific sign** that concern today are the cutaneous ones.

They are extremely variable and depend on the size of the vessels involved, the concerned plexus and the etiology.

We will therefore not go through all of them in the histopathological presentation but let say that the clinical sign within the anamnesis that will bring us to look more closely for vascular lesions if they are not obvious are: **ecchymoses, hemorrhages, necrosis, sharply demarcated ulcer, dermatopathy is oriented to the extremities.**

The other possible clinical signs are less specific: **Squamosis, alopecia, papules, pustules, and bulla.**

According to some, the lesions that have **a triangular shape or are sharply demarcated** may also suggest a primary vasculopathy.

HISTOLOGICAL CIRTERIA'S:

a) Vascular changes:

The terms vasculitis and vasculopathy are not standardized among pathologists. During my formation, the definition of vasculitis was rather rigid. In order to consider a vasculitis, at least four criteria's had to be observed within a single vessel. Among those criteria's, the presence of leukocytes within the vessel wall was mandatory. It appears actually that those criteria's will still be significant if met in different vessels. These changes may even be local within a vessel.

A new classification also arise :

***Cell-rich vasculitis**, vasculitis in which the inflammatory infiltrate is abundant and **cell poor vasculitis**, vasculitis with very little inflammatory component.*

The cell poor vasculitis may be also referred as vasculopathy because the inflammatory infiltrate is very scant but I prefer to use vasculopathy more largely. Hypertensive changes, atherosclerosis, arteriosclerosis, amyloid deposition…are also vasculopathy.

The criteria's to consider are:

♦**Endothelial cells hyperplasia,** is observed very early after any type of vascular insult and therefore is not pathognomonic of a vasculopathy

♦**Vessel's wall thickening** by an eosinophilic, hyaline, or fibrillar material that is local or diffuse, associated occasionally with fibrinoid degeneration. This fibrinoid degeneration is not as obvious as in human being.

♦**The presences of a leukocytic infiltrate** which could be abundant (cell-rich vasculitis) or minimal (cell-poor vasculitis). The infiltrate can be neutrophilic, lymphocytic, eosinophilic or histiocytic.

♦**Vascular extravasations** of blood and/or plasma and fibrin. Inducing microscopic or macroscopic hemorrhages (petechia, ecchymoses), or tissular oedema.

♦**A cellular necrosis: of the** endothelial, the leukocytes or of the vascular wall with picnosis or caryolysis. Leukocytoclasic vasculitis is rare in dog or cat.

♦**Presence of thrombi:** fibrin, cryoglobulin...

In more chronic lesions:

♦**Vascular proliferation**

♦**Dense hyalin thickening** of the wall,

♦**Rarefaction** or **fading of the endothelial cells**, decreased intradermal vessels.

b) <u>Changes within the other structures</u>

Vasculitis induces numerous changes within the epidermis in the annexae stroma and nerves that will be as much signal to look for vascular lesions.

They are not pathognomonic most of the time however.

1. **At the epidermal level**: coagulation necrosis, ulceration, intracellular œdema, spongiosis, basal vacuolation, occasional hypertrophy in acute

lesions and atrophy in ischemic vasculopathy. Pustules formation with hyperkeratosis, parakeratosis may sometime accompany vasculitis (hyper immune etiology). In drug reaction, bulla may form.

2. **At the level of the follicles:** cellular edema (at all level), individual cellular apoptosis, hyalinization and coagulation necrosis in the center of the follicle. In chronic lesions, atrophy with follicular fading will be observed. Occasionally the hair shaft will even be surrounded only by basal membrane only, without inflammatory reaction. In deep vasculitis, the fading of the follicle start from below. This may be quite pathognomonic of an ischemic dermatopathy.
3. Glands: cellular edema giving a ragged appearance to the apocrine glands, atrophy.
4. The stroma will be site of hemorrhages, œdema, fibrin deposition and collagenous hyalinization in chronic lesions. Occasionally a collagenolysis (German Shepherd dog vasculopathy). At the level of the ears, cartilage eosinophilia and necrosis.

The etiology may be suggested from those various signals.

For instance:

- A vasculitis at the level of the superficial plexus small vessels (capillaries, post-capillary veins) associated to epidermal changes such as spongiosis, neutrophilic exocytosis, pustules and superficial folliculitis suggest primary bacterial infection (staphylococci hypersensitivity).

- Post-capillary veins vasculitis of every plexus without epidermal response evokes an inside-out process such as systemic disease.

- Hyalin thrombi are a call for septicemia.

- Deep annexial fading suggests ischemic pathogenesis.

Classification:

Numerous classifications have been proposed: by the clinical aspect, by its distribution, by its morphological aspect...

The doctor C. Outerbridge de UC Davis recently proposed a classification by the etiology that will allow a better efficacy in the clinical follow-up and the patient therapy.

1) Infectious vasculitis: bacterial, mycobacterial, viral, mycotic, rickettsial, due to protozoan or helminthes.
2) Non infectious vasculitis:

a) Exogenous antigen
- drugs: vaccine, serum, hyposensibilisation, sulfa, l'itraconazole, isoquinolone derivatives…
- food additives

b) Endogenous antigen
- neoplasm's
- diseases such as: plasmocytic pododermatitis, ulcerative colitis, SLE, CDLE, dermatomyosis, rhumatoid arthritis

c) Unknown origin. We classified them then:

1) By the type of involved vessels, their size, location

3) By the inflammatory infiltrate.

Conclusions :

1. When must we consider the diagnosis of vasculopathy :

When at least four criteria's within the vessels are met but not necessarily within the same vessel. When the vessels lesions are more subtle, changes within the surrounding structures must be search to support the notion of primary vascular pathogenesis (more frequent in cell-poor vasculitis and ischemic dermatopathy).

2. Can we use as criteria of vasculopathy only the changes within the epithelium, the annexes and the stroma?

No. But when such lesions as punched out ulcer, annexal fading… are seen, a vasculopathy must be search within the biopsies and suspected to be the initial trigger of the dermatopathy. Even if the 4 initially required criteria's are not met. All the criteria's that are described for the surrounding tissue are non pathognomonic except maybe for the fading of the annexae. Be sure the biopsy is deep enough also. The vasculopathy may be within the subcutis (ischemic dermatopathy, post-vaccinal dermatopathy)

3. When must we consider that the vasculitis-vasculopathy is the primary mechanism of the dermatopathy?

When there is not in the surrounding tissue histopathological changes that may induce the vasculopathy by continuity (ulcer, mastocytoma, eosinophilic inflammation, inflammation induced by autoimmune, hyperimmune, infectious… inflammation).

Or when the vasculitis is the prominent lesion.

4. Does an IN-OUT process, an internal metabolic or neoplastic is often accompanied by vascular changes.

It is my experience but not systematically.

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FELINE MAMMARY CARCINOMAS AS AN ANIMAL MODEL IN COMPARATIVE ONCOLOGY

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1- Introduction

Mammary tumours are the third most frequent neoplasm of cat (Schmidt and Langham 1967, Dorn et al 1968, Patnaik et al 1975, Carpenter et al 1987). Feline mammary carcinomas (FMCs) represent between 80% and 96% of feline mammary tumours (Hayden and Nielsen 1971, Carpenter et al 1987, Moulton 1990), and they are commonly considered very aggressive because they grow rapidly and metastasise to regional lymph nodes, lung, liver and spleen (Hayden and Nielsen 1971, Hayes and Mooney 1985, MacEwen and Withrow 1996). The interval between feline mammary carcinoma (FMCs) detection and the death of cats may vary from less than 6 to 12 months (Hayden and Nielsen 1971, Hamilton et al 1976, Weijer et al 1972, Weijer and Hart 1983).

Several factors such as age, diameter of the primary tumour, histologically tumour-positive lymph nodes, mitotic figures, extent of necrosis, and completeness of surgical treatment evaluated histologically were found independently to influence the post-surgical survival time in FMCs (Weijer and Hart 1983). The type of surgery, conservative versus radical, was significantly related to the disease-free interval but not to the survival time (MacEwen et al 1984).

Based on these data, we have begun some years ago to study the ethio-pathogenetic factors of mammary carcinoma spontaneously occurring in cats. In this brief review, we have summarized the results obtained by our group and the future perspectives opened by the obtained data.

2 - Feline mammary carcinomas as a model

Spontaneous tumours in domestic animals represent an useful model to study of human cancer because many factors such as the relatively high incidence of some cancers, the large body size and the shorter overall life span. Furthermore, domestic animals are outbreed and live in the same human environment. These factors facilitate the comparison to human cancers and allow a more rapid determination of essential end point parameters such as post-surgical survival time and disease-free interval. However, up to some years ago only few data were available on many aspects of animals cancers.

a. Proliferation indexes

Proliferation indexes, such as the AgNOR and Ki-67 indexes, were studied in FMCs. The nucleolar organizer regions (NORs) are rRNA coding segments of DNA closely associated with nucleoli (Darnell et al 1990). They also contain a group of argyrophilic proteins which allow NORs to be visualized by means of silver staining techniques in histological sections (Ploton et al 1986). These regions are therefore commonly referred as argyrophilic-NORs (AgNORs). The quantity and distribution of AgNORs have been proved to be related to proliferating activity in different types of human neoplasms (Derenzini et al. 1990, Derenzini and Trerè 1991).

The Ki-67 antigen is a non-histone, highly protease-sensitive nuclear protein assembled by polypeptide chains with an apparent molecular weight of 345 and 395 kD (Gerdes et al 1991). This poorly characterised heterodimer was expressed in all phases of continuously cycling cells except G0, but it was not present in resting cells entering the cell cycle from the G0 to the early part of G1 (Gerdes et al 1984). The Ki-67 index obtained by immunostaining with specific monoclonal antibody correlated well with the bromodeoxyuridine labelling index (Moriki et al 1996) and therefore is generally considered a good marker of cell proliferation.

When applied to FMCs (Castagnaro et al., 1998a, Castagnaro et al., 1998b), both proliferation markers had showed values very similar to what reported in human breast cancer (HBC) and allowed the identification of two populations of mammary carcinomas with different post-surgical prognosis. The detection of FMCs with more aggressive course may also be used to test new therapies for the more malignant variant of HBC.

b. Grading systems

Morphological assessment of the degree of differentiation provides useful prognostic information in HBC (Bloom and Richardson, 1957; Black *et al*., 1975). A modification of the original Bloom and Richardson method by Elston *et al.* has been successfully applied to human mammary cancer, and its value as an independent prognostic factor demonstrated by a study of a large number of patients, with long-term follow-up (Elston and Ellis, 1991). We have attempted to adapt the human grading system to FMCs (Castagnaro et al., 1998). The histological grading was based on three main features: degree of tubule formation, nuclear and cellular pleomorphism, and accurate mitotic count obtained from a defined area. Immunohistochemical examination with an anti-actin antibody was used for the accurate detection of "in situ" carcinomas. The FMCSs were graded as well differentiated carcinomas (WDCs - 12.7%), moderately differentiate carcinomas (MDCs - 60%) or poorly differentiated carcinomas (PDCs - 27.3%). In HBC the range of percentages for each grade is 11-33 for WDCs, 23-52 for MDCs and 25-66 for PDCs (Bloom and Richardson, 1957; Elston and Ellis, 1991). These wide ranges clearly reflect the subjective nature of histological grading. Nevertheless, our results with FMCSs are in general accord with the ranges for HBC. Furthermore, the study showed an apparently "protective" effect of myoepithelial differentiation in tumour cells: all cats with MDC displaying a high degree of actin expression were alive after the first post-surgical year. Interestingly, human breast carcinomas with a biphasic cellularity (myoepithelial and epithelial components) are recognized as having a good long-term prognosis (Koss *et al.,* 1970; Ellis and Elston, 1995).

c. Molecular studies

More recently, our studies have been focused on the molecular features of FMCs with a particular interest on the steroid receptor status and oncogenes.

It is well known that estrogen receptor (ER) plays an important role in human mammary cancer biology but only recently it was shown that two ERs, ER-α e ER-β, are present (Mosselman et al. 1996). The expression of ER-α in a given tumor is positive a prognostic factor. However, the presence of multiple alternative mRNA ER-α isoforms has been claimed to play a role in the development of resistance to hormonal therapy in HBC (Fuqua 2001). Mouse

and rat animal models have been proved to useless because they lack of multiple isoforms of ER-α (Lu et al., 1999). We have recently sequenced the feline ER-α (Bargelloni et al. 2002). Feline ER-α shows a 94% homology with the human ER-α but, more interestingly, we were able to identify several alternative splicings of exon 2 (Δ2), 3 (Δ3), 4 (Δ4), 5 (Δ5), 7 (Δ7) and isoforms lacking of a segments of exon 2 and 4 and of the entire exon 4 (Δ2-4). The alternative mRNAs code for ER truncked or detective proteins that may play the some role hypothesized for HMC.

The studies on oncogenes carried out in collaboration with the Comparative Oncology Center, Institute for Cancer Research and Treatment, University of Torino, have been focused on RON (*stk)* and MET receptors. Constitutive activation of the RON kinase (obtained by replacement of the RON extracellular domain with a protein dimerization motif) induces a motile-invasive phenotype, characterized by constitutive cell dissociation, 'scattering', invasion of extracellular matrices and formation of aberrant epithelial tubular structures *in vitro* (Santoro et al, 1996). In HBC, a remarkable increased expression of the *RON* gene has been shown (Maggiora et al., 1998). Therefore we have attempted identify the *RON/stk* homologue gene in cats and to study its expression in FMC.

Feline *stk* sequences were found highly homologous to the *stk* and *RON* gene exons that encode the juxtamembrane and transmembrane domains of the stk and RON receptors (De Maria et al., 2002). Western blot and immunohistochemical analysis on *stk* expression and distribution in FMCS revealed a very close similarity to the human counterpart suggesting that these feline tumours are a suitable model to test innovative approaches to therapy of aggressive human breast carcinomas.

d. Conclusions

Our studies on proliferation activity, grading systems, estrogen receptors and *stk* expression indicate that FMCSs share many features with human mammary cancer. Although many other pathogenetic factors such as c-neu expression, other steroids receptors status and the role of oncosuppressors need to be investigated, FMCs may represent from now on a useful model to test new

therapeutic approaches like new chemotherapeutic regimens, gene therapy and the subsequent biological modifications.

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COMUNICAÇÕES LIVRES

EVOLUCIÓN DE LOS PARÁMETROS HEMATOLÓGICOS DE RODABALLOS (*Scophthalmus maximus*) INFECTADOS EXPERIMENTALMENTE POR *Enteromyxum scophthalmi*.

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El cultivo de rodaballo adquiere cada día mayor importancia debido a la gran demanda que existe de este producto y a la escasez de capturas de ejemplares silvestres. Diversas enfermedades afectan a la cría de este pez, condicionando la rentabilidad de su producción. Recientemente, las enfermedades parasitarias han obtenido una mayor relevancia, destacando el "síndrome de la cabeza hundida" o enteromixosis intestinal del rodaballo, observada por primera vez en granjas de rodaballos de Galicia y que cursa con una elevada morbilidad y mortalidad.

En el presente trabajo hemos analizado los parámetros hematológicos básicos de rodaballos normales y aparentemente libres de enfermedad, comparándolos con los parámetros de congéneres procedentes de la misma granja y criados en idénticas condiciones, que fueron expuestos a infección por *Enteromyxum scophthalmi*. Los objetivos de este trabajo son valorar el comportamiento de los distintos tipos celulares sanguíneos del rodaballo bajo infectados por *Enteromyxum scophthalmi*, y establecer un protocolo de toma de muestras y análisis fácilmente repetible.

Para la realización de este trabajo se diseñaron dos experimentos en los que la infección experimental se produjo por cohabitación. La toma de muestras sanguíneas se realizó bajo anestesia previa de los peces, mediante venopunción de la vena caudada. Con la sangre obtenida se realizaron microhematocritos y extensiones sanguíneas, en las que posteriormente se realizaron los recuentos de los principales tipos celulares sanguíneos de estos peces (linfocitos maduros, linfoblastos, células plasmáticas, granulocitos, progranulocitos, metagranulocitos, monocitos, macrófagos y trombocitos), interpretando los resultados mediante tratamiento estadístico.

Mediante el estudio hematológico, pudimos apreciar un claro descenso en el número de linfocitos, paralelamente al aumento del número de granulocitos en los peces expuestos a infección. Los valores microhematocritos de los animales control y los animales expuestos no difirieron entre ellos de forma notable.

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THE LEUCOCYTE RESPONSE OF SEA BASS (*Dicentrarchus labrax* L.) TO INTRAPERITONEAL VACCINATION AGAINST VIBRIOSIS AND PASTEURELOSIS

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The leucocyte mobilisation and the degree of recovery of the inflammatory status are dependent on the flogistic agent present at the inflammatory foci, including microorganisms and vaccine formulations injected. The peritoneal model was used to evaluate the non-specific cell-mediated immune response in sea bass (*Dicentrarchus labrax* L.). Monovalent (*Photobacterium damselae* subsp. *piscicida* or *Vibrio anguillarum*) and divalent (*Ph. damselae* and *V. anguillarum*) experimental vaccines formulations with different adjuvants were intraperitoneally injected. Formulations with the same adjuvants but without antigens were also used. The acute peritoneal cell immune response and the progression and establishment of a chronic status were evaluated by analysing quantitatively the peritoneal leucocyte exudates up to 30 – 60 days. The acute leucocyte response was characterised by an early (24-48h) mobilisation of neutrophils and macrophages, with phagocyte numbers dependent on the formulation, but no significant variations were observed in lymphocytes/small cells and EGCs numbers. Later on, a steady increase in lymphocytes/small cells and EGCs occurred. Groups i.p. injected with mineral oil adjuvanted formulations with antigen showed a high concentration in neutrophils and macrophages which was maintained up to 30-60 days. This was not observed in the groups i.p. injected neither with other adjuvants nor in non-adjuvanted formulations. The divalent mineral oil adjuvanted vaccine induced the strongest and the most prolonged inflammatory leucocyte responses. Moreover, this vaccine formulation was capable of inducing a chronic inflammation even without antigen.

PATHOBIOLOGY ASSOCIATED WITH *BUCEPHALUS* SP. (TREMATODA DIGENIA) METACERCARIAE IN THE GREY MULLET: HISTOLOGICAL ASPECTS AND PARASITE MOLECULAR IDENTIFICATION

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Trematoda Digenia parasites with complex life cycles affect aquatic organisms and cause great economic losses in aquaculture in various places in the world. Consequently, it is of great importance to understand the life cycle of this parasite and the pathologies it causes. Histological and morphological studies have focused on the bivalves (primary intermediate hosts), whereas the pathologies associated with the presence of these parasites in fish (secondary intermediate hosts) has received less attention. In teleost fish, the metacercarias cysts settled in various internal organs and in the muscles in a considerable number, causing serious pathological internal damage. It is difficult to identify the parasite using only histological methods and molecular biology is generally used in phylogenetic studies, the use of the latter technique can give additional information improving the identification. In this experiment, we have performed a histopathological analysis of the internal organs of Grey Mullets (*Mugil cephalus*) parasitized under natural living conditions, as well as a cytological and molecular characterization of the parasite.

Several Grey Mullets were caught in the river Douro estuary in May 2002. Necropsies were performed and liver, spleen and heart were collected for histopathological analysis. The tissues were fixed in neutral Formol (10%), included in paraffin and the sections stained with HE, PAS and Masson´s Trichrome. The liver was further prepared for electron microscopy and semi-thin sections carried out. Genomic DNA was extracted from the cysts and amplified through PCR using specific primers for the Digenia ITS1 region. The PCR product was purified, sequenced and analyzed.

The macroscopic analysis of the studied organs revealed great alterations in terms of morphology, colour and structure. Through the histopathological analysis, it was possible to confirm the presence of cysts in all analysed tissues. In the heart, large cysts were involved in a thick layer of fibrous tissue. In the liver smaller cysts were observed, disseminated throughout the hepatic parenchyma, normally associated with melanomacrophage centres. In the spleen, the parasites were involved by melanomacrophage centres and adipose tissue. The sequencing of the amplified

fragment revealed an ITS1 of 571 bases with 91 % similarity with the ITS1 sequence known from *Bucephalus polymorphus*.

COMPARISON OF CANINE MAMMARY TUMORS ROUTINE HISTOPATHOLOGICAL DIAGNOSES GIVEN BY DIFFERENT GROUPS OF PATHOLOGISTS AND APPLICABILITY OF THE 1999 WHO'S CLASSIFICATION

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During the last 40 years, several histological classifications of canine mammary tumors (CMT) have been proposed but none has been unanimously accepted. Nowadays, the most widely used, authoritative resources for the classification of tumors are the World Health Organization (WHO) classifications. The latest WHO International Classification of Mammary Tumors of the dog and the cat (Misdorp et al 1999) combines histogenetic and descriptive morphologic criteria, incorporating histological prognostic features that have been associated with increasing malignancy. However, doubts have arisen concerning its capability to identify some of the CMT with more than one tissue component (epithelial, myoepithelial, mesenchymal). The purpose of this study was to evaluate the level of applicability, acceptance and usefulness of the WHO International Classification of Mammary Tumors of the dog (Misdorp et al 1999) among Spanish and Portuguese veterinary pathologists in the routinely diagnosis of some particular histological subtypes of CMT.

Eighteen cases of routinely diagnosed CMT with more than one tissue component were selected from the archives of the Veterinary Pathology Laboratories of the Veterinary Schools of Madrid and Córdoba. After a call for participation, hematoxylin and eosin-stained tissue sections, together with a table for evaluation, were mailed to 13 Spanish (9 public Veterinary Faculties, 1 Animal Health Institute, and 3 private Veterinary Pathology laboratories), and 3 Portuguese (2 public Veterinary Faculties, and 1 Research Institute) institutions. In addition, 2 of the authors of the WHO Classification under evaluation (W. Misdorp and E. Hellmén), as well as 3 experts on the field (M. Castagnaro, M. Estrada, P. Roccabianca) were included in the study as positive controls. The individual diagnosis of the two authors were also included.
The evaluation of the results is expected to give an answer to the following questions, at least:

1. Are we all using the same name (diagnosis) for the very same tumor?

2. Is there a place for mixed tumors with only the epithelial component malignant (formerly called "malignant mixed tumor") in the new WHO Classification?
3. Is the carcinosarcoma subtype uniformly used by the diagnosticians?
4. When should the term of the new WHO Classification "Carcinoma or sarcoma in benign tumor" be used?
5. Is there a place for the differentiation between "complete" (both components are malignant) and "incomplete" (either the epithelial or the myoepithelial component is malignant) complex carcinoma?

EVALUATION OF INTERPHASE AgNOR QUANTIFICATION TECHNIQUE IN CANINE DIAGNOSIS OF HIGH AND LOW GRADE MALIGNANT LYMPHOMA.A STUDY OF 24 CASES

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The interphase AgNOR quantification, constitutes a simple and sensitive complementary diagnosis technique, which has been applied with excellent results in the study of several tumors, both in humans and animals.
This study comprised 24 malignant lymphoma (ML) (diagnosed at FMV's Pathology Service, between 1996 and 2002). In agreement with the "Histological Classification of Hematopoietic Tumors of Domestic Animals" (2002, WHO) they were classified as low or high grade ML.
Lymph nodes from four groups of animals were analyzed:

A - 1 Control case, normal lymphoid population.
B - 1 Leishmaniasis lymphoid hyperplasia.
C - 10 Low grade ML (1 B-cell lymphoplasmocytic; 3 B-cell lymphocytic; 3 T small cell; 1 small cell; 1 B-cell mantle type; 1 B-cell G1 centroblastic-centrocytic).
D - 14 High grade ML (1 B-cell G3 centroblastic-centrocytic; 2 G3 centroblastic-centrocytic; 1 B-cell centroblastic-monomorphic; 4 B-cell centroblastic-polymorphic; 2 B-cell immunoblastic; 1 immunoblastic; 1 T large cell; 2 B-cell Burkitt-type).

Silver staining of AgNORs was performed using Ploton *et al.* (1982), technique modified by Derenzini and Ploton (1991). Beforehand, we optimized silver nitrate and sodium thiosulfate reaction times for canine material, which were found to be 30 and 3 minutes, respectively. In each case a 100 cell AgNOR count was done.
Statistical analysis was based in a comparison of means by ANOVA's (Analysis of Variance) F-test with Fisher's LSD (Least Significant Difference) procedure, using StatGraphics Plus 5.0 (2000, Manugistics Inc.). There were statistical significant differences ($p<0.00001$) between normal control ($\overline{X}=1.07$; S.D.=0.355), lymphoid hyperplasia ($\overline{X}=1.51$; S.D.=0.904) and MLs (combining low and high grade) ($\overline{X}=2.89$; S.D.=1.378). The differences among mean counts for low grade ML's ($\overline{X}=2.73$;

S.D.=1.412) and high grade ML's ($\overline{X}$=3.00; S.D.=1.802) were also significantly different ($p<0.00001$).
These results demonstrate that interphase AgNOR quantification constitutes an important complementary technique in canine M L diagnosis, representing also a valuable tool in differential diagnosis among low and high grade ML's.

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A COMPARATIVE STUDY OF PROLIFERATION IN 3 CANINE T-CELL "CENTROBLASTIC" MALIGNANT LYMPHOMAS, USING Ki-67 IMMUNOHISTOCHEMICAL DETECTION

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Lennert (1992) studied human cellular differentiation, having established a direct association between certain cellular types (centroblasts, centrocytes, plasmo cells) and B phenotype. The clinical studies that stemmed from this classification outline revealed, in general, that B-cell malignant lymphoma (ML) has a better prognostic than T-cell ML, so this distinction is clinically important. In view of the scarce available knowledge about this association in dogs, this work aims to characterize the B/T phenotype of canine centroblastic ML and to determine whether they will fit Lennert's differentiation stages scheme.

This study comprised 36 ML (diagnosed in FMV's Pathology Service, between 1996 and 2002) whose histopathological classification was based in the "Histological Classification of Hematopoietic Tumors of Domestic Animals" (2002, WHO).

B/T phenotyping was performed by indirect immunohistochemical typing, using antibodies: BLA36 (Dako-M533), CD79αcy (Dako-M7051) and CD3 (Dako-A0452).

Out of the 36 cases, we identified 14 ML with centroblastic type cells. Among these we found 3 which revealed, unexpectedly, a T phenotype.

For better characterization of this atypical group, a cellular proliferation study using Ki-67 (Dako-M7240) indirect immunohistochemical detection was done, based on 1000 cells from each case (Soft Imaging System, Olympus DP-SOFT v3.00, 2000).

The comparative proliferation study included 4 groups:

A - 1 B-cell lymphoplasmoytic ML.
B - 11 B-cell centroblastic ML (7 polymorphic and 4 monomorphic).
C - 3 T-cell centroblastic ML (1 polymorphic and 2 monomorphic).
D - 2 B-cell Burkitt-type ML.

The statistical analysis was based in a comparative study of proportions using ANOM (Analysis of Mean) with StatGraphics Plus 5.0 (2000, Manugistics Inc.).

There were statistical significant differences ($p<0.00001$) between cellular proliferation of low grade ML (group A; Ki-67^{+}=3.0%) and high grade ML (groups B, C and D; Ki-67^{+}=69.6%).

Cellular proliferation between B-cell centroblastic ML (Ki-67^+=71.6%), T-cell centroblastic ML (Ki-67^+=62.7%) and B-cell Burkitt-type ML (Ki-67^+=71.1%) were not significantly different.
Based on the studied cases results, we conclude that:
1 - Centroblastic morphology can occur in T-cell malignant lymphomas.
2 - Cellular type (-blastes/-cytes) has a larger influence in the definition of cellular proliferation grade and eventual aggressiveness of MLs than its B/T phenotype.

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ENTEROPATHY ASSOCIATED T CELL LYMPHOMA WITH EPITHELIOTROPISM IN A HORSE

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A 16-years-old Percheron male was submitted for necropsy to the Veterinary Teaching Hospital from Complutense University (Madrid) due to a chronic diarrhea for the last 5 months. Clinical history revealed a progressive weight loss during the last 15 months with increased appetite; however, no other respiratory or digestive symptoms were observed. Private clinician administrated antibiotic therapy and vitamin supplementation but there was no response this treatment. The owners reported sporadic and acute abdominal pain (moderate colic), which were resolved by means of common analgesics (Fynadine and Nolotil). Preliminary diagnostic workup included a complete blood count and serum biochemistry profile. Hematological abnormalities included slight anemia, leucopenia and hypoproteinemia. In the last 15 days, the animal presented severe anorexia, loss 70 kg of weight, continuous abdominal pain and remain recumbence during long time. Due to the progressive clinical deterioration, the owners elected euthanasia.

At necropsy we appreciated a emaciated horse with pale mucous membrane, subcutaneous edema in the chest and abdominal region and moderate increased size of the lymph nodes. Upon opened of the carcass revealed the presence of the yellowish fluid in abdominal, thoracic and pericardial cavities. Abdominal lymph nodes were severe enlarged, overall mesenteric ones; cut surface showed a whitish and homogenous appearance of the parenchyma. Small intestine (mainly jejunum and ileum) showed a strong thickening of the wall (between 5-7 cm), associated with marked lymphangiectasis in mesenteric serosa (peritoneum membrane). Mucosa surface displayed a reddened, thickened folds and corrugate, similar to the "cerebroid aspect" observed in ruminant Johne's disease. Other additional pathological finding was a multiple cysts formation observed in the thyroid gland.

Histology examination revealed a strong diffuse infiltration of small and well differentiated lymphocytes in lamina propria and submucosa from bowel. This lymphoproliferative disorder adopted to patchy to diffuse growth pattern. Neoplastic lymphocytes are presented within the epithelial layer and along the epithelial basement membrane forming small groups or interspersed among enterocytes. The mitotic index was high. Na immunohistochemical study was performed to characterize the different lymphocyte populations: CD3 and CD79α. A predominant strong immunoreaction of

the tumoral cell against CD3 antibody indicated the round cell population was of T lymphocyte origin; for this reason we consider a high intestinal T cell lymphoma. On basis of the macroscopical, histopathological and immunocytochemical studies, the final diagnosis was equine epitheliotropic intestinal malignant T cell lymph.

Up to now, only 1 case of epitheliotropic T cell lymphoma has been reported in horses (Pinkerton et al., 2002). In both cases, small intestine is involved but we found also abdominal lymph nodes affected. Although a recent classification of equine malignant lymphomas has been published (Kelley and Mahaffey, 1998), this entity has no included. To complete the study of this neoplasm we have employed other primary antibodies (ki-67, p53, $p^{21WAF1/cip1}$ and $p16^{INKA}$) to obtain new information about the possible origin of this malignant neoplasm.

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LYMPHOMA LIKE LESIONS IN CALFS INFECTED BY *THEILERIA ANNULATA*

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The authors describe 12 cases of acute lethal infection by protozoan *Theileria annulata* in calfs with less than 6 months of age, in Alentejo, south of Portugal. The calves developed neoplastic lymphoproliferative lesions, similar to multicentric malignant lymphoma. In general, death occurred up to 15 days after the onset of the initial symptoms, and no improvement was obtained with any of the treatments applied. All animals were derived from farms free of Bovine Leukemia Virus. Calfs were emaciated, anemic, with exophtalmia, and presented generalized cutaneous nodules, petequiae in the oral, conjunctival and nasal mucosae, and external ear, together with enlargment of all superficial lymph nodes. Infection with ticks was intense especially by the *Hyalomma* genus.

In blood and lymph node smears forms of *Theileria annulata* were identified, the nature of the infection having been confirmed in one case by sequencing the V4 hypervariable region of the gene of 18S rRNA.

At necropsy, nodules varying between 0.2 and 3 cm, were identified in the skin, subcutaneous tissue, muscle and intraperitoneal fat, with an hemorrhagic halo or completely hemorrhagic, with dense homogenous cut surface. Similar lesions were observed in the tongue, pharynx, trachea, thymus, intestinal serosa, omentum, heart, hypophysis and retro-ocular tissue. All lymph nodes were enlarged. Histologically, nodules were formed by lymphoblastic-like cells, with reduced cytoplasm, increased nucleus/cytoplasm ratio, some nuclei showing indentations. Chromatin frequently formed large granules located in the nuclear membrane. Karyorrhectic cell figures were common. Occasionally, cells containing schizonts of *Theileria* were identified with the Giemsa stain.

Indirect immunohistochemistry was applied to various tissue sections with markers for leukocytes, B and T lymphocytes, natural killer cells and myeloid cells, showing consistent negative results. As all sera used were monoclonal for human markers, except for CD3, this may explain the lack of positivity, as positive controls were obtained in all cases.

Presently electron microscopic studies are being undertaken to help identify correctly these lymphoblast-like cells.

Scientific international reports describe the capability to produce neoplastic changes in lymphoid cells by *Theileria parva*. The same type of behaviour is not described for *Theileria annulata*. However, this protozoan parasite induces the clonal proliferation of macrophages (sometimes of B lymphocytes) from lymph nodes that drain the region that was bitten by the tick. On the other hand, infected cells induce the stimulation and proliferation of T lymphocytes.

IMMUNOHISTOCHEMICAL EVALUATION OF THE MITOGEN ACTIVATED PROTEIN KINASE ERK 1/2 SIGNALLING PATHWAY IN CONTAGIOUS RESPIRATORY TUMOURS OF SHEEP AND GOATS.

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Ovine pulmonary adenocarcinoma (OPA) and enzootic nasal adenocarcinoma (ENA) are two diseases grouped in the more general term contagious respiratory tumours of small ruminants. These tumours are associated with distinct betaretroviruses: jaagsiekte sheep retrovirus (JSRV) and enzootic nasal tumour virus (ENTV-1 in sheep and ENTV-2 in goats). These viruses are very similar and HYAL-2 has been identified as their cellular receptor. The mechanism of virus-induced cell transformation is one of the important questions to resolve in relation to the biology of these retroviruses. Expression of the JSRV/ENTV envelope protein is sufficient to transform rodent and avian fibroblast in culture. Phospho-protein kinase B (Akt) was detected in these cells following transfection and cell transformation assays. Mitogen activated protein kinases (MAPKs) and Akt activation was detected when human epithelial bronchial cell lines were transfected and transformed with these JSRV *env* expression plasmids. Phospho-44-42 MAPK (Erk1/2) activation was not found in rodent/avian fibroblast proliferation assays. Previous immunohistochemical detected Akt activated in few cells in naturally occurring nasal adenocarcinoma but not in lung tumours. This paper describes studies to detect the activation state of signalling pathway that may be relevant to oncogenesis in tissue sections of these contagious tumours. Several antibodies detecting activated form of several proteins of the Erk1/2 pathway (phospho-Raf-1, phospho-Mek1/2, p44-42, phospho-p44-42, phospho-p90Rsk, phospho-Elk-1 and phospho-c-myc (Cell signalling Technology)) were used as primary antibodies in a routine immunohistochemistry protocol (ABC kits, Vector laboratories). A rabbit polyclonal antibodies against SU and CA proteins of JSRV (Moredun Research Institute) were also used. Tumours from 6 natural cases of OPA, 3 experimentally induced OPA cases in young lambs, and 6 cases of ENA (3 goat and 3 sheep) were fixed in 10% buffered formalin and embedded in paraffin, by routine methods. Both CA and SU proteins were detected in all tumours examined. Both natural and experimentally induced OPA and all ENA tumours were clearly positive to most of the components of the p44-42 MAPK pathway. These findings support that MAPK Erk1/2pathway is clearly activated in contagious respiratory tumours of sheep and goats. The implication of the activation of this pathway in cell transformation *in vivo* is discussed.

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HUMAN LUNG ADENOCARCINOMAS CONTAIN PROTEINS RELATED WITH THE *GAG* GENE BUT NOT WITH THE *ENV* GENE PRODUCTS OF THE JAAGSIEKTE SHEEP RETROVIRUS

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Human lung adenocarcinoma is a lung cancer which shows many morphological similarities with a contagious lung cancer of sheep called ovine pulmonary adenocarcinoma (OPA, sheep pulmonary adenomatosis, jaagsiekte). OPA is associated aetiologically with a γ-retrovirus known as jaagsiekte sheep retrovirus (JSRV). Preliminary studies using a rabbit antiserum to the JSRV capsid (CA) protein detected only in the citoplasm of recognisable cancer cells in around 30% of the human lung adenocarcinomas. Recently new rabbit policlonal antisera detecting surface (SU) and matrix (MA) proteins of the JSRV have been generated. These two new reagents and a rabbit policlonal antibody detecting *gag* proteins of the human endogenous retrovirus herv-k (courtesy of dr m sauter. Universitätskliniken des saarlandes. Homburg. Germany) have been used in this study. Sections from 24 lung adenocarcinomas (18 adenocarcinomas, 6 bronchioloalveolar cell carcinomas) were obtained. Three squamous cell carcinomas were also tested. Sections from opa were used as positive controls. These new reagents were used as primary antibodies in a routine immunohistochemistry protocol (ABC kits, vector laboratories). Most proliferating tumours cells in OPA tumour tissue sections were positive using rabbit anti jsrv-su and jsrv-ma. All human lung tumours were negative with anti-JSRV-SU policlonal antisera but there was 80% of coincidence among tumours positive to CA and MA protein of the JSRV, and 55% coincidence using the rabbit anti-HERV-K. These results conclude that jsrv retrovirus is not present in human lung adenocarcinomas but the presence of cross-reactivity using anti JSRV-MA and anti gag-HERV-K keeps open the discussion about the implication of a retrovirus in human lung adenocarcinomas.

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HAEMANGIOMAS IN YOUNG HORSES: AN HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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Benign vascular tumours are relatively common in young horses. In the current literature, the terminology of these lesions is confusing and includes vascular hamartoma, haemangioendothelioma, vascular nevus, lobular capillary haemangioma, haemangioma and proliferative angioma. The clinical, pathological and immunohistochemical features of two cases of haemangioma in young horses are described in the present work. Case no. 1 was a one-year-old cross-bred male horse that showed three hyperpigmented and alopecic plaques ranging 26x15, 22x10 and 4x5 cm in diameter, located in the left leg, two of which showed deep ulceration. Case no. 2 was a 1.5 year-old crossbred female that showed a proliferative lesion in the metacarpus of the right hind limb. Histopathological examination of skin biopsies revealed the existence of multifocal, non-encapsulated proliferated closely packed small blood vessels, some of which showed irregular shape, whereas others were similar to capillaries, arterioles and venules. Some of these vascular structures contained erythrocytes in their lumina, but more frequently showed a small and empty lumen. Endothelial cells of these neoformed vessels showed prominent basophilic nucleus without evident nucleoli, and were surrounded by several concentrically arranged elongated cells with oval and euchromatic nucleus and eosinophilic cytoplasm with indistinct borders, similar to pericytes or smooth muscle cells. A loose collagenous stroma and variable numbers of plump fusiform cells with euchromatic nucleus and eosinophilic cytoplasm with indistinct borders were found among neoformed blood vessels, apparently unassociated with them. Proliferated cells showed no sign of atypia, and mitoses were very occasional. Mature fibrous tissue was observed between clusters of proliferated vessels. The immunohistochemical study demonstrated that endothelial cells of neoformed blood vessels and some plump fusiform cells apparently unassociated with vessels were vimentin and Factor VIIIar positive, suggesting an endothelial nature of these cells. Fusiform cells in the wall of small neoformed vessels similar to arterioles expressed desmin and muscle actin, whereas a small number of plump fusiform cells between vessels showed a weak staining for desmin and muscle actin. The similarities with juvenile haemangioma of human being and the differences with haemangioma of adult horses and other species are discussed.

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TUMORES PRIMITIVOS EN EL SISTEMA NERVIOSO CENTRAL DEL PERRO: CARACTERIZACIÓN HISTOPATOLÓGICA E INMUNOHISTOQUÍMICA DE DOS CASOS

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Caso 1: Masa intradural extramedular en la médula espinal lumbar de un perro Labrador Retriever de 1 año y medio. Histológicamente se describe una masa bien delimitada, que no infiltra al tejido nervioso, constituida por células poligonales con abundante citoplasma eosinófilo, destacando la formación de rosetas.

Caso 2: Masa intracraneal, intradural, localizada rostralmente comprimiendo al bulbo olfatorio y parte de la corteza frontal derechos, en un perro Pastor Alemán de 9 años. Histológicamente se describe una masa que infiltra al tejido nervioso, constituida por células poligonales o cilíndricas, caracterizada por la formación de abundantes rosetas y pseudorosetas.

Morfológicamente la presencia de rosetas en un tumor situado en el tejido nervioso sugiere un origen neuroectodérmico. En el diagnóstico diferencial de dichos tumores entran entidades cómo: Neuroblastoma, Meduloblastoma, Tumor espinal del perro joven o Nefroblastoma, Neuroblastoma olfatorio o Estesioneuroblastoma, Ependimoma, etc.

Se han realizado una serie de técnicas histológicas (HE, Masson, PTAH) e inmunohistoquímicas (ENS, NF- 200kD, SYN, GFAP, S-100, CK, VIM, PCNA, Ki68) para caracterizar la histogénesis de dichos tumores.

El diagnóstico de los tumores fue:
Caso 1: Tumor espinal del perro joven (Nefroblastoma).
Caso 2: Tumor neuroectodérmico primitivo con diferenciación ependimaria.

THE VALUE OF CELL PROLIFERATION AND OESTROGEN RECEPTORS IN THE BIOLOGICAL BEHAVIOUR ASSESSMENT OF BOVINE OVARIAN GRANULOSA CELL TUMOURS

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The granulosa cell tumour (GCT) is considered a common ovarian neoplasm in all domestic animals. Its biological behaviour is variable and, unfortunately, the histopathological features have proved unreliable in predicting the clinical course of those neoplasms. Other markers such as cell proliferation and oestrogen receptors (ERs) are being evaluated in human beings to assess their prognostic significance. However, there are no reports on the role of these two markers in bovine ovarian GCTs. For this reason, 11 GCTs from 1489 slaughtered cattle were examined immunohistochemically for proliferating cell nuclear antigen (PCNA) and ER in relation to histopathological features (growth pattern, nuclear atypia and mitotic count). Increased PCNA expression was significantly associated with nuclear atypia but not with histological growth pattern or mitotic count. Thus, the results indicated that PCNA expression may be of value in establishing the biological behaviour of bovine GCTs. With regard to ERs, a novel finding was the demonstration of ERβ but not ERα in tumour nuclei of bovine ovarian GCTs. The presence of ERβ in all of bovine GCTs examined suggests a role in the pathogenesis of this neoplasm of cattle; however, no relation among ERβ and the histopathological features examined was found and its relation with the biological behaviour of bovine ovarian GCTs has not been demonstrated in this study. A larger series of bovine GCTs should be examined to assess the prognostic significance of ERβ.

SQUAMOUS CELL CARCINOMA IN A *GORILLA GORILLA*: AND IMMUNOPATHOLOGICAL ANALYSIS

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Squamous cell carcinoma (SCC) is one of the most common types of skin cancer in many species. SCC is strongly related with light pigmentation, age and sun-light exposition. The aim of the study was to analyse the profiles of some cytokeratins (CKs), MHCclass II antigen as well as the inflammatory infiltrate in cutaneous SCC of the albine gorilla (*Gorilla gorilla gorilla*) from the Barcelona Zoo.
Material and methods: four formalin-fixed tissue samples from the tumoural mass obtained at necropsy (upper and deep areas) were used. Monoclonal antibodies RCK102, AE1/AE3, MNF116, (Dako, Glostrup, Denmark), K8.60, K2.342.7.4, (Progen Biotechnick GMBH), MCH II, CD3 and anti-IgG (Dako) were tested for the immunohistochemical study (avidin-biotin-peroxidase method).
Results: Histopathologically, the tumour showed areas of well differenciated SCC (Grade I) as well as moderately and poorly differenciated areas (Grades II and III, these areas mainly located in subcutaneous and among the muscles). Intra and peritumoral fibroplasia and linfoplasmocitary infiltrate was observed. Antibodies MNF116 and RCK102 (CKs 5,8) reacted similarly with the majority of basal cells of tumoural nests, and were more intense in deeply infiltrating tumour cells. AE1/AE3 reacted with squamous cells in areas well and moderately differentiated. Staining was intense and frequently outlined in the plasma membrane. Antibody K8.60 (CKs 1,10/11) reacted with squamous cell of the tumour nests, but the staining was moderate. The antibody K2.342.7.4 (CK2e) failed to detect this protein in the majority of tumour nests but reacted weakly with horny layers in well differentiated areas. MCH-II expression was occasionally observed in tumour cells and in some lymphocytes. CD3 (T cells) reacted with some lymphocytes and IgG was detected in many inflammatory cells.
In conclusion: This SCC retained the pattern of differentiation expected for keratinized epithelia and exhibited heterogeneous patterns for CKs associated with the cellular differentiation, similarly to those observed in SCC from other species. MCH-II expression both in tumour and in the infiltrate, as well as CD3 (T cell) and IgG and IgM immunoglobulins are also discussed in relation to the tumour progression.

IMMUNOEXPRESSION OF MHC CLASS II ANTIGEN IN CANINE CUTANEOUS HISTIOCYTOMA

AN HISTOLOGICAL AND ULTRASTRUCTURAL STUDY

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Canine histiocytic proliferative disorders include neoplastic diseases such as cutaneous histiocytoma. Their aetiology and pathogenesis are unknown.

Canine cutaneous histiocytoma (CCH) is a common, benign neoplasm of epidermal Langerhans cells, which appears as a rapidly growing, alopecic, erythematous, dome-shaped nodule.

Histiocytomas can occur anywhere on the body, but the preferential sites are the head and the extremities. The majority of histiocytomas occur in dogs between 6 months and 3 years of age; the incidence drops markedly in older animals.

The spontaneous regression of the histiocytomas makes this an attractive system for analysis of cellular immunity, and the increasing expression of MHC class II molecules on the cell surface might be a decisive factor for tumour regression.

In our study, we analysed the different patterns of expression of MHC class II antigen in neoplastic cells of 93 CCH, and its relationship with the lymphocytic infiltrate using immunohistology (CD3, Cd79 and HLA-DR) and immunoelectron microscopical (HLA-DR) methods.

The tumours were categorized in 4 groups, according to Cockerell and Slauson, 1979. We observed two principal patterns of MHC class II antigen expression: a focal juxtanuclear cytoplasmic reaction or a rim-like staining in the cell periphery, confirmed by the immunolectron microscopical study.

A significant association was observed between the different patterns of immunoreactivity for MHC class II antigen and lymphocyte infiltrate. The influence of CD3+ and Cd79+ lymphocytes will be discussed.

The maturation of Langerhans cells of CCH, with increase of MHC class II molecules in cell periphery, seems to be essential to the stimulation of an effective immune response and tumoral regression.

CANINE OCULAR MELANOMAS: FOLLOW-UP OF 15 CASES

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In dogs, tumors of melanocytic origin are tumors that affect ocular globe with a higher incidence. According to its location they are classified into epibulbar and intraocular. Epibulbar melanomas originate from melanocytes in the limbal stroma and are mainly benign and have high incidence. Intraocular melanomas are divided, in their turn, into anterior uveal melanomas and choroidal melanomas. The first ones can arise either from the iris or the ciliary body; most of them present a benign behaviour although the bibliography describes up to 5% of distant metastases. Choroidal melanomas present a low incidence and are generally benign. To determine the behaviour of these tumors one should take into account their location and histological features of malignancy. The mitotic index is, in the opinion of most of the authors, the most reliable predictor of behaviour. In this communication we revise the evolution on 15 primary melanomas of canine ocular globe. Regarding its location, 6 were epibulbar, 8 from the anterior uvea and 1 from the choroid. After 1 year minimum follow-up, just one of them gave rise to metastases on distant organs.

NORMAL AND HYPERPLASTIC CANINE PROSTATE - DNA PLOIDY CYTOPHOTOMETRIC DATA

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Hyperplasia of the canine prostate glandular epithelium is a benign pathological process. This is confirmed by histological (routine, immunocytochemical and AgNOR-related), clinical and epidemiological data. In the present study, this hypothesis is tested on a cytophotometric level.

DNA ploidy and G_1 and G_2 + M (proliferative index - PI) cell percentage were studied, using static cytophotometry. 22 cases of normal mature prostate (PNM), 25 cases of glandular benign hyperplasia (HBPG) and 24 cases of complex ("cystic") benign hyperplasia (HBPC) were analysed. Diagnosis was based on histological tissue sections stained with Ehrlich's hematoxilin -erythrosin.

Cell imprints of each case were fixed in 10% neutral buffered formalin, stained by Feulgen's method with 5M HCl hydrolysis during 60 minutes at room temperature (Soares *et al.*, 1991). DNA quantification was performed using an image analysis system with specific software (Ahrens ACAS). In each case 500 nuclei were assessed. The coefficients of variation of the histograms were inferior to 9,00%, with a mean (± standard deviation) and median of 6.95% ± 1.38% and 7.12%, respectively. Histograms were classified according to Auer *et al.* (1980) criteria; the diploid zone was set between 1,6 c and 2,4 c (Alanen *et al.*, 1992). PI was classified as low (<5%), medium (between 5 and 10%) and high (> 10%) - Ahlgren *et al.* (1997).

PNM - 22 diploid cases. 21 cases with low PI and 1 with medium PI. HBPG - 25 diploid cases. 23 cases with low PI and 2 with medium PI. HBPC - 24 diploid cases. 19 cases with low PI and 5 with medium PI. All histograms were, therefore, of type I, according to Auer *et al*. (1980) classification.

Group mean ± standard deviation:

	DNA ploidy	G_1	PI
PNM	2.086 c ± 0.116 c	98.38 % ± 1.38 %	1.62 % ± 1.38 %
HBPG	2.016 c ± 0.125 c	97.97 % ± 2.06 %	2.03 % ± 2.06 %
HBPC	2.030 c ± 0.105 c	96.90 % ± 2.84 %	3.10 % ± 2.84 %
HBP (G+C)	2.023 c ± 0.115 c	97.45 % ± 2.50 %	2.56 % ± 2.50 %

There were no statistical significant differences between DNA ploidy, G_1 and PI cell percentage in the four groups studied by an analysis of variance procedure.

It was also tested, for each group, if the average DNA ploidy would be significantly different from the theoretical DNA ploidy of benign cells, *i.e.* 2.000 c: pPNM = 0.0023; pHBPG = 0.5283; pHBPC = 0.1748; pHBP = 0.1693.

The significant difference of DNA ploidy of PNM group, in relation to the expected ploidy, points to two hypotheses:

a) The controversial subject of DNA aneuploidy in normal tissues (Malinin *et al*., 1988; Arber & Speights, 1991).

b) To consider that, in fact, PNM group is diploid; supporting this statement is the fact of non-existing statistical differences between means and medians of DNA ploidy among PNM, HBPG, HBPC and HBP groups, using an analysis of variance test.

We conclude that cytophotometric data on benign prostatic hyperplasia are concurrent with data of different natures (histological, clinical and epidemiological).

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THREE CASES OF CANINE PROSTATIC TUMOUR WITH PECULIAR FEATURES

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The incidence of prostatic carcinoma (CaP) in the dog (*Canis familiaris*) ranges from 0,2 to 0,6 % in necropsy studies (Morrison, 1998). The incidence of mesenchimal neoplasms in that gland is lesser.

We selected 19 necropsies and histopathologic examinations with a component of prostatic pathology from the files of the Laboratory of Histology and Pathologic Anatomy of the Departamento de Ciências Veterinárias of the Universidade de Trás-os-Montes e Alto Douro. We found three cases of prostatic neoplasm:

a) Case A – 10 year-old mongrel dog. Its prostate had essentially benign hyperplasia and large foci of alveolar atrophy with post-atrophic hyperplasia and some foci of purulent inflammation. Some alveoli were lined by a stratified epithelium with loss of cellular polarity and nuclei with prominent nucleoli (this lesion is suggestive of PIN – prostatic intraepithelial neoplasia – Waters et al., 1997). Among three of such alveoli, the stroma presented fusiform cells with basophilic cytoplasm and nucleomegaly; also, mitoses were present. The capsule was thickened by a tissue which cells were fusiform and showed isokariosis; the extracellular matrix was scant.

b) Case B – 5 year-old dog. There were PIN lesions in two subcapsular alveoli, one of which showed invasion of the stroma by loosely attached round, polygonal or fusiform cells with anisokaryosis (malignant tumour cells). Most of the section presented complex benign hyperplasia and there were some foci of lymphoplasmocytic infiltration of the stroma.

c) Case C – 9 year-old Boxer. The prostate harboured an adenocarcinoma with pattern 3B (small acinar) of the Gleason classification (Gleason, 1992); Gleason grading – 3 + 3 = 6 (moderately differentiated CaP). Neoplastic stroma presented desmoplasia and foci of osseous metaplasia.

Case A presents two different lesions: a focal intraprostatic malignant neoplasm, which ontogeny must be elucidated by immunocytochemistry (application of, at least, anti-cytokeratin, anti-vimentin and anti-desmin antibodies), and a possibly mesenchymatous

tumour of the prostatic capsule; the elucidation of the nature of this neoplasm demands DNA cytophotometric, immunocytochemical (intermediary filaments-related antibodies, for example) and cellular proliferation (mitosis counting, MIB-1 antibody) assessments.
Case B brings direct evidence about the malignant potential of PIN: this is a preneoplastic (and not a putative preneoplastic) lesion. The tumour is microscopic, but its location and its biologic behaviour predict a fast and fatal evolution. Most of the human CaP with little dimensions are diploid and assume a "benign" evolution.
Case C demonstrates that neoplastic stroma of the canine CaP plays a dinamic role, ***i.e.*** it is capable of active biological alterations, such as metaplasia.

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A CASE OF PROSTATIC MICROMETASTASIS OF HEMANGIOSARCOMA IN A DOG (*CANIS FAMILIARIS*)

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In the literature, prostatic metastases of canine malignant neoplasms are seldom-referred (*Canis familiaris*). The incidence of such tumours is unknown because, in many necropsies performing, the prostate isn't systematically examined.
The necropsy of a 14-old mongrel dog disclosed a hemangiosarcoma which possible primary location was the gastrocnemius muscle. There were widespread metastases: in the dermis/hypodermis (in the clinical examination, the diagnosis of hemangiosarcoma was based on the characteristics of a cutaneous nodule), fasciae, skeletal muscles, pericardium and myocardium, lungs, peritoneum, duodenum, liver and kidneys. Histological examination of tissue sections stained with Ehrlich's haematoxylin – erythrosin confirmed the diagnosis of hemangiosarcoma: Histopathological examination disclosed also a Sertoli cell tumour in a testicle and an intraprostatic microscopic neoplasm with malignant histocytologic features.
The immunocytochemical profile of this neoplasm was determined using monoclonal antibodies MNF116 (anti-cytokeratins 5, 6, 8, 17 and, maybe, 19), Vim 3B4 (anti-vimentin), BBS/NC/VI-H14 (anti-NSE) and F8/86 (anti-factor VIII) and polyclonal antibody anti-PSA (all of them from Dako). The immunocytochemical staining procedure was performed by TechMate ™ (Dako), in the Instituto Português de Oncologia Francisco Gentil – Section of Pathologic Anatomy (Lisbon). Epitope retrieval was made by high temperature in pressure cooker with 0.01 M citrate buffer at pH = 6.0 (1 minute) in the case of antibodies Vim 3B4, BBS/NC/VI-H14 e anti-PSA; in the case of antibodies MNF116 e F8/86, epitope retrieval was performed by enzyme digestion with 0.4 %pepsin (Sigma, P-7000) at 37 ºC, during 20 minutes.
Prostatic tumour cells were labelled by antibodies Vim 3B4 and BBS/NC/VI-H14 but weren't marked by MNF116 and anti-PSA. Sertoli cell tumour was labelled by BBS/NC/VI-H14 and Vim 3B4, but not by MNF116 and anti-PSA. Hemangiosarcoma cells from a renal metastasis were only labeled by. antibody Vim 3B4. Antibody F8/86 didn't act in canine tissues, as was stated earlier by DakoBase.
In the dog and cat, anti-NSE antibody proved to be an useful marker of normal and neoplastic Sertoli cells (Patnaik & Mostofi, 1993; Silva, 2000; Scudamore & Meredith, 2001). Though the immunocytochemical profile of the prostatic neoplasm points to a sertolian origin, the structural characters revealed by routine histologic exam set the final diagnosis of prostatic metastasis of a hemangiosarcoma. The positive labeling of

the prostatic tumor by anti-NSE antibody reveals the presence of intratumoral heterogeneity at immunocytochemical level. Besides neuroectoderm-derived cells, NSE may be found in smooth and striated muscle cells, megakaryocytes and plasma cells, for example (Zardawi, 2003), and in non-neuroectodermal tumours, as human hemangiosarcoma (Basten et al., 2002) and some kinds of human lymphoma (Nakatsuka et al., 2002).

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ANALYSIS OF P53 MUTATIONS AND POLYMORPHISMS IN DOG AND CAT SQUAMOUS CELL CARCINOMA

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The p53 tumour suppressor gene, plays a crucial role in the protection against carcinogenesis in a wide variety of tissues. It has come the forefront of cancer research because it is commonly mutated in human and animal neoplasias.
About 50% of all skin cancer in normal individuals exhibit p53 mutations. In several studies was identified the UVB part of the solar spectrum as the major epidemiologic risk factor for skin squamous cell carcinoma (SCC) of animals and human. The p53 mutations induced by UVB radiation were found in >90% of humans SCC. These type of mutations, attributed to the UVB radiation, are known as "UV signature". Only one study is known about p53 mutation spectrum in skin SCC of dog.
The purpose of this study was to analyse and identify the P53 mutation spectrum of canine and feline skin SCC.
Polymerase chain reaction products, from p53 exons 5 to 8 were screened by single strand conformation polymorphism analysis and DNA sequencing.
In our study, were detected different P53 mutations in tumours of the two different species. Tumour heterogenicity was found. The same tumour sample exhibited two distinct p53 mutations supporting the idea of two different p53 clones in the same tumour. Polymorphisms study showed a relationship between these and different breeds of dogs and cats.
The molecular analysis of canine and feline skin SCC provided evidences for the envolvement o p53 in the progression of this type of tumours in dog and cats

CLINICAL AND PATHOLOGICAL STUDY OF AN OUTBREAK OF OVINE FACIAL ECZEMA IN ASTURIAS

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Facial Eczema is a form of hepatogenous photosensitive dermatitis occurring mostly in sheep and caused by sporidesmin, a potent toxin carried by the spores of *Pithomyces chartarum*. This microscopic fungus grows in pastures composed of ryegrass (*Lolium perenne*) under presence of high levels of humidity, warmth and litter. This disease has been described in areas with an Atlantic climate and, in Spain, it has been only reported in the Basque Country.

In this work, we made a clinical and pathological study of an outbreak of Facial Eczema that occurred in Asturias, in a flock of 54 adult sheep grazed in a pasture composed of clover and ryegrass. In 2003, at the end of the summer, photosensitization was observed in 13 sheep and a detailed pathological study was made in 5 of them.

Grossly, four of the livers appeared firm at touch, with differences in the gross appearance: in one case, the liver showed jaundice and calcareous stones in the bile ducts; atrophy of the left lobe was observed in two animals and regenerative nodules in other two cases. The remaining sheep died due to a pregnancy toxemia and grossly the liver showed a marked fatty change. Microscopically, all the animals had necrosis of epithelium of the bile ducts with cholestasis, degeneration of hepatocytes, fibrosis and severe bile duct hyperplasia. In most chronic cases, besides these changes, hepatocyte regenerative nodules were seen. In all the cases a severe dermatitis characterized by ulcers and crusts located in the head (ears, eyelids, muzzle,…) was observed. Microscopically, ballooning degeneration of keratinocytes and the presence of inflammatory exudate formed by neutrophils, lymphocytes and plasma cells was seen. Cholemic nefrosis was also found in all the cases.

Laboratory analysis of serum samples showed an increase of gamma-glutamyl transferase (GGT) levels

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IMMUNOHISTOCHEMISTRY AND ELECTRON MICROSCOPY CORRELATIONS IN CANINE MERKEL CELLS

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Merkel cells are large, clear oval cells located in the basal layer of the skin epidermis, the bulge region in the hair follicle, and lower layers in mucosal epithelium, usually in groups of variable number of cells. The vast majority of Merkel cells are intimately associated with enlarged terminal endings of myelinated nerve fibres forming synapse-like contacts (Merkel nerve endings), which function as mechanoreceptors in the mammalian skin.

The aim of this communication is to show the correspondence between immunohistochemical and electron microscopy features of this cellular type in the dog. Skin and mucosa samples from several anatomic regions of adult animals were fixed in 10% buffered formalin, embedded in paraffin-wax and cut to 5 µm sections for histology and 3 µm for immunohistochemical study. Epithelial, endocrine and neural markers were used to label the cells. Samples from same locations were fixed in 2.5% glutaraldehyde for electron microscopy. Correlation between immunohistochemical and electron microscopy regarding the arrangement of the canine Merkel cells, their relationships with other structures, and intracellular distribution of the immunoreactive products is discussed. gramirez@becarios.ulpgc.es

FORENSIC PATHOLOGY IN LIVESTOCK INSURANCE REPORTS. EXPERIENCES IN CATTLE IN THE SOUTH-WEST OF ASTURIAS

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In this work, the experiences obtained along one year of working in cattle as a legal expert are presented, focused on the necropsy procedure in the field and the main lesions observed. The realization of post-mortem studies in a necropsy room or a place specifically conditioned has several advantages than cannot be used when necropsy is performed in the field, especially when they have to be done in a large number of cases of large animals, as cattle. Usually, necropsy is with the animals placed on their left side and, if it is performed by only one person and considering the heavy weight of the animal and organs, most of them cannot be totally extracted from the carcass. On the other hand, present legal dispositions state that the carcass has to maintain its integrity and incisions have to be sutured for allowing the dead body disposal. For these reasons, necropsies in the field have some individual peculiarities depending on the cases and situations.

In this presentation, the main pathological findings explaining causes of death, in cattle farmed in the SouthWest of Asturias are also presented together with some singular cases. Pneumonias or digestive processes, mainly enterotoxemia in young cattle, as well as traumatisms, parturition related pathologies and abomasal displacements are the most remarkable entities found. Sporadically, “fog fever”, blackleg disease or renal processes (nephrosis/nephritis) were also encountered. Wild animals attacks were also frequently seen in mountain areas.

PRELIMINARY RESULTS ON AN OUTBREAK OF MORTALITY IN GROWER IBERIAN PIGS ASSOCIATED WITH MYOCARDIAL AND ENCEPHALIC LESIONS

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Heart and brain pathology in swine is associated with several agents such as viruses (Aujeszky, encephalomyocarditis, enterovirus), bacteria, nutritional problems (deficiency of vitamin E and/or selenium) and poisoning. They, although are usually independent pathologies, in some cases, both can be caused by the same agent. This communication describes an outbreak of mortality in grower Iberian pigs with myocardial and brain lesions.

Pigs were reared in a semi-extensive system with sows and unweaned pigs kept separately from grower pigs by a metallic fence. Mortality was only found in grower pigs of several weights. Sudden death or nervous symptoms of short duration were the only clinical signs observed. Postmortem examination was done in 7 pigs. Samples for histopathology and microbiology were taken. Blood and serum samples were also taken for haematological and biochemical analysis.

At necropsy, significant lesions were only detected in the heart of three animals. Extensive pale areas in the myocardium, subepicardial haemorrhages, especially in auricles and serofibrinous fluid in the pericardium were seen. Lungs showed severe congestion and oedema, probably as a consequence of cardiac failure. Some clear fluid was also found in the thorax and the abdomen.

Histopathologically, heart lesions corresponded with extensive myocarditis and myocardial degeneration. Myocarditis was predominantly mononuclear with perivasculitis and haemorrhages. Some foci of neutrophilic infiltration, scattered giant cells and some eosinophils were also seen. A mild fibrinous pericarditis was also observed. Degeneration was characterised by hiperconcentration of muscle fibres, necrosis, vacuolisation, loss of myocardial cells and some fibrosis. Myocarditis predominated in one pig and myocardial degeneration in two. In three pigs with no gross lesions, moderate brain lesions were detected. They were mainly located in the cerebral and and cerebellar cortex and corresponded with mononuclear encephalitis with areas of encephalomalacia. Perivasculitis, prominent vessels with endothelial hypertrophy, astrocitosis and degeneration of neurons and neuronal were detected.

A virus infection, such as that associated with the virus of the encephalomiocarditis, or a nutritional deficiency (vitamin E and/or selenium) were considered as possible aetiologies and further studies are being carried out.

CHARACTERIZATION OF CIRCULATING T CELL POPULATIONS IN CANINE LEISHMANIOSIS

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Canine leishmaniosis is a systemic parasitic disease with a wide variability response to specific therapy. With treatment, some patients apparently improve, some of them relapse and others do not respond. It has been demonstrated that the immunitary protective response to leishmaniosis is mostly cellular. In animals that do not respond to convencional treatment, it has been described a decrease in CD4+ T cells, high titers of antibodies and a negative leishmanin delayed type hypersensitivity (DTH). On the contrary, in dogs that respond to treatment there is a high proportion of CD4+ T cells, variability in antibodies titers and a positive DTH. For this reason, knowing the proportion of circulant lymphocytes, especially CD4+, CD8+ and CD21+ subpopulations, has a marked interest when studying leishmaniosis immunology. The objective of this study was to follow up during one year the lymphocytes subpopulations from animals with leshmaniosis receiving treatment. An exhaustive evaluation of the animals at the moment of diagnosis and after 1, 6 and 12 months was performed. Titers of circulating antibodies (using the ELISA technique), response to DTH and lymphocyte subpopulations CD4+, CD8+ (using flow cytometry) were evaluated. Low proportions of CD4+ T cells were detected in those animals with high titers of antibodies and a negative DTH. However, CD4+ T cells counts raised in those animals that responded to treatment.

FAT EMBOLISM CAUSING HEMOTHORAX IN A CAT

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Hemothorax associated with fat emboli due to soft tissue injury is a rare case of pulmonary pathology presentation. Hemothorax is most often the result of traumatic rupture of blood vessels, but it can also be caused by clotting disorders and erosion of the wall of a vessel by an inflammatory, toxic or neoplastic process.
In this communication we presented a ten years old, castrated female European cat who died with an acute crises of respiratory distress. At the autopsy the findings consisted of hemothorax and multiple small nodular hemorrhages located predominantly in the subpleural regions. A large hematoma on the subcutaneous fat tissue was found in the left inguinal area infiltrating the adjacent muscles. Other lesions consisted of subcutaneous petechiae and a liver decoloration. The histological study showed hemorrhages in the lung, edema, microthrombosis and microatelectasis together with megacariocytosis and hemorragic foci in the subpleural areas.
Samples from lung, kidney, brain, liver and hematoma were stained with the special histological staining "osmium tetroxide method" for the detection of fat emboli in formalin fixed tissues. Fat emboli were detected in lung capillaries associated with stasis and perivascular inflammation and in the vessels of the hematoma.
Large contusion of subcutaneous fat tissue cause releasing of fat globules that rapidly penetrate into the circulation through the ruptured vessels of the injured tissue and reach the lung circulation. The fat droplets cause mechanical occlusion of lung capillaries followed by chemical changes associated with hydrolysis of the neutral fat to free fatty acids. The free fatty acids produce a toxic and inflammatory reaction resulting in pulmonary hemorrhage and consistently in hemothorax.

MAIN CAUSES OF CONDEMNATION IN WASTING PIGS AT SLAUGHTER. A STUDY FOR ONE YEAR.

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In the last years, it has become usual to find some animals with a deficient growing rate in pigs herds. While their partners of the same batch are sent to the slaughter, these animals stay in the growing unit because they are not able to reach the slaughter weight. This wasting pigs usually present a lot of pathologies, principally of a chronic course, being the reason of condemnation in the abattoir veterinary inspection in a high number of cases. The causes of official condemnation in Spain are legally described in the RD 174/93.

The present study has been performed in a local abattoir in Valencia (Spain), where wasting pigs from the Southeast coast of the country are slaughtered. A total number of 5 378 wasting pigs were slaughtered during one year and 396 (7.56 %) were totally condemned. The main causes for this condemnation were multiple abscesses (59.02 %), caquexia (25.85 %), osteomyelitis (10.24 %), arthritis (7.56 %), peritonitis (7.32 %) and pleuritis (7.32 %). In most cases, multiple lesions were found in the same pig simultaneously.

Thus, 20 000 Kg of meat were condemned. It would have reached a commercial value of 20 400 €uros.

LA DOCENCIA DE LA ANATOMIA PATOLÓGICA VETERINÁRIA EM ESPAÑA

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FUTURO DE LA DOCENCIA DE LA ANATOMIA PATOLÓGICA VETERINÁRIA EN ESPAÑA, EN EL MARCO DE LA CONVERGENCIA EUROPÉA

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HIGH PREVALENCE OF GLOMERULONEPHRITIS IN IBERIAN LYN (*LYNX PARDINUS*): HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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The Iberian lynx *(Lynx pardinus)* is the most endangered cat species in the world[1]. There are less than 200 animals left in Spain and Portugal[2]. There are very few published studies about Iberian lynx pathology. The aim of this work was to study, histopathologically and immunohistochemically, the renal alterations found in these animals. Between 1998 and 2003, necropsy samples of Iberian lynxes, found dead or that died in captivity, have been submitted fixed in 10% buffered formalin from Doñana National Park to the Veterinary Pathology Service of the Veterinary Faculty of Madrid (Spain). In this study, renal samples of 16 Iberian lynxes were embedded in paraffin, sectioned at 4µm and stained with H&E, PAS, Masson trichromic and silver stainings. IgM, laminin and collagen IV immunohistochemistry was performed using the streptavidin-biotin-peroxidase complex method. Focal to multifocal membranous glomerulonephritis was seen in all the animals studied. Focal glomeruloresclerosis was also seen in some cases. Glomerular membranous deposits expressed laminin and type IV collagen varying depending on the severity of the lesions, generally being more intense in chronic phases. IgM immunoreaction was present in membranous glomerulonephritis and absent in glomerulosclerosis. Our results reveal a high prevalence of immune-complex glomerulonephritis apparently not related to viral infections since the different tests used for feline immunosuppressor viruses (FIV, FeLV, FIP virus and Panleucopenia virus) were negative in most cases. In our series of cases, the glomerular lesions could be due to immune system alterations described in this species[3] and attributed to the consanguinity.

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PATHOLOGICAL FINDINGS IN WILD AND ZOO ANIMALS: A RETROSPECTIVE AND COMPARATIVE ANALYSIS

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This study describes the comparative and retrospective (1995-2000) analysis of pathological findings obtained in wild and zoo animals from the Zoological Park of Jerez and the Center for Rehabilitation of Wildlife (CRAS), which is located in the Zoological Park.. We analyzed 503 animals, 128 mammals (120 captive and 8 wild), 353 birds (117 captive and 236 wild) and 22 reptiles (4 captive and 18 wild).
Our results confirm that wild birds were the main and most diverse group analyzed, and that infectious diseases (such as salmonellosis, colibacillosis, aspergillosis and herpesvirosis) were the principal group of diseases in both, captive and wild birds in addition to systemic or respiratory mycotic diseases in wild birds. There were differences in lesional patterns observed in some captive and wild birds. Meningitis and nephritis were only seen in wild birds breeding in captivity, whereas septicaemias were most frequent in zoo animals. On the other hand, metabolic diseases (above all visceral gout and amyloidosis) and neoplasias were most often diagnosed in zoo birds, whereas parasitic diseases were in wild birds. Due to the low number of wild mammals studied, a comparative study could not be made, but in zoo mammals significant diseases, such as adenovirosis in Patagonian cavy (*Dolichotis patagona*), congenital goiter in Tiger (*Panthera tigris*) and peracute mortality in Giraffe (*Giraffa camelopardalis rothschild*) have been diagnosed. Finally, in reptiles coccidial enteritis have been diagnosed in zoo animals, and bacterial diseases and dystocias in wild animals
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ESTUDIO NEUROPATOLÓGICO DEL ENCÉFALO DE UN GORILA ALBINO.

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Gracias al convenio de donación de muestras entre el ZOO de Barcelona y el Banco de Tejidos Animales de Catalunya (BTAC, http://quiro.uab.es/btac/), el encéfalo, junto con otros tejidos, del gorila albino Copito de Nieve fueron recogidos y procesados para su posterior disponibilidad para la comunidad científica.

En el BTAC llevamos a cabo un control de calidad de todo el material recolectado y además realizamos estudios en profundidad de los casos de mayor interés. El hecho de que fuera un primate anciano (aproximadamente 38 años) y el interés social creado sobre este gorila promovió su estudio detallado.

Presentamos un estudio preliminar histopatológico e inmunohistoquímico del encéfalo del gorila. Hemos observado diversas lesiones generalizadas como son espongiosis, fibrosis vascular, cúmulos de lipofucsina y neuromelanina intraneuronal, formación de cuerpos amilaceos, entre otras.

De forma localizada en el *Globus Pallidus* (GP), además de la calcificación de diversos vasos sanguíneos, se ha observado numerosos esferoides asociados a un abundante depósito de hierro. En la *Substantia Nigra* (SN), el depósito de hierro era menor y se ha visto asociado a una gran densidad de cuerpos amilaceos.

Mediante técnicas inmuhistoquímicas no solamente hemos caracterizado la inmunoreactividad del contenido de los esferoides sino que también hemos detectado cúmulo de material amiloide en la pared de algunos vasos sanguíneos (angiopatia amiloidea).

Los cambios observados parecen estar asociados al proceso neurodegenerativo normal de primates de edad avanzada.

PATHOLOGIAL FINDINGS IN SAURIA PETS

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Sauria, o Lacertilia, is a suborder of reptiles divided into the families Iguanidae, Scincidae, Agamidae, Varanidae and Gekkonidae, which included many genera and species that have become popular pets. Nonetheless, for many pathologists, anatomy, physiology and diseases of these animals are still poorly known. Few reports about Sauria diseases, especially viral diseases, have been published.

This work describes the gross and histopathological findings obtained in 25 Sauria from private collections that were submitted (during one year) to Anatomic Pathology Service at Veterinary Faculty of Córdoba. The species studied include: 9 bearded dragons (*Pogona vitticeps*); 5 leopard geckos (*Eublepharis macularis*); 3 iguanas (*Iguana iguana*); 1 day gecko (*Phelsuma madagascariensis*); 1 teratoscincus (*T. microlepis*); 1 carrot tailed gecko (*Teratolepi. fasciata*); 1 water dragon (*Physignatus conscicinus*); 1 uromastix (*U. geiry*); 1 red tegu (*Tupinambis rufescens*); and 1 monitor (*Varanus acanthurus*).

Our results showed that infectious and parasitic diseases (mainly digestive ones) followed by storage diseases (visceral gout and vascular calcifications) were the most frequent. Thus, atrophic enteritis associated with *Cryptosporidium* sp infection (3 cases), acute bacterial enteritis (1) and chronic glomerulonefritis were diagnosed in leopard geckos. Adenovirus entero-hepatitis (2), gut adenocarcinoma (1), bacterial septicemia (1) and perinatal death (4) were diagnosed in bearded dragons. Metabolic or deposition diseases were also observed in 3 iguanas and same lizards. Moreover, gut impaction associated with lentils feeding in an uromastix, and hemi-penis necrosis in a water dragon were diagnosed.

Moreover, in various gecko species we observed an apparently structural osteocartilaginous tissue in the wall of the atrio-ventricular trigonus (to the authors knowledge, this is the first report in geckos).

Finally, clinico-pathological findings suggest that an inappropiated handling of these animals was in the origin of the healthy troubles.

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H-DAF TRANSGENIC PIG LIVERS PREVENTS HYPERACUTE REJECTION AFTER ORTHOTOPIC XENOTRANSPLANTATION IN BABOONS: IMMUNOPATHOLOGIC STUDY

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The purpose of this study was to analyse whether livers from pig donors transgenic for the human complement regulator hDAF would be protected from hyperacute rejection after been xenotransplanted into a baboon (OlxT) and to study the immunopathological phenomena that follow an h-DAF transgenic pig model hepatic orthotopic xenotransplantion into a baboon.

Six-orthotopic liver xenotransplantation from Landrace pig to baboon have been carried out. Four livers were from unmodified specific pathogen free pigs and two were from an h-DAF transgenic pig. The recipients were six baboons (*Papio anubis*).

Biopsies of the liver grafts were performed 2 hours after reperfusion and at the end of each experiment. hDAF expression was detected by a murine antihuman CD55 mAB (BRIC 216; IBGRL Research Products, Elstree, UK) and the pigs with high level of hDAF expression in the endothelium, arterial smooth muscle, and soft tissue of skin biopsies were selected for this study. Samples were stained with mouse anti-human monoclonal antibodies (mAb) to C3, C5b9, IgG, IgM, fibrin (DAKO), and rabbit anti-human policlonal antibody to C4 (Dako) to visualise the deposition along blood vessels. Intensity was assessed against a control section stained at the same time with Von Willebrand factor, a known endothelial marker. Phenotypic analyses of the cellular infiltrates were performed with murine anti-human monoclonal antibodies specificfor CD2, CD4, CD8, CD20, CD68 (DAKO). The average number of positively stained cells was counted per 5 random high power fields.

RESULTS: In all cases control hepatic xenotransplants showed massive haemorrhage, sinusoidal disruption, thrombosis, oedema and hepatocite vacuolisation. Immunohistochemistry demonstrated always endothelial deposits of the complement factors C3, C4 and C5b9, along with IgG, IgM and fibrin were observed. CD31 expression was seen in the portal spaces and at perilobulillar levels. A neutrophilic and macrophagic infiltration was also seen as the main round cell infiltrate in the xenotransplants.

In h-DAF transgeneic pigs, liver lobar architecture was preserved, with sinusoidal and liver plates integrity and absence of haemorrhage. h-DAF xenografts presents IgG, IgM and C4 deposits. However no fibrin, C3 and C5b9 deposits are observed after reperfusion. Therefore, h-DAF seems to have some degree of HAR immunoprotection of the liver from the alternative complement pathway and incomplete activation of the classical pathway. Both of our patients displayed CD31 staining at the portal spaces, perilobulillar levels and hepatic sinusoidal level.
The baboon that lived for up to 4 days displayed a round cell portal infiltrate with T cells, either CD4 or CD8, but also and to a lesser extent macrophages and B cells. However, our other case, surviving up to eight days, did not display at the post-mortem biopsy any type of round cell infiltrate. Anyhow, this animal developed a clinical case of delayed vascular rejection after 36 hours post xenotransplantation and it was successfully treated with boluses of methylprednisolone and an increment in the dose of CyP.
CONCLUSIONS: Our data suggest, throughout human CD55 expression in pig a blockade of the complement cascade (C5b9), and so forth the HAR. CD31 hepatic sinusoidal expression seen in both of our transgenically xenotransplantated animals will have to be surveyed for a prospective physiological role in our experimental model. Moreover, once HAR has been overcome, we have shown the progress of a CD4 and CD8 T-cell infiltrate. Future aims will be to determine whether these CD4 and CD8 populations constitute an effector population or not, and its role in xenorejection.

ACUTE GASTRIC DILATATION IN LABORATORY-HOUSED BABOONS

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Acute gastric dilatation (AGD) is a common condition to most primate clinicians. The aetiology of this process has been the subject of much speculation, but it is still poorly understood.

Clinico-pathological findings: Five cases of AGD were observed in adolescent or adult baboons over a 6 month period under captive conditions in our animal-house. One animal was saved due to a prompt veterinary action. The treatment consisted of evacuation of the stomach, correction of blood volume deficits and acid-base disturbances by administration of appropriate fluids, and supportive therapy for shock. Four animals were found dead in their cages without any premonitory clinical signs showing an arched back, a marked distending and tympanic abdomen, and pale mucus membranes. At necropsy, stomach was distended and replete of fermented food material. Stomach rupture of different dimensions was observed. Just in one case we observed alterations in the intestine and other abdominal viscera such as congestion and oedema. Peritonitis was not appreciated in any case. Several samples were taken from the stomach content and *Clostridium perfringens* was isolated.

After those experiences we have supervised the risk factors in our husbandry practices. Since we have observed a prolonged period of digestion with a progressively distending abdomen in the primate colony, we have evaluated gastric emptying times due to gastric motility has been suggested as possible causative factor for acute gastric dilatation. Our study shows a significant delayed of gastric emptying times. To avoid this problem we have used a prokinetic agent such as cisapride at a dose of 0.2 mg/kg PO before meal. None new AGD case has been observed since we have carried out this therapy.

We conclude that abnormal emptying gastric times observed in laboratory-housed baboons could be a main factor in the aetiology of acute gastric dilatation. The use of an appropriate prokinetic therapy has been proved to be a useful method to prevent this pathology.

CLINICO-LESIONAL STUDY OF EXPERIMENTAL MASTITIS CAUSED BY *MYCOPLASMA AGALACTIAE* IN GOATS

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Contagious Agalactia is an infectious disease of small ruminants characterized by mastitis, arthritis and keratoconjuntivitis, in which *Mycoplasma agalactiae* (Ma) is considered the main aetiological agent. Although the disease is endemic in most Mediterranean countries, the veterinary literature describes only few studies of experimental Contagious Agalactia associated with Ma in goats. The aim of this study was to investigate chronologically the clinical and lesional changes and the inflammatory response during experimental mastitis caused by Ma in goats. Fifteen lactating goats were inoculated via intramammary with Ma. Four goats were used as uninfected controls. Rectal temperature, California mastitis test, somatic cells (SCC) and differential leukocyte counts were carried out The animals were killed at 5, 15 and 45 days post inoculation (dpi) and tissues samples were collected for histological study. Clinico-lesional changes allowed to classify the infection into three phases**:** 1) Acute phase (0-7 dpi), characterized by agalaxia, severe mastitis, regional lymphoadenomegaly, and high levels of SCC and colony-forming units. Histologically, the inflammatory infiltration was limited to the glandular acini and intralobulillar ducts, and it was composed by macrophages and neutrophils; 2) Subacute phase (7-15 dpi) in which the inflammation of the mammary gland was marked reduced and the atrophic processes became remarkable. The lymphoadenomegaly, and SCC counts persisted increased. Histologically, suppurative galactophoritis with diffuse subepithelial lymphoplasmocytic infiltration, inter and intralobulillar fibrosis and discrete acinar inflammation were the main findings; 3) Chronic phase (15-45 dpi) that was characterized by severe atrophy of the mammary gland with regional lymphoadenomegaly. The SCC and bacterial milk excretion persisted high. The histological changes in this phase consisted of severe acinar atrophy and fibrosis, and galactophoritis, in which the subepitelial mononuclear infiltration was organized into lymphoid follicles.
The results of this study indicate an evolution of the lesions of the experimental Ma infection in goats from exudative inflammation in earlier phases to severe atrophy in chronic stages of infection. Moreover, this study represent the morphological substrate to further studies in order to stablish the immuno-inflammatory response of the caprine mammary gland to Ma infection.

PATHOLOGY OF BOVINE TUBERCULOSIS IN THE RED DEER AND THE WILD BOAR IN CENTRAL SPAIN, EVIDENCE FOR TRANSMISSION ROUTES AND EPIDEMIOLOGY OF THE DISEASE

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It is well known that bovine tuberculosis (*Mycobacterium tuberculosis* complex) may spread from domestic animals to wildlife, and those reservoirs may establish in wild animal species. High *Mycobacterium tuberculosis* complex prevalence have been found among wild ungulates, especially the red deer and the wild boar, in hunting estates in South Central Spain. In epidemiological studies distribution and type of pathologic lesions can give information about transmission routes and the role of a species in disease maintenance, especially when interpreted in the context of other information. Hunter harvested red deer and wild boar sampled in 76 sites as part of a nation-wide survey had mean prevalence of macroscopic lesions of 13.7% and 42.5% respectively.
In the wild boars, 39.8% with macroscopic Tuberculosis-compatible lesions (n=211) presented one single lesion. In 96.3% of them, this single lesion affected the mandibular lymph nodes, in 2.5% the thoracic viscera, and in 1.8% only the hepatic lymph node. The remaining 61.2% of the lesion-positive animals presented generalised lesions.
In the red deer, 42.9% of the macroscopically lesion-positive animals (n=98) presented lesions at one anatomical site: 40.5% in the retropharyngeal lymph nodes, 28.6% in the thoracic viscera, and 16.7% only in the mesenteric lymph nodes. The remaining 57.1% of the lesion-positive animals presented generalised lesions.
Routes of infection are indicated by the location of single site infections. In the red deer locations indicated that both the respiratory and the alimentary route are of importance, whereas in the wild boar routes of infection could not be differentiated, since mandibular lymph node lesions may reflect both routes. Histological lesions in the wild boar tend to be calcified and encapsulated, whereas lesions in the red deer have generally a less prominent fibrous tissue capsule, are rarely calcified and do frequently contain liquefied pus. This may indicate a higher resistance to the disease in the former and a higher susceptibility in the latter. Pulmonary lesions could be evidenced in 10% of the boar examined histologically, meaning that in these animal's excretion with respiratory secretion may occur.

EXPERIMENTAL INFECTION WITH *Mycobacterium avium* subespecies *paratuberculosis* OF CALVES PREVIOUSLY IMMUNIZED.

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Paratuberculosis is an infectious disease of ruminants caused by *Mycobacterium avium* subespecies *paratuberculosis* (Map) that occurs frequently all over Spain. In bovine species, a classification of the lesions associated with natural paratuberculosis, showing differences with those observed in small ruminants, has been proposed. However, it has not been validated in an experimental model. On the other hand, in sheep it is well known that vaccination, considered as the most efficient method for controlling the disease, does not prevent the infection but modifies the progression of lesions towards focal and latent forms. This fact has not been studied in cattle. With the aim of elucidate these topics, and experimental infection has been carried out using 18 Frisian calves, orally challenged with Map when 5 months old, after their immunization with a killed vaccine (Silirum®) two months earlier. The following groups were considered: i) Infected and non-vaccinated (n=6) (INV); ii) Infected and vaccinated (n=8) (IV); iii) Non-infected and vaccinated (n=2) (NIV); iv) Non-infected and non-vaccinated (n=2) (NINV). At 180 dpv (120 dpi) 5 calves were sacrificed (3 from IV and 2 from INV groups). The remaining animals were killed at 330 dpv (270 dpi). In all the cases, a complete histopathological study were carried out, focusing on the intestine associated lymphoid tissue. Humoral and cellular peripheral immune responses were assessed at different periods along the study, as well as the detection of Map DNA in blood samples by PCR.

The histopathological study confirmed the pathological classification proposed in natural cases. Focal lesions, located mainly in the lymph nodes, and multifocal an diffuse forms, harbouring higher numbers of giant cells than in sheep, were described. Concerning type of lesions according to the treatments received, in calves from group IV they were absent or focal or absent, except for one case having diffuse forms. However, in INV group, lesions varied from multifocal to diffuse, associated with high number of bacilli. Control animals did not show any lesion.

These results suggest that vaccination in cattle does not prevent the infection but induces a high number of animals having focal or latent lesions, with respect to non-vaccinated animals. However, protection is not absolute since a vaccinated calf had severe lesions.

Immunization induced a cellular and humoral peripheral immune response and DNA from Map was detected in INV animals at the beginning of the experiment, suggesting an initial bacteraemia.

ABSCESS DISEASE IN SHEEP: PATHOLOGY, DIAGNOSTIC AND USE OF AUTOVACCINES

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Abscess disease in sheep is a process that affects young animals, being caused by *Staphylococcus aureus*. Abscess disease has not been completely studied and many aspects of the process remain unknown.

In this work, we have studied two ovine farms dedicated to grow young lambs (males and females) for selling and replacement purposes. Up to 35% of the animals were affected in both farms. The animals spontaneously developed abscesses in the subcutaneous tissue of the neck, normally near or in the vicinity of the glottis. However, the abscesses could also be located in other subcutaneous regions such as in the limbs or in the chest. This abscesses could reach an important and remarkable size and contain variable quantities of a dense to semi-liquid yellowish-green material that was surrounded by a thick fibrous tissue. Lymph nodes showed marked chronic lymphadenitis and were always located near the fibrotic capsule of the abscess. Microscopically, the purulent material was formed by a vast majority of neutrophils but also macrophages, epitheliod cells, lymphocytes and plasma cells. Colonies of rounded bacteria could be seen in all cases, specially near the capsule. They were normally surrounded by an eosinophilic material (Splendore-Hoeppli material) and an intense inflammatory reaction. Microbiological studies demonstrated the presence of *Staphylococcus aureus,* without other agents. Autovaccines were prepared from the isolates and their use in the affected farms reduced the prevalence of the disease to sporadic cases.

In this communication, the findings will be discussed and we will stress the differential diagnosis with the subcutaneous abscesses caused by *Corynebacterium pseudotuberculosis*, the etiological agent of the ovine pseudotuberculosis that produces macroscopically-similar lesions. The possibility of the redefinition of this disease as a botryomycosis will be presented.

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DIFFERENT ROLES OF CD4 AND CD8 T CELLS *Chlamydophila abortus* IN IN A MOUSE MODEL OF PRIMARY INFECTION

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Role of specific cellular immune response has been well stablished in the infections induced by the family *Chlamydiaceae* members. However, the importance of each T cell subset seems to depend on species, since CD4+ cells are the main subset involved in the response *to Chlamydia trachomatis* (Morrison and Caldwell, 2002), while CD8+ cells have a crucial role in the resolution of *Chlamydophila pneumoniae* infection (Penttila et al., 1999). The aim of this study is to clarify the role of T cell subsets in the response to *Chlamydophila abortus* primary infection.

Female C57BL/6 mice were depleted of CD4+ or CD8+ cells by monoclonal antibody inoculation, and infected with the AB7 strain of *C. abortus*. At different post-infection days, mice were killed and samples were collected to carry out bacteriological, and histopathological analysis. In addition, spleen cell culture and IFN-γ quantification, immunohistochemical *C. abortus* antigen location and a TUNEL assay for apoptosis detection were performed.

T cell subset depletion displayed very different results: CD8-depleted mice showed a very high mortality after *C. abortus* infection (100% at day 8 post-infection) whereas CD4-depleted mice showed a decreased morbidity, expressed as weight loss. In relation with the chlamydial burden, CD8-depleted mice showed a significant increase compared with CD4-depleted and non-depleted mice. Additional analysis showed that CD8-depleted mice had a higher number of apoptotic cells in the inflammatory foci of the liver, an increase in the dissemination of the *C. abortus* infection in the lung, and an exacerbated IFN-γ production by spleen cellsafter specific stimulation**.**

All these data lead to the conclusion that CD8 T cells have a very important role in the control of primary *C. abortus* infection. This role could involve both a regulatory control of the CD4 T cell response and a direct cytotoxic or IFN-γ-mediated action on infected cells.

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DIAGNOSIS OF TUBERCULOSIS IN THE LYMPH NODES OF WILD BOARDS HUNTED IN LA RIOJA COMMUNITY

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At present, prevalence of cattle tuberculosis has been reduced significantly due to eradication programs and, in consequence, the role of wildlife as reservoir of the infection has gained attention. In the last years and in some areas of Spain, tuberculosis has been considered as a major disease among wild boards. In recent works carried out in Iberian pigs naturally infected with *Mycobacterium bovis*, lesions have been constantly identified in mandibular and retropharyngeal lymph nodes. A diagnostic protocol based on the examination of these lymph nodes was carried out in 101 wild boards coming from more than 30 hunts in La Rioja community. Lymph nodes were carefully trimmed and gross lesions were identified and classified according to caseous necrosis extension (A: miliary; B: nodular; C: diffuse). A total of 24 animals showed pathological changes (A: 14; B: 5; C: 5). Microscopically, two main types of lesion were identified. Type H1 was characterized by the presence of necrosis and mineralization, granulomatous inflammation and repair tissue. This lesion was seen in 10 of the animals, and acid-fast bacilli were identified in all the cases by Ziehl-Neelsen, immunohistochemistry against *M. bovis* antigens or PCR by the detection of the IS6110 insertion sequence of *M. tuberculosis* complex, in paraffin-embedded tissue sections. Type H2 lesions were depicted by the presence of "sulfur granules" in the necrotic areas, characterized by colonies of gram-positive bacilli surrounded by a zone of palisading, eosinophilic, club-shaped structures accompanied by an inflammatory exudate formed mainly by neutrophils, macrophages, lymphocytes and foreign body giant cells demarcated by a thick fibrous wall. This type appeared in 14 animals and mycobacteria was not detected in any of them. Type H1 lesions were associated with any of the gross lesions observed whereas H2 forms showed an A pattern. This work confirms the importance of wild board as a reservoir of tuberculosis, that was identified in a 10% of the cases and points out the importance of infections consistent with *Actinomyces* spp. (14%) in the retropharyngeal and mandibular lymph nodes. Lesions due to this bacteria could cause difficulties in the gross diagnosis of tuberculosis. The absence of mixed infections due to both pathogens is remarkable.

EVALUATION OF THE PATHOGENICITY OF SEVERAL STRAINS OF *Mycobacterium avium* subspecies *paratuberculosis* IN AN EXPERIMENTAL INFECTION OF LAMBS

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Paratuberculosis or Johne´s disease is a chronic granulomatous enteritis of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map). Infected animals show a variety of lesions, with differences concerning its type and location, between ovine and bovine species. On the other hand, it is well known that different strains can be recognized in Map, according to the animal species and microbiological properties. Their existence has been confirmed by recent molecular techniques. However, the pathogenicity of the strains and their ability to infect other animal species different from the one which they were isolated, it is not well documented. In this study, a method for the histopathological evaluation of lesions produced by Map infection has been used for assessing the sensitivity and pathogenicity of several strains of this bacteria, by means of an experimental infection carried out in lambs. A total of 30 animals, divided in 6 groups, were orally challenged with several strains of Map: groups 1 & 2: two bovine strains, with different genetic patterns (A and E); group 3 & 4: a bovine strain, directly purified from the intestinal mucosa of a clinical case, and the same strain grown in culture media; group 5: an ovine strain, directly purified from the intestinal mucosa of a clinical case; group 6: control lambs.
Throughout 5 months, humoral and cellular peripheral immune responses were evaluated as well as the existence of Map DNA in blood by PCR. At 150 dpi, all the lambs were humanely killed and a pathological study was made, focused on the intestinal lymphoid tissue. The presence of Map in the tissues was assessed by Ziehl Neelsen, immunohistochemistry and PCR, either in paraffin-embedded or in frozen samples. All the strains were able to infect the lambs, but differences were observed in the lesions. The ovine strain caused diffuse and severe lesions formed by epithelioid cells whereas bovine strains induced focal or multifocal lesions, composed of granulomas with a high proportion of giant cells and lymphocytes, although variations in the intensity were seen among groups. In the bovine groups, lymph nodes were the most affected organs whereas in group 5 lesions appeared in the intestine, mainly in jejunal Peyer´s patches. Map or its DNA were detected in tissues from all the groups, with

different sensitivity depending on the sample and technique used. In all the lambs, a peripheral immune response was induced, with variations among groups. In a total of 13 lambs, DNA from Map was identified in blood samples until 120 dpi. Its absence was associated with an increase in the cellular immune response. These results suggest that Map strains can have an effect on the pathological features of the infection.

MACROSCOPIC AND MICROSCOPIC LESIONS OF PARATUBERCULOSIS IN SHEEP AND GOATS IN THE AREA OF LISBON

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A Research Project for the control of paratuberculosis in herds of small ruminants on the area of Lisbon was initiated in mid 2002 and included a serological survey of a sample of 66 small ruminant herds in 5 counties in the area of Lisbon, identifying 18 herds with seropositive animals.

Nine seropositive sheep and goats, from 6 herds, with ages ranging from 3 to 7 years and showing emaciation, were selected for evaluation by necropsy followed by histopathology. Post-morten lesions were observed in 6 cases, 1 did not present lesions, and in 2 animals the histopathology analysis is still in progress.

Up to the present, bacteriological isolation of *Mycobacterium avium paratuberculosis* (*Map)* was positive in 6 samples of intestine from animals with lesions: in 1 case without lesions the bacteriology was negative and, in 2 animals, bacteriological analysis is still in progress. The bacteriological results had a 100% correlation with the suggested diagnosis of paratuberculosis raised by the necropsy lesions.

The 6 confirmed infected animals were goats and the common gross lesions were the typical granulomatous enteritis on the jejunum and ileum which involved, in 3 cases, the ileocaecal valve, and showed thickening of the intestinal wall and the presence of transversal folds giving to the mucosa a corrugated appearance. In 5 animals there was an hypertrophy of the mesenteric lymph nodes (LN) together with serosal lymphangitis.

Effusion of fluids into the body cavities occurred in 5 of the animals and, in 3 of these, it was also observed hydrocaquexia of the heart.

The histopathology included staining of sections of intestine and mesenteric LN with Ziehl Nielsen. In 5 animals, acid fast bacilli were found in both organs but were more abundant in the former. Acid fast bacilli were also observed in sections of LN without lesions but couldn't be found in the typical macro lesions of intestine and LN of an animal.

Our preliminary studies confirm that necropsy and histopathology can be considered valuable tools for the diagnosis of paratuberculosis as they provided, in a short period of

time, an effective presumptive diagnosis. Nevertheless, it is considered that the animals analysed were probably in a late phase of infection, and efforts are being developed to further increase the casuistic of the pathological studies in order to include several phases of infection.

VALORACIÓN DE LA PRUEBA DE LA INTRADERMOREACCIÓN PARA EL DIAGNÓSTICO DE LA TUBERCULOSIS EN ANIMALES INFECTADOS POR *Mycobacterium avium* subsp. *Paratuberculosis*

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La prueba de la intradermoreacción (IDR) es utilizada en los programas de erradicación de la tuberculosis (TB) en el ganado bovino. La interpretación de la misma en los animales con reacción dudosa es particularmente importante en áreas donde existe una baja incidencia de la enfermedad, debido en parte, a la existencia de reacciones cruzadas con otras micobacterias. En este sentido, cabe destacar la infección producida por *Mycobacterium avium* subsp. *paratuberculosis* (*Map*), que se caracteriza por ser una enteritis crónica granulomatosa de los rumiantes. El objetivo de este trabajo fue la valoración de reacciones cruzadas con esta micobacteria. Para ello, se investigaron 55 animales sacrificados en matadero (26 de ellos IDR+ y 29 IDR-) pertenecientes a 31 rebaños, y 926 animales vivos pertenenientes a 16 rebaños, de diferentes zonas de Asturias, oficialmente indemnes de tuberculosis en los últimos años y en los que se había diagnosticado paratuberculosis (PTB) por serología y por su cuadro clínico. De los 55 animales sacrificados se recogieron muestras para estudios histopatológicos de TB (linfonodos retrofaríngeos, mediastínicos y bronquiales) y PTB (válvula ileocecal y linfonodos ileocecal y yeyunal caudal), así como muestras para la valoración de la respuesta inmune humoral mediante la técnica ELISA frente a la TB y a la PTB. Asimismo, se realizaron estudios bacteriológicos para la identificación de *Map* mediante la Reacción en Cadena de la Polimerasa (PCR). A los 926 animales se les realizó la IDR para la detección de TB, y una valoración de la respuesta inmune humoral mediante la técnica ELISA TB y PTB. Asimismo, se estudió la respuesta inmune celular mediante la técnica del gamma-interferón (gINF) comparada en los animales positivos y dudosos a la IDR.

En el primer estudio, en los animales IDR+, se detectaron lesiones tuberculosas en 11 animales. En cambio, se encontraron lesiones de PTB indistintamente en los dos grupos de animales. De los 15 animales IDR+ que no presentaron lesiones tuberculosas, 2 aparecían sin lesiones de PTB, 10 presentaban lesiones de tipo focal y 3 de tipo multifocal, pudiendo identificarse *Map* en 6 de ellos. 7 de estos animales resultaron negativos al ELISA TB y PTB; y 8 positivos o dudosos a ambas pruebas. De los 926 animales vivos estudiados 7 resultaron positivos a la IDR (> 4 mm), 15 dudosos (2-4 mm) y 904 negativos (< 2 mm). Los 7 animales positivos a la IDR resultaron negativos

al ELISA de TB, 3 dudosos al ELISA de PTB, y 6 positivos "aviares" al test de gINF comparado. Los 15 animales dudosos a la IDR simple fueron positivos "aviares" en el test de gINF comparado. De los 904 animales negativos a la IDR, 54 fueron positivos, 95 dudosos y 755 negativos al test ELISA TB; en cambio, 99 fueron positivos, 166 dudosos y 639 negativos al ELISA PTB. Dos animales con cuadro clínico de PTB y negativos a la IDR simple, fueron necropsiados y realizados estudios histopatológicos, observándose una enteritis granulomatosa difusa grave de tipo multibacilar en un animal y de tipo intermedio en el otro. El primer animal había resultado negativo al ELISA TB y positivo al de PTB. En cambio, en el segundo, el resultado al test ELISA TB fue positivo débil y al test ELISA PTB dudoso.

Los resultados obtenidos indican que las reacciones inespecíficas a la IDR ocurren principalmente en animales que presentan lesiones paratuberculosas de tipo focal, latentes y subclínicas. En este sentido, la realización del test gIFN comparado en vacas con reacciones dudosas o positivas débiles en la IDR simple, estaría recomendado para la diferenciación de reacciones cruzadas a esta última prueba debidas a infecciones por otras micobacterias diferentes a las de la TB, como *Map*. Asimismo, los tests serológicos no tendrían una utilidad en la discriminación de estos animales. Ello sería debido a que la respuesta inmune celular se produciría en los estados iniciales, latentes y subclínicos de la infección por *Map*, y la respuesta humoral en la mayoría de los estados finales. Por lo que el hecho de que los animales positivos y dudosos a la IDR presenten una respuesta inmune celular alta y mayor frente a la proteína aviar, es indicativo de que las reacciones cruzadas podrían aparecer principalmente en animales subclínicos asociados con lesiones de paratuberculosis de tipo focal, y de difícil detección post-mortem.

Myoporum laetum POISONING IN CATTLE

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The poisonings for plants are common in extensive rearing bovine. Some of them provoke serious hepatic injuries, with or without associate photosensitization. *Myoporum laetum* is a plant of the *Myoporaceae* family, introduced in Spain as ornamental shrub, developing of natural form in some zones of Andalusia and Estremadura. In other countries, the consumption of this plant has provoked serious poisonings in sheep and cattle, with hepatic injuries and sometimes photosensitization. In the present communication a case of poisoning for *Myoporum laetum* in limousin cattle happened in the province of Cadiz is described, process not known up to this moment in our country. In a group of 30 cows that consumed big quantities of the above mentioned plant, discouragement, anorexia, ruminal atony, lateral decubitus and death of twelve animals in the course from five until ten days later to the consumption was observed. In the rest of the animals four abortions took place, and all of them showed signs of photosensitization in the skin of the knob. The autopsy of three animals was carried out, and histopathologic studies of liver, kidney, heart and brain were realised. Extensive haemorrhages in subepicardial zone, intestinal serosa and mesentery were observed, as well as erosions and ulcers in reticulum and abomasus. In the rumen great quantity of Myoporum laetum was appearing. Histopathological, as more relevant injuries, phenomena of fibrinoid focal necrosis of the arteriole's wall of the haemorrhages areas, and in the liver, intense biliary hiperplasia, periportal necrosis and vacuolar degeneration of medial and centrolobular zones was appreciated. The clinic and the observed injuries are, in general, coincidental with described previously in this type of poisoning. The possibility of poisoning for *Myoporum laetum* should be considered in the differential diagnosis of poisonous processes that should show serious hepatic injuries with necrosis and biliary hiperplasia, as well as in unspecific hemorrhagics states with high mortality in sheep and cattle, principally in zones of Andalusia and Estremadura, where Myoporum laetum seems to have been naturalised.

STUDY OF PORCINE RESPIRATORY DISEASE COMPLEX ASSOCIATED TO CIRCOVIRUS TYPE 2 IN POSTWEANING PIGS IN A PIG FARM IN GRAN CANARIA

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This work reports a clinical case occurred in a pig farm of 130 breeding sows, in Gran Canaria Island. Poor weight gains, anorexia, jaundice, cough, periorbital edema and 50 % mortality were found in affected postweaning piglets (60-90-days old). Clotted and unclotted (EDTA) blood samples from affected (n=14), 60-90-days old non-affected (n=17), 35-days old (n=6), 17-days old (n=6) pigs, and breeding sows (n=20) were collected for serologic test (ELISA) and molecular biology (PCR-ELISA). Fourteen affected pigs were euthanised and standard necropsy was performed. Samples of tissues for histopathological, bacteriological and molecular biology analysis were taken. Gross lesions consisted of systemic lymphadenomegaly, hepatomegalia, splenomegalia, fibrinous-purulent poliserositis, interstitial pneumonia and central nervous system congestion. Microscopically, the examinations revealed mixed meningitis and non-suppurative encephalitis. Serologic studies revealed ADV antibodies in all tested animals and PRRS antibodies in affected pigs. A DNA fragment of porcine circovirus type 2 (PCV 2) was successfully amplified from sows and euthanised pigs, by PCR-ELISA. *Streptococcus suis, Haemophilus parasuis, Arcanobacterium pyogenes* and *Pasteurella multocida* were isolated from lung, tonsil, and fibrinous-purulent exudate. Antibiogram showed that amoxicillin and penicillin were the most effective antibiotics. This study showed that symptoms and lesions were related to porcine respiratory disease complex (PRDC). Several references have demonstrated the association of PRDC with PCV 2, and PCV 2 with other viruses and bacteria as the ones isolated in this work. In this cases, bacterial infection is considered the most probably cause of death.

ETIOPATHOGENIC STUDY OF NATURALLY INFECTED PIGS WITH PORCINE CIRCOVIRUS

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Concerning the pathogenesis of porcine circovirus (PVC), it is known that posweaning multisystemic wasting syndrome is a new porcine disease affecting mainly nursery and early growing pigs. The aim of these communication was to investigate the distribution of PCV antigen in the tissues and blood of naturally infected pigs.

We studied 43 piglets. As to the clinically non-affected, their ages were: 17 (n=6), 35 (n=6), and 60(n=17) days old. The clinically affected piglets (n=14) were all 60 dys old. Multiparous (n=10) and nulliparous (n=10) breeding sows were investigated. Clotted and unclotted (EDTA) blood samples were collected. The affected animals group were euthanised and complete necropsies were performed. Tissues samples were fixed in 10% buffered formalin, and embeded in paraffin. Immunohistochemistry (IHQ) identification of PCV in tissues was performed in 32 selected samples. Twenty nine samples were frozed at –80ºC, for virus detection by ELISA-PCR. Antigen PCV were detected in blood samples of animals from the group 35-60 day old. In tissues samples of the animals, two and four animals were negative respecting detection of the antigen PCV by ELISA-PCR and IHQ technique, respectively.In breeding sows, the antigen PCV was detected in blood samples from 70% of the multiparous and from 40% of the nulliparous..

The results of PCV distribution in tissues was observed according to a decreasing range starting at the lymphoid system, passing the lungs and, finally, terminating in the tonsils. The distribution of antigen, in blood and at the same time in tissues (in group of animals with PMWS), suggest that the infections herds occurs before 20 weeks of age and that the role of breeding sows as source infection of PCV can be a fact.

SUSCEPTIBILITY PATTERNS OF INBRED MICE TO EXPERIMENTAL VIRUS INFECTION SUGGEST A SIGNIFICANT INFLUENCE OF THEIR GENETIC BACKGROUND

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The mouse type-1 parainfluenza virus (Sendai), a widespread natural respiratory pathogen of mice, is currently used as a model to study host-pathogen interactions. In this context, the present study aimed at establishing whether and how the genetic background may influence the course of a standardized experimental infection.
Twenty 13-wk old female mice of six different inbred strains (Balb/c, DBA/2, SJL/J, 129Sv, C3H/HEN and C57BL/6J), were inoculated intranasally with 50µl of a viral suspension containing 1000PFU, a volume and an infecting dose that had been previously shown to result in an homogeneous distribution of infection throughout the lungs. Body weight and pulmonary function values were monitored daily for two days before and seven days postinoculation (pi) by double-chamber plethysmography in 10 mice of each strain. At 5, 6, and 7 days pi, sets of 5,5, and 10 mice of each strain were euthanised. The left lungs were homogenized in PBS and a 10% suspension of each was prepared for virus titration, by plaque assay.
The functional, morphological and virological data collected unequivocally suggest the existence of extreme phenotypes in terms of resistance/sensitivity to Sendai virus infection. We intend to use these data as tools for genetic mapping, through crossbreeding studies between susceptible and resistant strains, attending to highlight one or more of the following putatively monogenic phenotypic traits: (i) *sver* for « sendai virus early restriction», (ii) *svel* for «sendai virus elimination (fast vs. slow lung clearance), (iii) *svir* for «sendai virus inflammatory response» (granulocytic bronch(oli)itis vs; agranulocytic pneumonitis) et (iv) *svep* for «sendai virus epithelial permissiveness» (bronch(iol)otropism vs. pantropism).

PRELIMINARY RESULTS IN THE EPIDEMIOLOGICAL STUDIES OF THE JAAGSIEKTE RETROVIRUS INFECTION IN A NATURALLY INFECTED FLOCK

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Ovine pulmonary adenocarcinoma (OPA, sheep pulmonary adenomatosis, jaagsiekte) is a contagious neoplastic disease of the lung which occurs naturally in sheep. Alveolar type II cells and Clara cells proliferate developing a characteristic lung adenocarcinoma. A retrovirus known as jaagsiekte retrovirus (JSRV) is the cause of the neoplasia. The virus establishes a disseminated infection and can be detected in white blood cells by PCR, either in diseased animals or in contact sheep with no evidence of neoplasia. However there is no detectable immunoresponse. Thus, the only way to evidence the presence of infection in life animal is a PCR procedure which detects the viral DNA in peripheral white blood cells. Previous studies determinated that this PCR is specific for JSRV. We selected a flock with 200 Rasa Aragonesa breed and crosses with a long history of OPA and we have bleed all the animals, obtaining the first prevalence of the infection. Results have indicated a percentage of 28.6% for the positive animals. These were detected in all ages, but were more prevalent around two years of age with other peak at 6 years of age. No differences have been observed in relation with breed. A group of ewes and ewe lambs have been selected and monitorized. There is no clear correlation between positive ewes and JSRV infection in the progeny. Investigation in the sheep culled or died from the flock indicated a proportion of 30% positive to the PCR blood test: ½ of these animals developed OPA lesions and 1/3 of them developed clinical signs of the disease. Uterine and perinatal transmission studies are also being carried out.

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RELATIONSHIP BETWEEN ACUTE PHASE PROTEIN LEVELS AND LESION SCORES IN PMWS CLINICALLY AFFECTED PIGS

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Postweaning multisystemic wasting syndrome (PMWS) associated with porcine circovirus type 2 (PCV-2) has now become a global swine disease problem. The disease primarily affects pigs between the ages of 5-18 weeks, and is clinically characterized by progressive weight loss, tachypnea, dyspnea, anemia, diarrhea, and jaundice. Characteristic microscopic lesions are lymphoid depletion, granulomatous interstitial pneumonia, hepatitis and nephritis. Several studies have demonstrated enhanced disease and lesions with experimental co-infection of PCV-2 and other infectious agents, such as porcine reproductive and respiratory syndrome virus (PRRSV). Determination of serum acute phase proteins (APPs) has been demonstrated to be an useful tool for productions animals not only for identification of individual animals with disease but also as a means to categorize the level of any sub-clinical disease present.

The objective of this study was to compare the concentration levels of three APPs (C-reactive protein, serum amyloid A and haptoglobin) between PMWS clinically affected pigs and specific pathogens pigs (SPF) at the same age to establish a control concentration level in healthy pigs, and determine the relationship between severity of lesions and concentration levels of APPs in PMWS clinically affected pigs and the possible influence of the co-infection of PCV-2 and PRRSV.

Seven, 16 to 18-week-old conventional PMWS clinically affected pigs were selected for this study. Seven, 16 to 18-week-old SPF healthy pigs were bled to establish a control APPs concentration level in healthy pigs for each one of the APPs studied. Acute phase proteins were measured in serum of the pigs by commercial kits previously validated for porcine specimens at our laboratory. PCV-2 and PRRSV infections were determined by PCR in different tissues. The concentration levels of APPs, the score of interstitial pneumonia and granulomatous inflammation in lymphoid tissues, and the presence or absence of bacterial pneumonia, hepatitis, nephritis and meningo-encephalitis were compared between PMWS clinically affected pigs to establish possible statistic differences. There were significant differences in the concentration levels of all APPs studied between SPF and clinically affected animals. A trend toward statistical

significance ($p=0.072$) was found between the lung lesions (interstitial pneumonia and bacterial pneumonia) and the concentration level of serum amyloid A protein.

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE HUMORAL IMMUNE RESPONSE ON PARAFFIN-EMBEDDED TISSUES OF PIGS INOCULATED WITH THE CLASSICAL SWINE FEVER VIRUS

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The aim of this study was to characterize the humoral immune response during Classical Swine Fever. 32 Large White x Landrace pigs of 4 months old were inoculated intramuscularly with 10^5 $TCID_{50}$ of the virulent isolate "Alfort 187" and slaughtered in groups of 4 animals from 2 to 15 post-inoculation days (pid). Four animals were used as uninfected controls. Samples of spleen and thymus were fixed in 10% buffered formalin and Bouin's solution, and were routinely processed. For the immunohistochemical study, avidin-biotin peroxidase complex (ABC) method was used to immunolabel activated B-cells (λ-chains, Ig-M and Ig-G). Positive cells were counted and tested for significance ($P \leq 0,05$) by Student's *t*-test.

Despite the lymphoid depletion observed in B and T areas of spleen and thymus respectively, B-lymphocytes and plasma cells λ-chains (+) showed a significant increase in spleen from the initial stages of the disease, peaking at 14 pid, whereas a slight increase of λ-chains (+) cells was observed in thymus from 4 to 9 pid. A significant increase of Ig-M (+) cells was observed in thymus and spleen, peaking at 7 and 11 pid respectively, while the presence of Ig-G (+) cells was observed occasionally from 11 pid onwards.

The evolution of λ-chains (+) cells throughout the disease was associated, spatially and temporally, with the presence of antigen positive cells. So, the evolution of monocytes-macrophages population and the cytokines levels released by them, would make clear the interaction of these cell populations with B-lymphocytes and could be involved in the proliferation and differentiation of B-cells to plasma cells. The presence of immunoglobulins released by plasma cells would be related with the establishment of a typical humoral immune response, appearing an increase of Ig-M (+) cells in early stages of the disease and the presence of a small number of Ig-G (+) cells in late stages, according with the γ-globulins serum levels of our study.

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PATHOLOGICAL CHARACTERIZATION OF THE NERVOUS FORM OF OVINE MAEDI-VISNA IN NATURAL CASES

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The nervous form –or Visna- of Maedi-Visna disease was described for the first time in Iceland in the fifties and the pathological features of the disease were characterized in sheep naturally or experimentally infected. Since then, this form has been reported only sporadically in several countries but no detailed pathological studies have been carried out. Taking into account the high number of sheep diagnosed as having Visna in our laboratory and that not all of them fit with the pattern described in the Icelandic cases, in this work a pathological study was carried out, focused on the distribution and type of lesions and presence of viral antigen in the tissues assessed by immunohistochemistry.

A total of 62 sheep showing neurologic signs and a non-suppurative meningoencephalitis, in which Maedi-Visna virus was demonstrated either by immunohistochemistry or PCR, were examined. Histopathological evaluation was made at different leves of the Central Nervous System.

In 56 of the cases, lesions were found in more than one of the examined locations whereas in the remaining six lesions was solitary (in three of them located exclusively in the spinal cord). The site in which pathological changes appeared more frequently (82%) were in the pons and in cerebellar peduncles. Lesions were severe and bilateral but they did not show a periventricular distribution, considered as characteristic of Visna in the Icelandic studies. In this case, inflammatory changes appeared in the cerebellar peduncles and extended to the vestibular nuclei region and cerebellar white matter. The corpus callosum level was also a frequent location, showing bilateral and moderate to severe lesions. Most of the cases of choroiditis were found in this site. Other areas as mesencephalon or medulla oblongata were more rarely affected and changes were mainly mild. It is remarkable the number of sheep having lesions in the spinal cord, in some cases as unique changes. Lesions varied from a severe mononuclear cell infiltrate, with a perivascular pattern, to cases where, besides this, malacia and demyelination werethe most outstanding features. These results would suggest a vascular progression of the lesion instead of a ventricular dissemination. By immunohistochemistry, positive cells were only found in association with lesions. These cells had different morphology and their number varied from scarce to abundant in the tissues, depending on the cases, but without any relationship to the intensity of the lesions.

PATHOLOGICAL CHARACTERIZATION OF A PORCINE-ADAPTED BSE PRION

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Prion diseases are neurodegenerative disorders that affect humans and animals, the most common lesions of which occur in the Central Nervous System (CNS). After the extensive outbreak of BSE in cattle occurring in the UK in the late 1980s, a potential spread of BSE to other domestic animals, such as pigs, was pointed out. Although transmission of prions between certain pairs of species is governed by a strong barrier to infection (Pattison and Jones, 1968; Prusiner, 1990; Kocisko et al., 1995), the possibility of BSE prion transmission to porcine has been fully addressed. The bovine/porcine species barrier to BSE infection was explored by generating transgenic mouse lines expressing the porcine prion protein gene (poTg). In this work, we describe the histopathology (neuronal loss, spongiosis and astrogliosis), the presence of PrP deposits (amyloid plaques and deposits of different sizes) and glial reaction in transgenic mice expressing po-PrP gene inoculated with BSE inoculum (first passage) and porcine-adapted BSE inoculum (poTgBSE) (second passage). Histochemical and immunohistochemical techniques were performed to study lesions, PrP^{res} (mAbs 6H4 & 2A11) and GFAP; and tomato lectin-histochemistry to study microglial cells. In agreement with the biochemical data, histopathological and immunohistological studies showed a low percentage of mice that were successfully infected in the first passage studies. This mice showed granular neuronal cytoplasmic and perineuronal PrP^{res} labelling and punctate neuropil PrP^{res} labelling appeared as the most common histological pattern, occasionally as plaque-like deposits. Severe vacuolation was always accompanied by astrocytic gliosis and microgliosis. Astrocyte prolongations enveloped the vacuoles and plaque deposits. Although second passage animals presented similar histopathological pattern and IHC findings, it was possible to detect the presence of the infectious agent in 100% of the mice and it could be detected much earlier than the first passage.

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POSTERS

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INHIBICIÓN TEMPORAL DE APOPTOSIS NEURONAL EN EL GANGLIO TRIGÉMINO DE CERDOS INFECTADOS CON EL VIRUS DE LA ENFERMEDAD DE AUJESZKY

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Los virus con un ciclo de replicación complejo, como los herpesvirus, necesitan impedir la muerte de las células infectadas para producir una abundante progenie vírica. Esta habilidad se ha demostrado para el virus de la enfermedad de Aujeszky (VEA), capaz de bloquear la muerte por apoptosis de las neuronas infectadas del ganglio trigémino (GT). Puesto que las neuronas que sostienen la replicación del VEA están rodeadas por numerosas células inmunitarias, cuya función es eliminar a la célula infectada mediante inducción de apoptosis, un aspecto interesante sería comprobar si el bloqueo vírico sobre el proceso apoptótico es definitivo o temporal. Para investigar esta cuestión utilizamos GT de cerdos infectados intranasalmente con el VEA y sacrificados a las 24, 48 y 72 horas postinoculación. La infección del GT se evaluó con la detección de antígenos y ADN vírico mediante inmunohistoquímica e hibridación in situ, mientras que la presencia de células apoptóticas fue detectada mediante la técnica TUNEL, inmunohistoquímica frente a caspasa 3 activa y microscopía electrónica de transmisión. Los resultados obtenidos en este trabajo sugieren que el bloqueo vírico de la muerte neuronal es temporal y no definitivo, puesto que en los últimos estadios de la infección se produce activación de la cascada apoptótica en neuronas infectadas por el VEA y se identifican neuronas con características morfológicas de apoptosis en los focos de neuronofagia del GT.

EVALUATION OF MAEDI-VISNA DIAGNOSTIC METHODS IN SHEEP SHOWING THE NERVOUS FORM OF THE DISEASE.

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Maedi-Visna is a chronic infectious disease of sheep caused by a lentivirus, that is widespread in Spain. In the last years, we have recorded a high number of cases showing the nervous –Visna- form. This presentation was studied in detail in Iceland but, in the rest of the countries, it has been only sporadically reported. In this study, the main clinical and pathological features of Maedi-Visna nervous form are presented and several diagnostic methods of the disease are assessed. A total of 79 sheep showing nervous lesions consistent with Visna were examined. An immunohistochemical technique using monoclonal antibodies against the virus was employed for its demonstration on tissue sections. In 36 of the sheep, serological studies were performed by Agar gel immunodiffusion test (AGID) and ELISA. A LTR-PCR technique was used on paraffin embedded tissues of 16 animals.

The most common, but not exclusive, clinical sign was ataxia and paresis of the hindlimbs. Lesions, characterized by a non-suppurative meningoencephalitis and choroiditis, were classified according to their intensity in mild, moderate or severe. In a 73% of cases showing moderate or severe lesions, positive cells by immunohistochemistry were identified. However, they only appeared in one case with mild lesion. All the sheep but three having mild forms, were positive to ELISA. This technique showed a higher sensitivity than AGID being five animals with severe lesions negative to the latter. Only 5 cases –all having severe lesions- in which PCR was performed were positive, but in 10 cases it was not possible to extract DNA from the tissue. This was probably due to the long duration of formalin fixation in those samples. The absence of positivity in most cases, would suggest that mild lesions could be either non-specific or associated with initial phases of Maedi-Visna infection, showing a low viral loading, undetectable by the techniques employed in this work. It is remarkable the good agreement among immunohistochemistry, serological methods –especially ELISA- and moderate or severe lesional forms.

CLINICAL SIGNS AND PATHOLOGY OF FOOT AND MOUTH DISEASE INFECTION IN SWINE: A PRELIMINARY STUDY

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Foot and mouth disease (FMD) is a highly contagious picornavirus infection of all cloven-hoofed mammals that often causes rapidly spreading epizootics, especially among cattle, pigs and sheep. The disease is characterised by the formation of vesicles in the mouth and on the feet, causing inapetence, lameness and a drastic drop in production. Other vesicular diseases are described in swine such as swine vesicular disease, vesicular exanthema of swine and vesicular stomatitis and these diseases are identical clinically. In this work we study the clinical signs and pathology of pigs inoculated intradermally (coronary band) with a high dose of FMDV serotype C. Animals developed pyrexia from 2 dpi coinciding with viremia and showed clinical signs such as inapetence and lameness. Vesicular lesions appeared from 2-3 dpi firstly in the inoculation site and lately in the rest of the limbs, tongue, and snout. Animals were slaughtered at 9-10 dpi and only skin lesions were found at necropsy. Histopathological findings consisted mainly in skin vesicles, mainly in the sratum spinosum, where the vesicles begin to be formed. High rates of death keratinocytes were found and acantholysis and intercellular oedema were frequently observed. The stratum basale remained intact in most of the cases. Moreover, other organs were studied by histopathology and interestingly, we found mononuclear infiltrates in the liver and kidney and lymphoid depletion in lymph nodes, Peyer´s patches and spleen, where reactive macrophage sheets were also found. The lungs showed severe hyperemia and alveolar edema. Immunohistochemical detection of FMDV was standardised in paraffin embedded tissue sections by using monoclonal antibody SD6. FMDV was present mainly in the skin and lymphoid tissues. The pathology of FMD has been widely studied in cattle but not in swine. A new experiment with tissue samples from animals slaughtered at different dpi will help us to improve the knowledge of the pathogenesis and the immune response of this important disease
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PCV1 AND PCV2 IN A CASE OF PORCINE DERMATITIS AND NEPHROPATHY SYNDROME FROM A PRRS-FREE FARM

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Porcine Dermatitis and Nephropathy Syndrome (PDNS) was firstly described in the U.K. in 1993. The pathogenesis of the disease is unknown but a type III hypersensitivity reaction is supposed to be the base of the pathological findings. The disease has been associated to PCV-2 infection and some authors have pointed that the association with PRRSV could also be related to the syndrome. In this work we describe a case of PDNS in a pig with skin lesions (haemorrhagic cutaneous infarcts) in a farm free of PRRS. This pig was painlessly killed and at necropsy, we observed kidney petechiae and swollen and purple-coloured superficial inguinal lymph nodes. Histopathological findings consisted mainly on necrotising vasculitis, fibrinous glomerulonephritis, and the presence of giant multinucleated cells and intracytoplasmic inclusion bodies in the lymph nodes and Peyer's patches. Fraser-Lendrum technique was performed to study fibrin deposition and we found positivity in the kidney and the skin. Immunohistochemical detection of PCV-2 was also performed and we found positive cells only in the lymphoid tissues. Different uniplex and multiplex PCR were performed on samples from the pig to detect nucleic acid of African swine fever virus, Classical Swine Fever virus, PRRS virus and Porcine circovirus type 1 and type 2. Samples from different tissues showed positive results to PCV1 and PCV2 and negative for PRRSV, ASFV and CSFV. Other 3 pigs from the same batch without signs of disease were killed and no lesions were found. Samples from healthy pigs showed PCR positive results to PCV2 but not to the rest of virus tested. Due to the results obtained, we think that PDNS must not be related to the presence of PRRSV infection. PCV1 has been described to be non-pathogenic virus and maybe the presence of PCV1 in the pig with PDNS is only a sporadic finding. The study of the new cases of PDNS appearing in this farm and other ones with similar characteristics could help to improve the knowledge of this syndrome, especially related to the co-infection of PCV1 and PCV2 as a possible aetiology.

CYTOKINE PROFILE IN SERA FROM PIGS INOCULATED WITH AFRICAN SWINE FEVER VIRUS (ISOLATE E-70)

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African swine fever is a viral disease that affects members of the family Suidae. The disease is characterised by lymphopaenia and haemorrhages. The disease is acute with highly virulent isolates and the infected animals die at 5-10 dpi. The role of different cytokines in the pathogenesis of this disease is not very clear, but the one for proinflammatory monokines has been reported by our group and related with the apoptosis of lymphocytes and the haemorrhages. In this work, we inoculated 4 pigs with the highly virulent isolate E-70 to study the serum profile of cytokines during the disease. Blood samples were taken daily and the level of IL-1β, IL-2, IL-4, IL-8, IL-10, TNF-α and IFN-γ was measured using ELISA commercial kits. Pigs developed the typical signs of the disease and die or were slaughtered at 6-7 dpi. At necropsy, we observed the typical lesions of acute ASF such as haemorrhagic splenomegaly, haemorrhagic lymphadenitis, mainly in gastrohepatic and renal lymph nodes, petechiae in the kidney and septal oedema in the lungs. Samples for histopathology and immunohistochemical detection of ASF viral protein 73 were fixed in 3.5% formalin and embedded in paraffin wax. Viremia was evident from 1-2 dpi specific antibodies were undetectable during the experiment. TNF-α, IL-β and IFN-γ increased in sera from the first dpi, while IL-2, IL-4, IL-8 and IL-10 increased from the 3-5 dpi. These changes suggest the TNF-α and IL-1β might be related to the lymphocyte apoptosis and haemorrhages. The increase of IL-2 might be enhanced by the macrophage secretion of IL-1 and could promote the proliferation of T lymphocytes and lythic activity of NK. IL-4 and IL-10 are mainly produced by CD4+ Th2 lymphocytes. The increase of their expression might be related to B and T cell differentiation and the inhibition of the hyperproduction of proinflammatory cytokines. The expression of chemokines such as IL-8 could induce neutrophil attraction, phenomena observed in different tissues such as lungs in acute ASF.

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PRRS VIRUS INFECTION INDUCE APOPTOSIS IN LYMPHOID ORGANS

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In this work we study the apoptosis in different lymphoid tissues and organs during PRRS virus infection with a South American isolate and the implications in the pathogenesis of this disease. Four groups of three 3-week-old piglets were used in this experiment. One group was used as control and slaughtered at the beginning of the experiment, the other three groups being inoculated with a strain of PRRS isolated in Chile and killed at 7, 14 and 21 post inoculation days. Samples from tonsils, medial retropharyngeal and mediastinal lymph node from all animals were fixed in 10% formalin being routinely processed for light microscopy, immunohistochemistry and TUNEL studies. The immunohistochemical study was performed employing the ABC method with the monoclonal antibody SDOW-17 against PRRS virus. The tonsils of the infected animals showed a progressive lymphoid depletion during the disease, affecting mainly the interstitium and lymphoid follicles, but also the perifollicular areas, although with less intensity. Lymphoid depletion was also observed in medial retropharyngeal and mediastinal lymph nodes, being more prominent in the cortical zone. TUNEL technique revealed an increase in the number of apoptotic cells, most of them lymphocytes, in the three organs studied. These apoptosis phenomena coincided with the apparition of lymphoid depletion, and were located in a higher degree in the interstitium and follicles of the tonsils and in the follicular and interfollicular structures of the lymph nodes. These results are related to those found in other organs where apoptosis phenomena are also observed during PRRS infection.

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RHODOCOCCUS EQUI INFECTION: COMPARASION BETWEEN INMUNOHISTOCHEMISTRY AND ELISA TEST DIAGNOSES

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Rhodococosis is a disease that affects mainly the respiratory system of young foals, under 6 months of age. Rhodococcus equi is an important patogen that causes suppurative broncopneumonia, enteritis, lymphadenitis and abdominal abscesses. The most important reservoir for this patogen is the horse bowel, being faecal-contaminated soil the principle source of infection. Inhaled particles of virulent strains of R. equi are the main pathway of infection.This disease is usually diagnosed by macroscopic, microscopic and immuno histochemical examination of samples taken from the necropsies of affected foals.

This study proposes a diagnostic method for live animals, performing an enzyme-linked immunosorbent assay (ELISA) with serum samples of suspicious animals and its comparation with the results obtained with immunohistochemical techniques.

Blood samples from 45 foals were submitted to the Pathology Service of the Veterinary Faculty of Madrid. 32 animals were suspicious of suffering rhodococcosis and 13 samples came from healthy animals that were used as negative controls. Serological studies by ELISA were conducted in the Department of Animal Health of our Faculty using the antigen-ATCC6939 generously provided by profesor Shinji Takai. This technique was performed with two different antibodies, anti-horse IgG and anti-horse IgM.

Immunohistochemistry technique was performed in 9 tissue samples also submitted to the Pathology Service, using streptavidin-biotin-complex peroxidase method after a high temperature antigen unmasking protocol.

Anti-horse IgG was positive in 4 of the 45 serum samples with Optical Density (OD) values ranging from 0.26 to 1.12. 3 samples were considered doubtful because the OD values were ranged from 0.11 to 0.26. The remaining 38 sera were negative. The mean value of anti- R. Equi IgG antibody in foals with suspected infection (0,18) was significantly higher ($p<0.05$) than that of normal horses (0,05) and afected horses with treatment (0,10) .

Anti-horse IgM was no positive in any of the 45 serum samples. The mean values of anti-R. Equi IgM antibody in foals with suspected infection, treated foals under treatment and normal horses were almost the same.

The mean values of the titles observed of anti-R. equi IgG antibody in foals with clinical suspected infection, without and under treatment were in both cases significantly higher than on negative horses. On the other hand, there were no significant differences between normal foals and foals with clinical suspected infection with and without treatment in the mean values of anti-R.equi IgM antibody.
The study also confirms that the ELISA test is a rapid, reliable tool for detecting R. equi infection in foals. The relation between the results obtained in immunohistochemistry in comparation with ELISA test is under evaluation.

PORCINE POSTWEANING MULTISYSTEMIC WASTING SYNDROME IN PIGS: DETECTION OF PORCINE CIRCOVIRUS 2 INFECTION BY IMMUNOHISTOCHEMISTRY AND POLYMERASE CHAIN REACTION-ELISA

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Porcine postweaning multisystemic wasting syndrome is a disease associated with presence of circovirus type-2 (PCV-2). These porcine viruses belong to Circoviridae family. The aim of this work was to test two different diagnostic methods, immunohistochemistry in paraffin sections and PCR-ELISA, for detection of porcine circovirus type-2 in natural infected animals. Sensibility of both methods was also estimated.

Eleven animals with clinical signs suggestive of PMWS were euthanatized and standard necropsy was performed. Samples of tonsils, lungs and superficial inguinal lymph nodes were taken for histopathological (fixed in 10% buffered formalin and paraffin-wax embedded) and molecular biology (stored at -80ºC) analysis. For histopathological study, 36A9 monoclonal antibody (Ingenasa, S.A., culture supernatant, 1:200, ABC method) was tested in the 32 selected samples. For PCR-ELISA, an easy-read kit (Ingenasa, S.A.) was used. These results showed that porcine circovirus antigen was detected by IHQ in a total of 15 from 32 (47%) samples tested. A DNA fragment of circovirus was successfully amplified from 17 of the 29 (58%) samples tested by PCR-ELISA. Finally, both methods were compared to evaluate statistical correspondence.

MYCOTIC DERMATITIS CAUSED BY *MUCOR RAMOSISSIMUS* IN CANARIES (*Serinus canarius*)

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Mycotic dermatitis in birds is relatively rare. The etiologic agents most frequently isolated are pathogenic dermatophytes belonging to the genera *Trichophyton* and *Microsporum*, but *Aspergillus* spp, *Candida* spp, *Mucor* spp, *Rhizopus* spp among others have also been isolated from the skin and the feather follicles of clinically affected birds. Approximately 15 % of the animals of a group of 80 canaries presented loss of feathers in the legs extending to dorsum, neck and head and hyperkeratosis in the feet. Skin scrapings were negative for parasites. After some days of illness and due to the poor condition of the animals, three of them were sacrificed. Fresh samples of affected skin were taken and submitted for microbiological cultures. Samples from different organs of the animals were fixed in 10% buffered formalin and sent to the Department of Comparative Pathology. Tissues were embedded in paraffin wax, sectioned at 4 μm and stained with haematoxylin and eosin (HE), periodic acid-Schiff (PAS) and Grocott's methenamine silver stains (GMS).

The histopathology study revealed an ulcerative dermatitis with a slight to moderate interstitial infiltration of heterophils in the underlying dermis. Numerous rounded to oval fungal spores on the corneal stratum of the epidermis and within feather follicles were observed. In the mycology study, *Mucor ramosissimus* was isolated and identified from the skin samples. Bacterial cultures were negatives.

Mucor ramosissimus is considered a fungal opportunist and only a few cases of human cutaneous zygomycosis caused by this agent have been reported. As far as the authors know, this is the first report of dermatitis caused by *Mucor ramosissimus* in canaries.

IMPLICATION OF PULMONARY INTRAVASCULAR MACROPHAGES IN THE PATOGHENESIS OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

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One of the most controversial questions in the pathogenesis of Porcine Reproductive and Respiratory Syndrome (PRRS) is the role of pulmonary macrophages in the origin of the pulmonary lesions observed in the disease. In order to clarify this matter, four groups of three 3-week-old piglets were employed. One group was used as control and slaughtered at the beginning of the experiment, the other three groups being inoculated with a strain of PRRS isolated in Chile and killed at 7, 14 and 21 post inoculation days. Lung samples from all the animals were fixed 10% formalin and in glutaraldehyde, being routinely processed for light microscopy, immunohistochemistry and ultrastructural studies. The immunohistochemical study was performed employing the ABC method with the monoclonal antibody against PRRS SDOW-17.

The inoculated animals showed a progressive thickening of the alveolar septa throughout the infection, histopathological lesion characteristic of the disease, and that was associated to an increase in the number of pulmonary intravascular macrophages and alveolar macrophages from the initial dates of infection. This increase was related to the frequent observation of mitotic figures of these cells and with the apparition in the lung of numerous cells of smaller size and fewer organelles in their cytoplasm, cells considered immature macrophages. The increase in the number of these cells was related to a decrease in the number of cells infected by the virus, fact that could be correlated to the natural resistance of monocytes and immature macrophages to PRRS virus.

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USE OF AN HISTOPATHOLOGICAL METHOD IN THE ASSESSMENT OF THE EFFICACY OF VACCINATION AGAINST BOVINE PARATUBERCULOSIS IN A FIELD STUDY

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Paratuberculosis is a chronic disease of ruminants caused by *Mycobacterium avium* subsp *paratuberculosis* (Map) that causes severe economical losses due to a decrease in productions and death of animals. Although this is a controversial topic, previous studies have pointed out that vaccination in cattle is an efficient method for controlling this mycobacterioses by reducing the number of clinical cases of the disease and the faecal shedding of Map. In this study, the efficacy of vaccination with a killed vaccine in cattle has been assessed through the histopathological evaluation of paratuberculosis-associated lesions in culled animals. A previously proposed pathological model, made in natural cases of bovine paratuberculosis, that relates the lesions with the several phases of Map infection has been used.

The study has been carried out in a herd of 350 adult Frisian cattle, showing clinical cases of paratuberculosis. A 70% of the animals was vaccinated with a killed vaccine and the remaining 30% was kept as unvaccinated control. Along a period of 18 months, all the culled cattle, with or without clinical signs of paratuberculosis, were recorded and in 65 of them, samples from the intestine and related lymph nodes were taken for histopathological examination. Lesions were classifed as diffuse, associated with severe clinical forms of the disease, and focal or multifocal, related to latent of initial forms of Map infection.

The percentage of culled animals in any of the three periods in which the study was divided (0-6, 6-12 y 12-18 months post-vaccination), was higher in the control than in the vaccinated group. The difference was increasing along the study (11.8% at the beginning and 19.6% in the latest period considered). The histopathological study showed a higher proportion of severe and diffuse forms, associated with large amounts of mycobacteria, in control culled cattle than in the vaccinated group. In the latter, a decrease was observed in the number of cattle with lesions, with a majority of focal or latent forms.

ANALYSIS OF THE POLYARTHRITIS ASSOCIATED TO SUSCEPTIBLE FORMS OF THE CANINE LEISHMANIOSIS FOR LEISHMANIA INFANTUM

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Active forms of canine leishmaniasis (LC) include a pathological complex process present in latent and patent status of the disease. The diagnosis based on the parasite identification and the antibody response in sera, must be carried out together with a clinical study that allows a prognostic evaluation and the determination of possible concomitant pathologies.
The case corresponds to a seven year old- Dutch hound dog with acute signs of prostration, anorexia, dehydration, yellowish mucosa and locomotion problems with joint cracklings, inflammation and acute pain at abdominal level in the hepatic projection area. The analytic results showed a normal hemograma and biochemical serum changes: BUN (48,8 mg/dl), AST (88 UI/L), Alkaline Phosphatase (1125 U/L) and proteinemia (9 g/dl) among others. The diagnosis of the parasite disease was performed by lymph node biopsy, and also by sera analyses using IFAT and ELISA techniques. Once leishmaniasis was confirmed, the animal was euthanasied according to the owner's decision.
The post-mortem study included macroscopic and histopathological findings, mainly in the joints, which were reddish and inflamed. Histopathological study showed an abundant cellular infiltrate basically constituted by plasmatic cells and infected macrophages with high number of amastigotes forms inside. In this infiltrate, infected fibroblasts, some polimorphonuclear cells and lymphocytes were also observed. In the joint fat tissue, an inflammatory infiltrate with plasmatic cells and macrophages with parasites were also present.
In the same way, other lesions such as lymphocytic encephalitis, glomerular sclerosis, interstitial nephritis, hepatitis and acute pancreatitis appeared in this active form of the canine leishmaniasis, and they were described and discussed.
The high canine incidence of this parasitic zoonoses and the complexity of the evolution and variability of the disease, allow to evidence not well-known lesional areas in different organs and tissues.

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BLUETONGUE, SEPTICAEMIC PASTEURELLOSIS AND PATHOGNOMONIC HAEMORRHAGES

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Bluetongue is an orbiviral disease of sheep that has reappeared in recent years in certain parts of Greece, France, Italy and the Balearic islands in Spain. Presumptive diagnosis can be done by clinical features and lesions, the appearance of haemorrhages at the base of the pulmonary artery being considered pathognomonic[1,2]. We have observed the appearance of such lesions in a flock of sheep that was affected by an unusually-aggressive form of septicaemic pasteurellosis. A group of 150 ewes kept indoors for lambing and lactation was affected. A total of 24 adult ewes were affected during a three-week period and 16 of them died. The animals showed fever, they were reluctant to move and finally became prostrate and died. The clinical course was short, not longer than 24-48 hours maximum. Other clinical signs compatible with bluetongue were not observed. Post mortem demonstrated the appearance of multiple haemorrhages in the subcutaneous tissue, lung, tracheal mucosa, heart, spleen, kidney, gallbladder and, remarkably, the digestive tract, which showed massive haemorrhages. Haemorrhages of the *vasa vasorum* of the pulmonary artery and aorta were seen in at least 5 cases. Histologically, no evidence of inflammatory reaction was seen in any affected organ, massive perivascular haemorrhages and bacterial emboli being the most important alterations. Bacteriology demonstrated the presence of *Mannheimia haemolytica*, *Pasteurella trehalosi* and *Pasteurella multocida*. A group of seven affected ewes were tested by ELISA for bluetongue virus antibodies, with negative results. It was concluded that this outbreak was caused by an unusual combination of Pasteurella and Mannheimia that possibly gained virulence due to bacterial synergistic action and/or weather conditions although other factors can not be ruled out. However, the appearance of haemorrhages in the base of the pulmonary artery, as well as in other main blood vessels must be underlined. If these lesions had been considered pathognomonic for bluetongue, the case could have been misdiagnosed at the post mortem level, even knowing that the only recent outbreak of bluetongue in Spain had been in the Balearic islands, which are more than 500 kilometers East from the place were this Pasteurella outbreak was detected. We therefore consider that the appearance of the haemorrhages at the base of the pulmonary artery can not be any longer considered pathognomonic for bluetongue because they can also appear in common bacterial diseases such as septicaemic pasteurellosis.

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CYTOKINE PROFILE IN SERA FROM PIGS INOCULATED WITH CLASSICAL SWINE FEVER VIRUS (ALFORT 187 STRAIN)

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Classical swine fever (CSF) is a viral haemorrhagic disease of swine caused by an enveloped RNA virus belonging to the genus Pestivirus (Flaviviridae), which typically produces haemorrhage, thrombocytopenia and lymphoid depletion. In this study, we inoculated 4 pigs with the virulent strain Alfort 187 of the CSF virus. Blood samples were taken daily from the jugular vein and the level of IL-1β, IL-2, IL-4, IL-8, IL-10, TNF-α and IFN-γ was measured using ELISA commercial kits. Animals developed clinical signs characteristics of acute CSF, showing persistent pyrexia (40.5-41.5ºC), varying degrees of anorexia and ocular discharges associated with conjunctivitis. From 6 dpi onwards animals developed yellowish diarrhoea and from 10 dpi nervous signs consisting mainly in ataxia of the forelimbs and tremors. Animals were painlessly slaughtered at 23 dpi or culled before because of ethical reasons. Gross lesions were typical of acute CSF: splenic infarcts, petechiae in the kidney and gall bladder, necrosis in the tonsils, and lymphadenitis. Histopathological study showed the typical lymphoid lesions as well as perivascular cuffing in the central nervous system, periportal infiltrates, bronchopneumonia, etc. Viral antigen was widely distributed using a monoclonal antibody against E2 glycoprotein by immunohistochemistry. Proinflammatory cytokines were highly expressed in sera of infected animals from the beginning of the experiment, decreasing in the case of IL-1β in latter stages of the disease. The concentration of IL-2 in sera displayed a significant increase at 2 dpi; thereafter it decreased up, showing no significant differences from the concentration before inoculation. IL-4 and IFN-γ peaked at 7 dpi and decreased up to the end of the experiment. These results suggest that proinflammatory cytokines secreted by macrophages play an important role in the typical lesions observed in acute CSF. The latter increases of IFN-γ and IL-4 suggest the role for T lymphocytes in the immune response against CSFV and might induce the decrease in the serum level of some proinflamatory cytokines from 6-8 dpi.

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INCIDENTAL INFECTION BY SARCOCYSTS IN A HORSE OF THE SOUTH OF SPAIN

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Sarcocysts in horses is a very common disease in EEUU and Central Europe, however, it is considered an infrequent disease in the South of Europe, a data that contrasts with the high prevalence of Sarcocysts infection in small and large ruminants in this geographical area.

Samples from striate muscle, including heart, skeletal musculature and tongue, from thirty horses, were fixed in formalin and routinarily processed for histopathological study from 2001 to 2004. By light microscope, only in a male horse of 7 years old, typical sarcocysts packed with bradyzoites were found. Sarcocyts were localized in the muscular fibres of the tongue, which additionally showed multiple erosions.The cysts were circular in section and were enclosed in the muscle. The cyst wall was thin and generally smooth though some small identations of the wall were visible. There was no host tissue reaction around the cysts. The number of intramuscular cysts observed varied from 1 to 6 cyst per field with the 10x lens.

Electron microscopy revealed the cysts were composed of tightly packed groups of bradyzoites and occasional metrocytes within a parasitophorous vacuole. The cyst wall was compound of villa formed by evaginations of the parasitophorous vacuole´s primary wall into the sarcoplasm of the host myofiber. For this reason the parasite was identified as *Sarcocysts fayeri,* that matchs the most common parasite describe in Central Europe.

The life cycle of this parasite requires a carnivore as definitive host, so the low incidence of *S. fayeri* observed in our study could be related to the lack of consumption of horse meat in our geographical area, so that the evolutive cycle of the parasite would be interrumpted.

This work has been supported by grants from PAI (AGR 137).

CHANGES OF UTERINE INTRAEPITHELIAL GRANULATED LYMPHOCYTES IN PREGNANT EWES EXPERIMENTALY INFECTED WITH *Chlamydophila abortus*.

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The ruminal uterine mucosae have a population of ntraepithelial granulated lymphocytes (IGLs) which increase in their number and activity from mild to late gestation, however their function is still unknow. Since IGLs have been reported to be originate in several species from natural killer cells, it has has been hyptotehized that they can acts as a first line of defense against foreing pahtogens. Nevertheless, recent studies have demonstrated that ovine trophoblastic cells are generally resistant to the lytic action by NK cells. The immunophenotype of IGLs is similar to the γ/δ T lymphocytes in sheep. The production of cyotkines such as IL-12, TNF-α and IFN-γ has been reported in bovine $WC1^+$ γ/δ T lymphocytes (Collins et al., 1996; Baldwin et al., 2000, Fikri et al., 2001; Pollock y Welsh, 2002). These cytokines could be important in the early defense against intracellular pathogens and lead to the development of Th1 response. *C. abortus* is an obligate intracellular bacterium, etiologic agent of ovine enzootic abortion, whose main target organ is the placenta. During the last third of gestatión the multiplication of *C. abortus* in the troplobast produce a suppurative placentitis and lead to abortion or stillbirth. To contribute to a better understanding of the role of the uterine IGLs, we have studied the changes of this subupopulation in a *C. abortus* induced abortion model based on the experimental infection of ewes at day 75 of gestation. At day 105 gestation IGLs are numerous in the uterine intercotyledonary areas. In the following days the number of IGLs declined, coinciding with the multiplication of *C. abortus* in the placenta. At day 130 some degenerative or apoptotic ILGs were observed, similar to those that predominate in the uterus after abortion o parturition when the number of IGL dramatically declines. The relation and hipotethical rol of the IGLs in the pathology of the placental infection in the enzootic abortion are discussed.

CUTANEOUS LEISHMANIOSIS IN A HORSE IN PORTUGAL

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The authors describe a case of cutaneous leishmaniosis in an eight year old Lusitano breed horse for the first time in Portugal.
The lesion was found in the abdominal region, near the groin, appearing as an ulcerated nodule.
Histologicaly they observed lesions of difuse/nodular dermatitis with lymphocytes, plasma cells and macrophages (some multinucleated) infiltration; numerous amastigot forms of *Leishmania* were found in the macrophage citoplasm.
The diagnosis was confirmed by imunocitochemistry

GRANULOMATOUS PNEUMONIA ASSOCIATED TO *SALMONELLA* TYPHIMURIUM INFECTION IN A COMMON PIGEON (*COLUMBIA LIVIA)*

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Salmonella infection has been described in pigeons, more often after routine faecal microbiological analysis. Although salmonella transmission from pigeons to human beings has been reported, recent studies suggest that most pigeon isolates are highly host adapted. Pathological descriptions are scarce, being arthritis, hepatitis and enteritis the most often reported lesions. This case describes a granulomatous pneumonia associated with *Salmonella* Thyphimurium infection in a common pigeon (*Columba livia*).

The pigeon proceeded from a massive capture of city pigeons for the study of several diseases. No symptoms were detected in the clinical analysis. At necropsy, multifocal irregular green nodules, about 0.5 to 3 mm, were observed in the lung. At section, the nodules were also seen in the pulmonary parenchyma. A moderate splenomegalia was also observed. Samples for histopathological and microbiological studies were taken.

Microscopically, a granulomatous pneumonia with necrotic foci surrounded by giant cells, macrophages, lymphocytes and mild fibrosis was observed. Gram negative bacterias were observed in the necrotic area. Neither fungus structures nor acid fast bacilli were observed with PAS and Ziehl-Neelsen stains, respectively. No lesions were detected in the remaining organs, except for a moderate lymphoid hiperplasia in the spleen. *Salmonella enterica* serotype Typhimurium was identified by standard methods. Cultures for fungi and mycobacteria were negative.

Respiratory disease in pigeons is a multifactorial problem with mycoplasmas, chlamydias and herpesvirus being the major infectious agents involved. Less often several bacterias and certain fungi have been reported to cause pneumonia. To the best of our knowledge this is the first pathological description of a granulomatous pneumonia associated to *Salmonella* Typhimurium.

CLASSICAL SWINE FEVER: VARIATIONS IN THE EXPRESSION OF PROINFLAMMATORY CYTOKINES AMONG DIFFERENT POPULATIONS OF FIXED MACROPHAGES

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Interaction of classical swine fever virus with macrophages induces the production of proinflammatory cytokines, Interleukin (IL)-1α, IL-6 and Tumor Necrosis Factor (TNF)-α, in the course of the disease. As a different pattern of production of chemical mediators in response to stimuli has been described in macrophages depending on their anatomical location, the aim of this study is to determine if there exists any difference in the percentage of expression of theses cytokines in the distinct populations of fixed macrophages of lung and liver, pulmonary intravascular macrophages (PIMs), alveolar macrophages (AMs), Kupffer cells and hepatic interstitial macrophages.

Fourteen Large White x Landrace pigs were inoculated intramuscularly with 10^5 $TCID_{50}$ of the "Alfort 187" strain of CSF virus. The animals were killed in pairs at 2, 4, 7, 9, 11, 14 and 17 post-inoculation days (pid). Two additional animals were employed as controls. Samples from liver and lung were fixed in formalin and Bouin's solution for immunohistochemical studies, performed with the ABC technique employing antibodies against viral antigen Gp55(E2), SWC3, IL-1α, IL-6 and TNF-α. The number of immunolabelled cells for each antibody and macrophage population was calculated by a cell count of 10 consecutive field of 1 mm^2 of lung and liver parenchyma per animal, being considered de number of immunostained cells against SWC3 the total number of macrophages in order to calculate the percentage of cytokine expressing cells.

Although the results of our study showed that there was an increase in the percentages of expression of IL-1α, IL-6 and TNF-α in all the populations in the infected animals, but AM, that diminished their percentage of IL-1α expressing cells, there were differences in the percentages of expression of each cytokine in the different populations of macrophages. PIMs showed the highest percentage of expression of IL-1α and TNF-α, while IL-6 expression occurred mainly in hepatic interstitial macrophages in the course of the disease. Additionally, the percentage of infected cells reached higher values in hepatic macrophages than in pulmonary, although infection in lung macrophages was more persistent throughout the disease.

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CLINICO-PATHOLOGICAL FINDINGS IN SHEEP GIARDIASIS NATURALLY INFECTED

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Two cases of sheep giardiasis diagnosed in the histopathology unit of Cardenal Herrera University are described. The affected farm is in Albacete and had 200 Manchega breed ewes in semintensive production. Close to 10% animals showed a malabsorption syndrome. A progressive weight loss and a poor hair coat were the most evidents signs. Five animals were studied, two of one year old, and the rest of 3-4 years old. A systematic necropsies were carried out in all animals and mainly focused to digestive system. Histopathological studies were realized in order to confirm a macroscopic thickened mucosa. At the same time that the necropsy are being done some mucosal smears were taken. After a Diff-Quick stain it was revealed many *Giardia lamblia* trophozoites -we have arrive to count aproximately between 20-30 protozoa per 40x microscopic field. A mild mononuclear infiltration was detected in the lamina propia with massive presence of a intraepithelial lymphocytes population. An hypertrophy of crypts an increased production of cells were another features in animals in younger animals-1 year old-. Applied by their concave surface to brush border of epithelial cells and between the villi are ussually evident as crescent shapes *Giardia* trophozoites. In adults ewes an atrophy of villi was observed, but no trophozoites were detected. After microscopic study no other pathological findings related to chronic digestive disease were observed. Finally, this comunication tries to attract attention about this flagelated parasite in sheep as agent to malabsorption syndrome, by the other hand very commun in the majority of wild and domestic animals and of course in humans, specially in childrens, as well.

GRANULOMATOUS HEPATITIS BY *Salmonella typhimurium IN A* SPUR-THIGHED TORTOISE (*Testudo graeca*)

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Reptiles are an important natural reservoir for *Salmonella* spp**.** Mostly they are latent carriers, although can show clinical signs. Commonly, the infection in reptiles is caused by pathogenic serotypes of sub-group I *(S. enterica)* and consists of acute enteritis, although may cause septicaemia with lesions in various internal organs, hypovolemic shock and death. Foci of necrosis in liver are the main lesion found in internal organs. Studies of prevalence of faecal excretions of *Salmonella* spp. in tortoises in captivity have shown rates of 80%, most of them *S. enterica* serotypes. In only few cases other different serotypes as *S. arizonae* species have been isolated. In *Salmonella* spp. infections described in tortoises, the lesions observed are limited to alterations in the intestinal tract, and no cases of hepatic lesions have yet been found.

A fifteen-year-old male spur-thighed tortoise (*Testudo graeca*) which shared its habitat with other tortoises and birds of prey in the "El Valle" Wild Fauna Recovery Centre of the Region of Murcia (Spain) presented severe clinical signs of infection and was necropsied. Numerous well-demarcated nodular areas that varying in size from 1 mm to 0.5 cm in diameter, yellowish in colour and soft to the cut were observed in liver. Liver samples were collected in 10 % neutral buffered formalin, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin-eosin, Gram and Giemsa techniques. A microbiological study was performed using one gram of affected hepatic tissue. The Salmonella isolated were tested against to the O (somatic) antigens and the H (flagella) antigens. Microscopically, numerous foci of necrosis were observed in the liver that varying in extension from few necrotic hepatocytes to large areas of necrosis where the structure was partially conserved, surrounded by mixed infiltrations of mononuclear cells. Microbiologically, the *Salmonella* isolated were *Salmonella* t*yphimurium*.

In the present case, the granulomatous hepatitis described is attributed to *S. typhimurium* and it may be supposed that the source of the infection was the contact of the tortoise with birds of prey.

PREVALENCE OF ASPERGILLUS FUMIGATUS IN FREE-RANGING BIRDS IN NORTHWESTERN SPAIN

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During 1999-2002 we studied necropsy results from carcasses of 288 birds which died in the Wildlife Animal Recovery Center of Cotorredondo (Pontevedra). We analyzed the prevalence of *Aspergillus sp* in free-ranging birds, in order to examine any differences that may exist in relation with the birds' habitat (aquatic, terrestrial, diurnal, nocturnal), age and sex.

Samples were fixed in 10% buffered formalin and processed by routine histological methods. Once histopathological lesions were studied, immunohistochemical techniques were performed to find Aspergillus antigens.

Fungi of this genus were identified in 34 of 288 birds, what makes this the most frequent natural disease in birds admitted in this Center. The species with biggest prevalence were: Goshawk (*Accipiter gentilis*, 27.27%), Northern Gannet (*Morus bassanus*, 22.22%), Razorbill (*Alca torda*, 18.18%), Grey heron (*Ardea cinerea*, 16.67%), and Tawny owl (*Strix aluco*, 16.67%).

The disease did not show notable differences regarding the sex of the animal; however, age and class seemed to alter the prevalence of aspergillosis. Younger birds seemed prone to suffer aspergillosis. Regarding their habitat (aquatic or terrestrial) there were differences on the prevalence of the disease. A 15.70% of aquatic birds were positive in comparison with an 8.98% of those that live in a terrestrial habitat.

21 birds diagnosed as positive to *Aspergillus sp* showed a concomitant problem which may be the actual cause of death, but in 13 birds there was no external reason for their admission to the Center.

We can conclude that aspergillosis is a major nosological entity that must be considered as an element contributing to or directly causing the death of 11.81% free- ranger birds admitted in the Wildlife Animal Recovery Center of Cotorredondo.

EXPERIMENTAL FASCIOLOSIS IN SHEEP: COMPARISON BETWEEN SECONDARILY SINGLE AND TRICKLE INFECTIONS

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The aim of this work was to compare the effects of small repetitive doses (trickle infection) and two large doses of *Fasciola hepatica* metacercariae (mc) in sheep. Group 1 (7 sheep) was orally infected with a dose of 200 mc at week 0 and reinfected with a dose of 200 mc at week 18. Group 2 (7 sheep) was infected with 7 daily doses of 25 mc each at week 0 (trickle infection) and reinfected with other 7 daily doses of 25 mc each at week 18. Both groups were killed at 30 weeks post-primoinfection. A control group (4 sheep) was used as uninfected. The number of hepatic flukes recovered was lower in group 1 (54±16) than in group 2 (88,7±27,8). Similarly, hepatic lesions such as portal fibrosis, chronic fibrous tracts, bile duct hyperplasia and granulomata to parasite eggs were mild to moderate in group 1 and severe to very severe in group 2. Cirrhosis was severe in group 2, whereas it was not observed in group 1. In the hepatic lesions the infiltrate of eosinophils, $CD3^+$ T lymphocytes, $B\text{-}B4^+$, $CD79\alpha^+$, B cells and IgG^+ plasma cells was very severe in group 2 and moderate in group 1. The ratio CD4/CD8 was 2.1 and 3.2 in groups 1 and 2, respectively. The hepatic lymph nodes (HLN) showed very severe (group 1) and moderate (group 2) hyperplasia of lymph node follicles and medullary cords. Hyperplasia of paracortical areas was moderate in both groups. The density (percentage) of immunolabelled cells marked with the different antibodies, particularly CD3 and CD8, decreased, mainly in group 2, with respect to the control group. By contrast, the density of $CD4^+$ T lymphocytes, $CD79\alpha^+$, $B\text{-}B4^+$ and IgG^+ cells was increased in comparison to the control group. This increase was more pronounced in group 2 than in group 1. These results strongly suggest that trickle infections induce more severe hepatic damage and a greater local cellular and humoral immune response in sheep. The increase of $CD4^+$ over $CD8^+$ T cells, as well as the increase of B cells and IgG^+ plasma cells in the HLN was proportional and more pronounced in group 2 than in group 1, supporting the hypothesis that trickle infections induce a more severe humoral response. However, this strong local immune response is unable to provide enough protection to prevent hepatic damage in subsequent infections, as revealed by the number of hepatic parasites and the hepatic lesions found in group 2.

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SITUACIÓN DE LA INFECCIÓN NATURAL POR *Mycobacterium avium* subsp. *paratuberculosis* EN LOS GAMOS (*Dama dama*) DE ASTURIAS

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La paratuberculosis es una enteritis crónica granulomatosa causada por una bacteria denominada *Mycobacterium avium* subsp. *paratuberculosis.* La enfermedad se ha detectado prácticamente en todas las especies domésticas de rumiantes, así como en una gran variedad de animales en cautividad y de vida salvaje del orden *Artiodactyla* (*cervidae*, *antilocapridae* y *bovidae*). La primera descripción en Asturias de la paratuberculosis en una especie no domestica fue realizada en un gamo en el año 1997 (S. Lavin, 1998). El gamo es una especie introducida en Asturias, que en la actualidad solamente se encuentra en la Sierra del Sueve, con una población que oscila en torno a los 1.000 individuos. Con el fin de valorar la importancia actual de la enfermedad en estos animales, hemos realizado un estudio serológico e histopatológico en un total de 192 gamos, a los que se recogieron muestras durante los años 1999, 2000, 2001, 2002 y 2003, siendo la distribución por años de 63, 43, 42, 33 y 11 animales respectivamente. En todos los casos los animales fueron abatidos en cacerías bajo el control de la Consejería de Medio Ambiente. Se realizaron estudios serologicos en la totalidad de los animales y análisis histopatológicos en los 11 recogidos en el año 2003.

La seroprevalencia más elevada fue la del año 1999 con un 23,8% de animales positivos, seguido de 2000 con el 16,27%, de 2002 con el 12,12% y de 2001 con el 7,14%. La media de las seroprevalencias fue del 15%. Los resultados de los análisis histopatológicos mostraron lesiones compatibles con paratuberculosis en cinco de los gamos estudiados, estas lesiones fueron del tipo focal, en el que aparecen pequeños granulomas formados por macrófagos, no observándose en ninguna de las muestras bacilos ácido alcohol resistentes. El ELISA fue positivo en dos de estos animales. En uno de los gamos, abatido por presentar un mal estado de carnes y con síntomas de diarrea, se observó una enteritis con presencia de células tumorales (adenocarcinoma), siendo en este caso el ELISA positivo.

En el presente trabajo se constata que la paratuberculosis tiene una amplia distribución entre los gamos de Asturias. Todos los animales positivos por técnicas histologicas mostraron lesiones focales, características de animales subclínicos sin síntomas aparentes. Algunos de los animales positivos por histología fueron negativos al ELISA.

ROLE OF MONOCYTE-MACROPHAGES AND DENDRITIC CELLS IN LYMPHOCYTE APOPTOSIS OF LYMPH NODES IN EXPERIMENTAL CLASSICAL SWINE FEVER

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In previous studies, we have reported a massive lymphoid depletion caused by lymphocyte apoptosis in the ileocecal lymph nodes of pigs infected with Classical Swine Fever (CSF) virus. The monocytes-macrophages (m-MØ) were identified as the main target cells of virus. Infection of dendritic cells was also reported, being these cells located in lymphoid follicles and interfollicular areas of lymph nodes, probably corresponding with follicular dendritic cells and interdigitating cells respectively. As the direct action of CSF virus on lymphocytes plays no more than a minor role in the massive lymphoid depletion caused by apoptosis, the purpose of this study was to determine the role of m-MØ and dendritic cells in lymphocyte apoptosis.

32 Large White x Landrace pigs were inoculated intramuscularly with 10^5 $TCID_{50}$ of the virulent CSF viral isolate "Alfort 187" and slaughtered from 2 to 15 days post-inoculation (dpi). 4 pigs were used as uninfected controls. Samples of ileocecal lymph nodes were fixed in 10% buffered formalin and Bouin's solution for structural and inmunohistochemical studies. Avidin-biotin-peroxidase method was used to immunolabel m-MØ and dendritic cells (MAC387 and HLA-DR) and cytokines (TNFα, IL1α, IL1β, IL6). Positive cells were counted and tested for significance (P≤0'05) by student's *t*-test.

A significant increase in the number of m-M∅ inmunolabelled with both markers (MAC387 and HLA-DR) was noted from 2 dpi. So, follicular dendritic cells and interdigitating cells marked against HLA-DR showed a significant increase from the initial stages of the disease. Dendritic cells have not been identified as the main target cells CSF virus. For this reason, the presence of viral antigen on dendritic cells could be related with their antigen-presenting cells function. Therefore, lymphocyte apoptosis may not be related with the changes suffered by dendritic cells during the differentiation mechanism of lymphocytes, but the numerical increase of m-MØ and the high tissue concentrations of TNFα, main chemical mediator released by these cells, could play an important role in the apoptosis of lymphocytes.

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SLAUGHTERHOUSE PREVALENCE OF URINARY BLADDER LESIONS IN GRAZING ADULT CATTLE; IMPLICATION OF BPV-1 and BPV-2

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The ubiquitous bracken fern (pteridium spp.) is the only higher plant known to cause cancer naturally in animals. Its consumption by cattle produces cancer in the gastrointestinal tract and in urinary bladder. The clinical syndrome is called bovine enzootic hematuria (BEH) due to the existence of urinary bladder carcinomas and mixed tumors. Brackern fern contains Ptaquiloside (PT), a potent carcinogenic principle. The mutagenicity, clastogenicity, teratogenicity and carcinogenicity properties of PT have been convincingly demonstrated. The involvement of bovine papillomaviruses 1 and 2 (BPV-1 and 2) and its synergism with bracken fern in the development of the tumors is also well known. Several publications aware about the risk of this bovine disease on human health. Epidemiological studies have shown a close association between bracken consumption and cancers of the upper alimentary tract in humans. The prevalence of BEH in Spain is unknown, although it is supposed to be low due to the production system and predominant dry climate. The aim of this research was to know the prevalence of urinary bladder lesions in adult grazing cattle exposed to bracken fern in the slaughterhouse, and to study the macroscopic and histological characteristics and their association with BPV-1 and 2. Urinary bladder opening and inspection (non usually required in the slaughterhouse) of grazing adult cattle from a region in Spain with possible ingestion of Bracken fern (Extremadura) sacrificed in a slaughterhouse (n=127) (Bilbao) has been carried out for one year (April 2002 to April 2003). Only samples with macroscopic alterations were submitted for histopathology (n=47). Urinary bladder samples from necropsies performed in a livestock with known Bovine Enzootic Hematuria (BEH) (Extremadura) were also included (n=6). Samples of 4 normal bovine urinary bladder were used as controls. Samples of 35 bladders (8 neoplasms and 27 inflammations) were conserved in RNA-Later. DNA was extracted from all samples using standard techniques. PCR amplification of papillomaviruses (BPV-1 and BPV-2) was carried out in all genomic DNA samples using different primers. Plasmids containing the entire BPV-1 or BPV-2 genomes isolated from skin bovine papillomas were used as positive controls. The prevalence of urinary bladder lesions in the slaughterhouse, after histological confirmation, was 37% (47/127): chronic cystitis

34,6% (44/127) and tumors 2,4% (3/127). All the animals had been exposed to bracken fern consumption. Genomic BPV DNA was detected in most of the samples. The prevalence of urinary bladder lesions (chronic cystitis and tumors) in the slaughterhouse was higher than expected according to the supposed low clinical prevalence of Bovine Enzootic Hematuria in Spain. Considering the possible risk for human health, the opening of the urinary bladder of adult grazing cattle in slaughterhouse inspections should be recommended. Our results indicate that bovine papillomaviruses play an important role in the development of our cases.

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NEUROPATHOLOGICAL FINDINGS OF BSE-INFECTED TRANSGENIC MICE WITH ONE EXTRA REPEAT OCTAPEPTIDE INSERT MUTATION

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Insert mutations within the repetitive octapeptide region of the prion protein gene (Prnp) are often associated with familial spongiform encephalopathies in humans. Transgenic mice expressing bovine PrP (bo7ORTg mice) bearing an additional octapeptide insertion to the wild type (seven octapeptide repeats instead of six) were generated and inoculated with BSE brain homogenates to check if the introduction of this mutation would render mice more sensitive to infection . In this mice, the course of infection progressed more rapidly in comparison with boTg mice expressing six-octapeptide protein (bo6ORTg mice). We show results of neuropathology, after performing histopathological and immunohistochemical (IHC) studies in paraffin embedded tissues along with WB analyses. In accordance with the lack of an interspecies barrier to BSE infection, we detected the typical CNS spongiform degeneration by histopathological analysis and the presence of the bovine prion PrP^{res} by immunohistochemistry. The histological pattern consisted mainly in vacuolization of the neuropil mostly observed in the brain stem, hippocampus and cerebellar white matter. In addition, several patterns indicating immunopositivity and correlating with Western blot patterns of PrP^{res} appeared. The most common were fine granular and punctuate neuropil labelling, and stellate labelling foci, which seemed to be associated with glial cells, but we also observed granular staining in the neuronal bodies and around neurones, and occasionally as a plaque-like deposits. However, this labelling pattern was identical for the bo6ORTg and bo7ORTg lines. On the other hand, bo7ORTg mice showed a higher sensitivity than bo6ORTg mice for detecting prion infection and, in the absence of clinical signs of disease, 7OR-PrP(res) could be detected as early as 120 days after inoculation by immunohistochemical and Western blot analyses.

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EXPRESSION AND SIGNIFICANCE OF p53, p21$^{Walf/Cip1}$ AND p16^{INK4A} PROTEINS IN GASTRIC TUMOR-LIKE LESIONS AND NEOPLASIAS IN THE DOG

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INTRODUCTION: Neoplasias of the alimentary system are the second most frequent tumor in small animals. The incidence of gastric tumors in dogs rates around 1% of all canine neoplasias. Today oncology research is focused on the detection of the different regulator proteins which play an important role in the cell cycle. Molecular studies have demonstrated a variable expression of supressor proteins which could help give a correct prognosis of the tumors. The genes encoding tumor supressor p21$^{Walf/Cip1}$, p16 and p53 proteins have been postulated to contribute to the pathogenesis of gastric neoplasias in humans and experimental animal models. At the moment, there are no previous studies in veterinary medicine which determine how these proteins may participate in the development and progression of the canine gastric proliferative lesions.

O**BJETIVE:**

1) To detect the presence of ki-67, p53, p21$^{Walf/Cip1}$ y p16^{INK4A} in canine gastric proliferative lesions (dysplasia, hyperplastic polyp, adenomas and carcinomas), establishing a different index for each histopathological type.
2) To compare the expression of the cell cycle regulator proteins and establish their possible prognostic value.

MATERIAL AND METHOD: A 9 years retrospective study revealed 28 proliferate epithelial lesions of canine stomach. We used the WHO histological classification of tumors of the alimentary system of domestic animals (2003) to classify these tumor-like lesions and neoplasias. Immunocytochemical detection was done by means of streptoavidin-biotin complex, using as primary antibodies against ki-67 (Master Diagnostica), p53 (Novocastra), p21$^{WAF1/Cip1}$ (DAKO) and p16^{INK4A} (Novocastra).

RESULTS AND DISCUSSION: Histopathological classification of the proliferative lesions resulted as: 4 dysplasias (3 moderate grade and 1 severe grade), 7 hyperplastic polyps, 7 adenomas (1 papillary type, 4 tubular type, 2 tubulopapillary), 10 carcinomas (5 tubular type and 5 signet-ring cell type). Immunohistological examination revealed immunoreaction against p53, p21$^{Walf/Cip1}$ and p16^{INK4A} antibodies in canine gastric pre- and neoplastic lesions. Nuclear staining brown-yellow as positive was observed for p53 and p21$^{Walf/Cip1}$, whereas p16^{INK4A} showed nuclear and cytoplasm immunostaining. According to Gerardts et al. (2000), Go et al. (2001) and Koening et al. (2002) we

established the criteria for the Ki-67, p53, $p21^{Walf/Cip1}$ and $p16^{INK4A}$ indexes. Ki-67 index was a useful tool to differentiate between hyperplastic polyp (ki-67<30%) and adenoma (ki-67>30%). One papillary adenoma with a focal moderate dysplasia revealed a high ki-67 index (85%). P53 protein expression was more significant and constant in gastric carcinoma (grade 2 and grade 3) in comparison with the absence in hyperplastic polyps and adenomas, and scarce presence in dysplasias (grade 1). The proliferative lesions displayed immunorreaction against $p21^{Walf/Cip1}$, however the percentage of positive cell was minor than 10%. Only in three dysplasias the p21 index was considered positive. In contrast with Go et al. (2001) we did not find correlation between p21 index and proliferating index. Expression of $p16^{INK4A}$ protein was detected in normal gastric mucosa, hyperplastic polyps, adenomas, displastic gastric mucosa and gastric carcinomas of dogs. We were able to appreciate a decrease of $p16^{INK4A}$ protein (p16 index < 50%) in gastric carcinomas. The lack of expression of $p16^{INK4A}$ is usually indicates poor prognosis since it is associated with lymphatic invasion and distal metastasis.

LIPID-RICH CARCINOMA IN A MALE DOG

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A 6- year-old Yorkshire Terrier male dog was presented at a private veterinary clinic bearing two non-ulcerated nodules in the left caudal thoracic and in the right cranial abdominal mammary glands. No other lesions or distant metastases were found in the clinical exam. The dog did not suffer any other disease and it was not under any treatment. After surgical excision, both masses fixed in 10% buffered formalin and submitted to the Veterinary Pathology Service (Veterinary School, Madrid). The two nodules were diagnosed as A) complex adenoma with ductal carcinoma *in situ* and B) lipid-rich carcinoma, following the criteria of human pathology (containing more than 75% of lipid secreting cells). Immunophenotyping and proliferation rate of the tumors were assessed by immunohistochemistry (cytokeratins, vimentin, actin, estrogen receptorα, progesterone receptor, androgen receptor and Ki-67). Tumor A did not differ histologically or immunohistochemically from the characteristics of the female counterpart. Tumor B was infiltrative, moderately cellular, arranged in nests, tubules and cystic structures which contained two types of neoplastic cells: elongated large undifferentiated basal cells and round to polygonal more differentiated large cells containing lipid droplets. Cytological features of malignancy were evident. Mitotic rate was low. No invasion of lymphatics was seen. Basal cells were cytokeratin+, vimentin+, actin+ and lipid rich-cells were cytokeratin+, vimentin+ and actin-. Ki-67 proliferation rate was moderate. The expression of steroid receptors was ER-, PR+ and AR+. Mammary tumors in male dog are rare and there are few reports published[1]. Lipid-rich carcinoma is a very rare type of mammary neoplasm in humans[2] and dogs[3,4]. Only one case of lipid-rich mammary carcinoma has been reported in humans, although the steroid receptors status was not evaluated[5]. To our knowledge this is the first report of lipid-rich mammary carcinoma in a male dog.

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CORRELATION AMONG E-CADHERIN EXPRESSION, TYPE AND HISTOLOGICAL GRADE OF CANINE MAMMARY TUMORS

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The recognition of an important role of cadherins in the determination of epithelial phenotype, in the cell's migration and differentiation and in the tumor dissemination has stimulated a huge interest in this family of adhesion's molecules. In the human species, E-cadherin is observed in epithelial cells of adults' mammary gland. Considering the similarities between mammary tumors of human and canine species, the present study correlated the E-cadherin expression on neoplasms (benign and malignant) of canine's mammary, employing the criterion of human diagnostic and classification. Materials and methods: 23 tumors have been studied. 6 of those benign (3 mixed tumor and 3 adenomas), 17 malignant (10 carcinoma with metaplasia and 7 ductal carcinoma) arranged histologically in grade I and grade II. To analyse the E-cadherin expression, immunohistochemical and the statistical analysis (Kruskal-Wallis text and Spearman) were done. Results and discussion: Comparing the groups, was found more expression of E-cadherin in the benign tumors than in ductal carcinoma ($p<0.001$), more in the carcinoma with metaplasia that in ductal carcinoma ($p<0,05$) and more in grade I tumors than in grade II tumors ($p=0,01$). Thus, the association between the lower expression of E-cadherin and more aggressive behavior was observed, because the ductal carcinoma, that have the worst prognostic, showed lower expression of E-cadherin, just as the tumors of grade II showed, as compared to the tumors of grade I.

SURVEY OF CANINE MAMMARY TUMORS IN THE VETERINARY HOSPITAL OF THE EV/UFMG

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A clinical-anatomopathological survey was conducted using 556 tumoral lesions of mammary gland obtained in dogs, diagnosed by the Veterinary Hospital of UFMG, between 11/97 and 02/04. The epidemiological characteristics and conditions of the animals had been reviewed, identifying the breed type and the age of the animals and evaluating the main macro- and microscopic characteristics of the pathological alterations, as well as the frequency of their occurrence. Studying the case history of the animals, it was possible to find the age and breed type of 309 and 350 females, respectively. The animals presented an average of 9 years of age. In the identification of breed, a predominance of animals without a definite breed was observed, (30%), followed by 21% of animals of the poodle breed. These results correlate with what is found in the literature. Regarding the location of the tumors, 330 animals were identified: 12.7% having tumors in the thorax, 26% in the abdominal glands and 24% in the inguinal mammary glands. Around 35.5% of these females displayed tumors with multi-centric characteristics, that is, with formations of tumors in more than one gland. Regarding the size of the tumors, 191 cases could be evaluated, of which, 38.74% were smaller than 3 cm, 50% of them diagnosed as malignant neoplasms. Tumors between 3 and 5 cm and larger than 5 cm represent 23.03% and 38.21% of the neoplasms, respectively. Among the tumors larger than 5 cm, 82.19% of these were malignant. These results demonstrate that there is a positive relation among tumor size and its potential to be malignant. A microscopic examination reveals that 6.47% of the diagnostics were benign, non-neoplasic alterations, with the majority being mixed tumors, representing 40.82% of the cases. The tumors with malignant characteristics make up 52.69% of the total neoplasms. Histological analysis of regional lymph nodes was possible only in 74 cases, among those characterized as malignant neoplasms. 28.4% of these showed metastasis. The results found in this study are similar to those described in the literature, confirming the importance of clinical characteristics and anatomopathological exams to understand the evolution of mammary gland tumors.

MIXED TUMORS AND METAPLASTIC CARCINOMAS OF THE MAMMARY GLANDS OF THE FEMALE DOG: COMPARATIVE ASPECTS WITH HUMAN SALIVARY GLAND TUMORS. A NEW MODEL FOR STUDY

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A strategy that is gaining ground in comparative pathology is the use of animal models for understanding the responsible factors for cancers in humans, so that these can be identified, controlled or eliminated. Canine mammary tumors are especially interesting because of their similarities with human breast cancer. Mixed tumors are uncommon lesions in the human breast, but they are the most frequent in the mammary glands of the female dog and in the human salivary glands. The pleomorphic adenomas (PA) and carcinomas ex-pleomorphic adenomas (Ca ex-PA) resemble mammary gland tumors of the female dog in several aspects: as in the mammary tumors of the female dog, these tumors are spontaneous and the malignance probability is inversely proportional to the size of the gland. Mixed tumors of the mammary glands of the female dog can suffer malignant transformation just as occurs in PA for Ca ex-PA. Our objective was to analyze mixed tumors and metaplastic carcinomas of the female dog and to compare them to PA and Ca ex-PA using histological and immunohistochemical methods. Immunohistochemistry was performed using streptavidin-biotin-peroxidase complex technique. The antibodies AE1-AE-3, vimentin and p63 were used in 10 samples of each tumor (mixed tumor, metaplastic carcinoma, PA and Ca ex-PA). In our results, the benign and malignant tumors of both species analyzed shared clinical characteristics, such as age of emergence and size of affected gland, as well as histopathological aspects. The antigenic phenotype for p63 showed significant differences between malignant tumors but, AE1/AE3, vimentin and p63 expression were similar among the benign tumors of the two species. Taken together, these data suggest that the mammary gland neoplasms of the female dog can be used as study model for the human salivary gland neoplasms.

IMMUNOHISTOCHEMICAL STAINING OF METALLOTHIONEIN IN CANINE MAMMARY TUMORS: BETTER SURVIVAL WITH HIGHER EXPRESSION

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Metallothioneins (MT) are small proteins able to strongly bind and therefore neutralize heavy-metals, free-radicals and other genotoxic compounds. Additionally, a close relationship has been shown between MT and zinc-finger motif-dependant transcription factors, with implications for cell proliferation and survival. Human malignant neoplasms (e. g. breast cancer) usually present worse prognoses with increased MT content. We therefore evaluated the immunohistochemical expression of MT for a group of fifteen canine mammary gland tumors. There was no relationship between MT immunostaining and histological type or malignancy grading of the lesions. Comparison of MT immunolabelling with the overall survival of the animals revealed that MT over-expression was related to better prognosis, a finding that contrasts with the human counterparts. We considered that differential expression of MT genes could be responsible for this variation, as observed for some human neoplasms of distinct embryonic origin, but further investigation is required to elucidate this topic.

STUDY OF THE INFLUENCE OF HYPERTHYROIDISM IN ASSOCIATION WITH CASTRATION IN THE DEVELOPMENT OF EHRLICH TUMORS

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In the present work the effect of hyperthyroidism was studied in the solid form of the Ehrlich tumor, inoculated in castrated adult female mice. Hyperthyroidism was induced by the consumption of thyroxin in the drinking water by the animal. In the experiment, 40 mice were divided into four groups: castrated hyperthyroid, intact hyperthyroid, castrated euthyroid and intact euthyroid. The mice had received a subcutaneous injection into the left footpad of 0.05ml of the cellular suspension, containing 2.5 x 10^6 neoplastic cells. The tumor growth curve was determined by measuring the inoculated footpad during 10 days after which autopsies were done on the animals. In accordance with the estimated consumption of thyroxin, castrated hyperthyroid and intact mice consumed on average 20.49µg and 13.96µg of thyroxin/mice/day respectively, with a significant difference between the groups. The morphology of the thyroid gland in mice treated with thyroxin confirmed the action of the drug in the induction of the hyperthyroidism. A difference was not observed between the body weight averages of the mice. From the beginning to the end of the experiment, a significant difference was observed in the food consumption when the hyperthyroid and euthyroid groups were compared. After tumor inoculation there was a decrease of this consumption in the euthyroid groups. The thyroid hyper function significantly increased the size of the solid tumor in the non-castrated animals. Less intense alterations were observed with the associated action of the hyperthyroidism and castration.

MALE MAMMARY NEOPLASM IN MANED WOLF (*Chrysocyon Brachiurus*): A CASE REPORT

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Neoplasm of male mammary gland is an uncommom phenomenon. A benign neoplasm - papilloma was diagnosed in a 9-years-old male maned wolf (*Chrysocyon brachiurus*) born at Fundação Zoobotânica, in Belo Horizonte, Minas Gerais. The tumor mass, approximately one cm diameter, detected in the left inguinal mammary gland was surgically excised. The histologic sections revealed an orderly proliferation of ductal epithelium on well-defined fibrovascular stalks. A myoepithelial layer, between a single epithelial layer and the fibrovascular stalk enabled the diagnosis of intraductal papilloma. Immunohistochemical staining was positive for p-63 and AE1-AE3 as expected and also for estrogen receptors. The maned wolf (*Chrysocyon brachiurus*) is a wild wolf that lives in the South American grasslands and scrub forests of Brazil, northern Argentina, Paraguay, eastern Bolivia and southeastern Peru. As it is a typical animal found in the grasslands of Minas Gerais, Brazil, this kind of wolf is also bred in captivity in our zoos.

MAMMARY NEOPLASMS IN THE BRAZILIAN JAGUAR (*Puma concolor*) AND PANTHER (*Panthera leo*)

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The mammary tumors of some domestic species, like dogs, represent the most frequent type of neoplasms in females. In other species these tumors are frequent only in felines. Information about these in the wild animals is hard to come by. This study describes two cases of mammary gland neoplasms diagnosed in a Brazilian jaguar (1) and in a panther (2) from the Zoological and Botanical Foundation of Belo Horizonte/MG (Brazil). **1)** During the annual physical exam of the adult jaguar, capt in captivity for more than twenty years, two tumoral masses were detected in the second right-side mammary gland and another one spreading from the third right-side gland in the direction of the third left-side one. In its case-history, the animal had undergone two estrogen hormonal implants . The decision was made to surgically remove the smallest tumoral mass. Macroscopic examination revealed the size of the tumor to be 7.5 x 4.5 x 3.0 cm, weighing 40.42 g. The histopathological exam enabled a diagnosis of papillary invasive carcinoma. Approximately one month after the surgery the animal was re-evaluated, showing a large growth of the tumoral mass, which had reached the both right and left-side inguinal glands and the second left-side gland. Euthanasia of the animal was administered, followed by autopsy and extraction of samples for histopathological examination. The microscopic exam determined the size of the tumor to be 22.0 x 24.0 x 5.0 cm, weighing 1.4 Kg, with several cystic areas revealed when opened. The microscopic exam confirmed the diagnosis of invasive papillary carcinoma with metatastasis in the retro-mammary lymph nodes and lung. **2)** In the annual physical exam of an adult female panther, a nodule in the left inguinal mammary gland was identified. The nodule was excised. Macroscopic exam determined the tumor to be 1.7 x 1.5 x 1.2 cm in size, weighing 1.73g. The microscopy showed growth of multiple ramified papillas, showing an axis of connective tissue covered with epithelial and myoepithelial cells, compatible with the diagnosis of papiloma.

GRANULATION TISSUE-TYPE HEMANGIOMA (PYOGENIC GRANULOMA) IN *Puma concolor*

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The so-called pyogenic granuloma is a polypoid form of capillary hemangioma on skin and mucosal surfaces. The inflammatory changes that often accompany these tumors may be so pronounced that the lesions bear a striking resemblance to granulation tissue. In a 9-years-old female puma (*Puma concolor*) from the Zoological and Botanical Foundation of Belo Horizonte/MG, the routine annual exam found an ulcerated exophytic tumoral lesion at the base of the tongue. This was surgically excised and the material sent for anatomopathological exam. The microscopy revealed the ulcerated surface of the lesion, covered with fibrinous exudate and consisting of fibroblasts and of dilated and congested new small blood vessels. Both acute and chronic inflammatory cells were scattered throughout the lesion. Some purulent exudate and occasional, isolated, foreign body – type giant cell, with strange forms and related to refractive brown material were encountered. The adjacent epithelium was acanthotic. The lamina propria was also shown to be edematous and with non specific inflammatory infiltrate. There were no signs of malignancy, or others specific findings. These results were compatible with granulation tissue type hemangioma (pyogenic granuloma).

P63, A NEW MARKER FOR STEM CELLS/MIOEPITHELIAL CELLS OF MIXED TUMORS AND CARCINOMAS IN MIXED TUMORS IN CANINE MAMMARY GLANDS

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Mixed tumors are the most frequent benign tumors in canine mammary gland and are characterized by epithelial and mesenchymal elements. Carcinomas in mixed tumors frequently originate from a malignant transformation of a benign tumor. Despite the existence of mioepithelial characteristics, there is no a consensus about the origin of these tumors yet. Recently, new markers have been developed to evaluate the presence of mioepithelial cells. The codified product of the gene p63 has been considered a valued immunohistochemistry marker of mioepithelial cells and stem cells, with excellent sensibility and high specificity, as compared with other markers like calponin and smooth myosin muscle high chain (SMM-HC). This study has the goal of evaluating the profile of immunohistochemistry expression of p63 in mixed tumors and carcinomas in mixed tumors in canine mammary glands, trying to establish a possible correlation between this expression and the histogenesis of these tumors. Twenty samples (10 benign and 10 malignant) were obtained in archives of the Laboratory of Comparative Pathology (LPC), ICB-UFMG. They were revised and subjected to immunohistochemistry techniques using streptavidin-biotin-peroxid method, using the antibody anti-p63. The staining was positive in 100% of samples, ranging in intensity according to tumor's undifferentiation. This result brings new evidence in favour of a mioepithelial origin of this heterogeneous group of neoplasms, like as suggested in human breast neoplasias.

LYMPHANGIOMA: CASE STUDY OF A BRAZILIAN JAGUAR (*Panthera onca*)

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Benign tumors of the lymphatic vessels are rare, especially when compared to corresponding tumors in blood vessels. Lymphangiomas are comparable to hemangiomas, except that the vascular spaces are connected to the lymphatic system and contain lymph instead of blood. In a male feline (***Panthera onca***), 11 years-old from the Zoological and Botanical Foundation of Belo Horizonte/MG, the routine annual exam showed an increase in volume in the inguinal area, approximately 20 cm in diameter, with partial mobility and firm consistency. The tumoral mass was surgically excised and sent for anatomopathological examination. Macroscopically, the mass displayed a smooth surface, and when opened, released a large quantity of yellowish liquid with no scent. The microscopic examination revealed proliferation of lymphatic channels without proliferation of blood carrying channels. Lymphangioma is a rare benign tumor, which can be the result of congenital malformation (hamartoma) or can spontaneous develop in adults.

STUDY OF TWO CASES OF CANINE LIPID-RICH MAMMARY CARCINOMA

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Lipid-rich mammary carcinoma is an extremely uncommon tumour in animals, which has been only described in dogs. Clinicopathologic and immunohistochemical findings of two cases of this rare variant of mammary carcinoma are described. These tumours were diagnosed in a collection of 223 canine mammary tumours sent to the Pathological Anatomy Section of the Veterinary Faculty of León, which represented an incidence of 0.89%. The predominant histologic feature in both tumours was the presence of at least 80% of tumour cells with intracytoplasmic vacuoles which stained positively with Sudan IV but not with alcian-blue PAS (Periodic Acid-Schiff) method, criteria used to recognize lipid-rich mammary carcinoma. However, one of them was composed of the proliferation of tumour signet-ring cells arranged in lobular pattern, as cited in dogs, while the other neoplasm showed an intraductal growth of tumour cells with a fine vacuolated cytoplasm, which has been described in human. Besides, while most vacuolated cells were positive for cytokeratin (CK) 8 in the first tumour, indicating a secretor epithelial immunophenotype; in the second tumour, most vacuolated cells showed expression of CK14, alone or in combination with CK5, suggesting a myoepithelial immunophenotype. There was no clinic or cytological signs associated with possible metastases in both cases.
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MULTIPLE UTERINE LEIOMYOMA IN A FREE-LIVING IBERIAN HARE (*Lepus granatensis*)

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The Iberian hare is a popular game species and an important alternative food source to the rabbit for many endangered wild carnivores. The claimed decrease of this species has generated an increasing interest in its diseases. In this respect a large scale study on the pathology of hunted individuals as well as animals found dead in the field is under way in collaboration with the Spanish Hunters Federation. In the context of this study an agonizing female hare was found by hunters during a hunting drive in february and submitted for analysis.

Necropsy findings included a generalized fat atrophy and multiple small 1 to 2 mm sized white foci in the lung. In the uterus, multiple whitish nodules of 1 to 5 mm in diameter arising from the uterine wall were observed. Only one of these communicated with the uterine lumen. The animal did not appear to be in gestation although the mucosa of one of the uterine horns showed congestion. In addition, two irregular nodules of 1.8 and 2.8 cm in diameter respectively, one next to each ovary were detected in the mesovary that were attached to the uterus by a small pendicle.

Microscopically, all nodules were identified to be leiomyomas. Among the other organs only the liver presented pathologic lesions that consisted in hepatic necrosis with Kupffer-cell activation and proliferation of the epithelium of the biliary ducts.

Uterine leyomiomas have been described in the European brown hare (*Lepus europaeus*), but to the authors' knowledge, this is the first description in a free-living Iberian hare (*L. granatensis*).

IMMUNOLOCALIZATION OF CALPONIN AND CYTOKERATIN 14 IN APOCRINE AND MODIFIED APOCRINE GLAND TUMORS OF THE DOG

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Tumors of the apocrine sweat glands are rare in the dog, accounting for approximately 2% of all canine skin tumours. Carcinomas appear to predominate, making up from 50% to 90% of cases in dog. Two modified apocrine gland tumors are described: 1) Tumors of the ceruminous glands of the ears, and 2) Tumors of the anal sac. Little information is available on the biologic behavior of apocrine sweat gland carcinomas. Biologic behavior may be analogous to mammary gland neoplasms, in which the metastatic incidence closely correlates to histologic subtype (simple, complex and mixed tumors).
The aims of this study were twofold: 1. To analyze the value of two myoepithelial cell markers (calpopin and cytokeratin 14) in the selective staining of the myoepithelial cell component in the normal apocrine sweat gland and its tumors; and 2. To study the morphogenetic role of the myoepithelium in these neoplasms.
Formalin-fixed tissues from 60 benign and malignant tumors (35 also containing surrounding normal skin) have been evaluated. Five different types of calponin-immunoreactive myoepithelial cells were identified: hypertrophic myoepithelial cells, fusiform cells, stellate myoepithelial cells, rounded (myoepithelial) cells and chondroblasts. Differences in staining intensity and staining pattern among these five types of cells suggested a transition of myoepithelial cells to chondroblasts. Cytokeratin 14 immunoreaction was observed in the same cells but the reaction was less in both number of positive cells and intensity of staining than with the anti-calponin antibody. Furthermore epithelial cells with squamous differentiation was also stained with anti-cytokeratin 14 antibody. Stromal myofibroblasts showed calponin immunoreactivity, but they did not react with the cytokeratin 14 monoclonal antibody.
In conclusion, calponin appears to be a very sensitive marker of normal and neoplastic myoepithelium in the canine apocrine sweat gland and its identification in different cell types of complex and mixed tumours of the apocrine sweat gland of the dog suggests a major histogenetic role for myoepithelial cells.

PROGESTERONE RECEPTOR EXPRESSION IN CANINE FEMALE GENITAL TRACT TUMOURS

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Vulvar and vaginal tumours account for 2.4% to 3% of canine neoplasms. They are the second most common canine female reproductive tumour after those of the mammary gland. The vast majority are benign, arise from smooth muscle, are found in intact female dogs and affect dogs from 2 to 18 years of age, with an average of 10.8 years. Benign smooth muscle tumours of the canine female genital tract are variably referred to as leiomyomas, fibroleiomyomas, fibropapillomas, fibromas, and polyps, and vary only in the amount of connective tissue present. Subjective data indicate that leiomyomas may be hormone dependent, but the presence of ovarian steroid hormone receptors has not been analysed. In the present study, 36 canine female genital tract tumours routinely diagnosed as leiomyomas (5 uterine, 16 vaginal, and 10 vulvar), fibroleiomyomas (2 vaginal and 1 vulvar), fibropapillomas (1 vaginal), polyps (1 cervical) and leiomyosarcoma (1 vulvar) were examined to determine the occurrence of progesterone receptor (PR) expression. The presence of smooth muscle differentiation was analysed through the expression of the smooth muscle terminal differentiation marker calponin. Control tumours included urinary tract leiomyomas (n=2) and leiomyosarcoma (n=1), cutaneous fibroma (n=1), and hepatoid gland adenoma (n=1). Archival formalin-fixed, paraffin wax-embedded tissue samples were stained with the Avidin Biotin-Peroxidase complex (ABC) technique using the anti-human PR antibody PR10A9 (Immunotech) and the anti-human calponin antibody (Dakocytomation) as specific primaries.
Ninety-two percent were intact female dogs from 5 to 15 years of age, with an average of 11.2 years. Seventy five percent of dogs were pure breed. Eighty three percent of the genital tract tumours expressed PR. Progesterone receptor-positive tumours included 80% of leiomyomas, and all cases of fibroleiomyoma, fibropapilloma, polyp and leiomyosarcoma. All these PR-positive tumours were calponin-positive as well. Control tumours lacked PR expression with the exception of 1 urinary tract leiomyoma, which indicates that its tissue of origin was most probably wrong. PR-negative genital tract leiomyomas were all located in the vagina and 2 of them lacked smooth muscle differentiation. The vast majority of canine female genital tract tumours with smooth muscle differentiation expressed progesterone receptors. Vaginal tumours were exceptional in that one third of them lacked PR even though they had smooth muscle

differentiation. These findings may have therapeutic implications because PR antagonists could be used in intact female dogs with PR-positive tumours to prevent recurrence after surgery as well as to treat extraluminal vulvar tumours with difficult surgical approaches.

ANAPLASTIC TRANSITIONAL CARCINOMA IN A DOG

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The clinical, pathological and immunohistochemical features of a dog suffering an anaplastic transitional carcinoma leading bilateral hydronephrosis and hydroureter are described. A 7-year-old crossbred female dog was vomiting, mildly dehydrated and had abdominal pain. A bilateral ovariohysterectomy had been carried out two weeks before. Blood and urine analysis revealed chronic renal failure and urinary tract infection. Ultrasonographic examination revealed bilateral hydroureter, hydronephrosis, and an irregular surface of the urinary bladder wall that showed two small masses protruding into the lumen. Cytological examination of the urine sediment revealed and admixed cell population composed by abundant neutrophils, some normal epithelial cells, and different size epithelial cells showing marked signs of malignancy such as different nuclear/cytoplasm ratio, anisocytosis, anisokaryosis, multinucleation, coarse nuclear chromatin and mitotic figures. A transitional cell carcinoma was diagnosed. Despite the bad prognosis the owners decided to administer chemotherapy and antibiotherapy that induced an improvement of the clinical signs during two months, but a month later the dog presented severe anorexia and vomiting and it was euthanasised. At necropsy, the kidneys showed severe enlargement, when opened, the renal pelvis occupied most of the kidneys and contained abundant yellowish fluid, the parenchyma was reduced to a small portion in the poles. Urinary bladder showed several 2 to 5 mm in diameter whitish or reddish nodules protruding in the lumen, whereas the wall was markedly thickened. The iliac lymph nodes were enlarged and contained abundant purulent fluid. The lungs showed severe congestion, alveolar oedema and irregular areas of a red-brown colour. The histopathological examination revealed an anaplastic transitional cell carcinoma than often infiltrated the submucosa, tunica muscularis and serosa of the urinary bladder, with abundant fibroplasia and numerous emboli in lymphatic and blood vessels, with frequent intratumoural necrosis. The ureter walls were not invaded by tumour cells, but the ureter opening into the bladder were comprised by tumour cells and

associated fibroplasia. The lungs showed multiple tumoural emboli and metastases of highly anaplastic cells that were isolated or in small nests. The iliac lymph nodes showed multiple metastases of highly anaplastic cells with abundant lymphatic and vascular emboli and intratumoural necrosis. Tumour cells were keratin positive (AE1-AE3 and RCK 102 antibodies), vimentin negative, S-100 negative, and desmin negative.

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E-CADHERIN IMMUNOHISTOCHEMISTRY ANALYSIS IN MAMMARY NEOPLASIA IN FEMALE DOGS

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Mammary tumors studies in female dogs turn out to be an excellent model for the clinical, pathological, diagnostic and prognostic investigation of mammary neoplasia that affect human beings. The aim of this study was the use of e-cadherin to determine a good prognostic for the patient with cancer. The e-cadherin is a protein which provides adherence to the cells and it is of great value on epithelial differentiation. High e-cadherin levels shows that the adhesion between cells is preserved and, thus, the risk of distance metastasis is considerably low, because the possibility that the neoplasic cells reach the blood stream is nearly null. In this study, 50 (fifty) female canines with mammary neoplasia were brought to the UNIRP's Animal Hospital "Dr Halim Atique", from January to June 2003. The dogs underwent a tumor excision and these fragments were sent to histopathologic exam and immunohistochemistry procedure. The immunohistochemical study was done by using the NCH-38 (Biogen®) clone. The antigenic unmasking was done in wet heat (around 90°C), the amplification of reaction was done with kit Dako® LSAB+, Peroxidase – Universal and for revelation diaminobenzidin (DAB). The results showed that the expression of this marker was strongly positive on benign neoplasias and negative or positive in up to 25% of the cells in the malignant neoplasias studied. Considering that the absence of expression of this marker is linked up to little adhesion between the cells, what increases the odds of distance metastasis, the results confirm the consulted literature. Yet, the result of this work shows that the use of this antibody may contribute for a more discerning tumor prognostic, leaving aside the complex and inconclusive tumor classification as the only answer for the search of a better and longer lifetime for the patient with cancer. Moreover, the little number of researches on Veterinary Medicine, seeking new prognostic and predictive markers for mammary neoplasia show an immense research field to be explored.

AN IMMUNOHISTOCHEMICAL STUDY OF E-CADHERIN AND CELL PROLIFERATION IN CANINE SPONTANEOUS HEPATOID GLAND TUMOURS

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Hepatoid gland tumours are among the most common types of skin tumours in the dog, particularly in males. Most are benign, and recurrence after incomplete removal is uncommon.

Aim of the study: The aim of the present study was to investigate the expression of E-cadherin (E-cad) and the proliferative index assessed by the MIB-1 (Ki-67) labelling index (LI) in hepatoid gland tumours, and study the relationship to histological tumour type.

Material & Methods: A total of 61 canine hepatoid gland tumours (27 adenomas, 7 epitheliomas, and 27 carcinomas) were included in this study. The tumours were classified using the diagnostic criteria proposed by the World Health Organization classification of tumours in domestic animals. Immunohistochemical analysis was performed by the avidin-biotin complex method (ABC), using commercially available monoclonal antibodies against the adhesion molecule E-cadherin and nuclear antigen Ki-67. E-cad was evaluated as the percentage of membrane labelled cells and the MIB-1 immunostaining /MIB-1 labelling index) was scored by counting 1000 cells in 10 to 20 fields per histological section, depending on their cellularity. The chi-squared statistical test of association was used to determine the significance of the relationships studied. P<o,05 was considered to be significant.

Results: Twenty out of 27 benign tumours had more then 75 per cent of positive cells, whereas 13 of the malignant tumours had less than 25 per cent and 20 had between 25 and 75 per cent of positive cells. The expression of E-cad on the begnin and malignant tumours was significantly different ($p<0.001$). Hepatoid gland carcinomas showed a marked reduction in the expression of E-cad. The malignant tumours had higher rates of cellular proliferation than the benign tumours ($p<0.001$).

Conclusions: The results of this study suggest that there is an association between reduction in the expression of E-cad and the malignancy of canine hepatoid gland tumours.

FIRST DESCRIPTION OF FELINE INFLAMMATORY MAMMARY CARCINOMA: CLINICOPATHOLOGICAL CHARACTERISTICS AND HORMONAL STATUS OF THREE CASES

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Inflammatory mammary carcinoma (IMC) is a special type of locally advanced mammary cancer, associated with a particular aggressive behaviour and poor prognosis. For the moment, it has been described only in humans[1] and dogs[2,3] as a spontaneous disease.

The objective of this work is to describe clinicopathologic and hormonal findings of three cats with IMC, and to compare them with those previously described in the dog with IMC.

Materials and Methods. Three cases of female cats with clinical signs of IMC were prospectively collected during a period of three years. Clinicopathological evaluation, steroid receptor status (estrogen receptorα ERα, progesterone receptor PR, androgen receptor AR) and serum hormone profile (dehydroepiandrosterona, androstenedione, testosterone, progesterone, 17ß-estradiol, estrone sulphate) were performed.

Results. All three animals presented secondary IMC (post-surgical) characterized by a rapid onset of erythema, severe edema, extreme local pain and firmness, absence of subjacent mammary nodules, and affectation of extremities. Rejection of the surgical suture was observed in two cases. Histologically, highly malignant papillary mammary carcinomas, dermal tumor embolization of superficial lymphatic vessels and severe secondary inflammation were observed. Euthanasia was performed at 10, 15 and 45 days after diagnosis. Metastases were detected in regional lymph nodes and lungs in the two animals necropsied. All tumors had a high Ki-67 proliferation index and were ER, PR and AR positive. Serum hormone levels were elevated in all three cases compared to normal cats.

Conclusions. Like the dog, the cat can suffer spontaneous IMC. Feline IMC has a very low prevalence (lower than canine IMC[3]); only secondary IMC has been found in this species and it is frequently associated with a moderate inflammatory reaction with surgical suture rejection. Prognosis of feline IMC is also very poor, but slightly better than canine IMC[3]. As in canine IMC[4], massive embolization of dermal lymphatics is the histological hallmark for the diagnoses. Furthermore, serum hormonal profiles are similar in feline and canine IMC[5].

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TWO HEAD CARCINOSARCOMAS IN DOG

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Carcinosarcomas are defined as tumors composed admixed malignant epithelilial and mesenchymal elements, their incidendence in dog is very low and they are described mainly in the mammary gland of the bitch.
We report two cases located in different areas of the head of two dogs (both elder than 10 years old): one in the retrobulbar region and another in the frontal area. Histologic and immunohistochemical examination were performed. In both cases, histologic examination showed biphasic neoplasm composed of both malignat epithelial and malignant mesenchymal element although the ratio epithelial/mesenchymal malignant cells was different in each case. The epithelial elements were diagnosticated as squamous cell carcinoma and adenocarcinoma while the mesenchymal element was osteosarcoma in both cases. Differential diagnostic was considered with carcinoma with osseus metaplasia Immunohistochemical studies showed that carcinoma cell were positive for cytoqueratin and negative for vimentin, desmin, S-100 protein and smooth muscle actin, the malignant mesenchymal cells only were positive for vimentin. In agrrement with our findings both tumours were diagnosticated as carcinosarcomas. The review of the literature only mentions one case of extramammary canine carcinosarcoma located in the lung. The histogenesis of these tumours remains unknown

SPLENIC MYELOLIPOMA IN A SOUTH AMERICAN SEA LION (*Otaria flavescens*)

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Few tumors have been described in sea lions. Most of them have been seen in California Sea Lion (*Zalophus californianus*), probably attributable to their popularity as zoo and aquarium display animals. Tumors described are adenocarcinomas, squamous cell and urogenital carcinomas, adenomas and meningiomas. Myelolipoma is a rare benign tumor of mesenchymal origin. It is composed of mature adipose tissue and normal hematopoietic elements including mature and immature cells of all hematopoietic cell lines. Adrenal glands are by far the most common location, although myelolipomas have been described in liver, spleen and lung.

We describe the first case of splenic myelolipoma in a 28-year-old male South American Sea lion (*Otaria flavescens*) that lived in captivity in an aquarium from birth.

At necropsy, a well-demarcated nodule of 3 cm in diameter and projecting above the surface was observed in the spleen. Cut surface was whitish to grayish in color and relatively soft to the touch. Spleen samples were collected in 10 % neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-eosin. Microscopically, the nodule consisted of adipose tissue well differenced mixed with hematopoietic elements including myeloid cells, erytroid cells, megakaryocytes and lymphocytes. Adipose tissue supposed the 80% of the mass tissue. Thin layers of connective tissue were observed inner and surrounded partially the tumor. A diagnosis of splenic myelolipoma with few hematopoietic elements was reached based on histological characteristics.

Myelolipomas must be taken into account as a differential diagnosis of splenic tumors in Sea Lions.

DERMATITIS WITH INVASIVE CILIATED PROTOZOA IN A STRIPPED DOLPHIN (*Stenella coeruleoalba*) STRANDED IN MEDITERRANEAN COAST IN THE SOUTH OF SPAIN (MALAGA)

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Dermatitis with intradermal ciliated protozoa have been reported in bottlenose dolphins and other cetaceans. A database search of the American Forces Institute of Pathology (AFIP) archives over 37 years, found this type of dermatitis in 20 animals from about 1000 stranded cetaceans studied, being the incidence much greater during morbillivirus epizooties. We reported a case of dermatitis with invasive ciliated protozoa in a stripped dolphin (*Stenella coeruleoalba*) stranded in the Mediterranean Coast in Malaga (South of Spain) in May 2004. The animal stranded, died during the travel to the Recovery Center (CREMA). Necropsy showed several skin lesions. These lesions were usually characterized by discrete ulcers with subjacent dermal, and often, subcutaneous, necrosis and inflammation. Within the dermis and subcutis, there were variable numbers of organisms among numerous degenerate neutrophils and a few macrophages. Organisms were irregularly oval to spindle shaped, and had a macronucleus, vacuolated cytoplasm, and tufts of cilia. The cytoplasmic vacuoles often contained eosinophilic globules. Other lesions appreciated were suppurative pneumonia and severe hepatosis. The other organs were very congestive with no other lesions. Recently, ciliated collected from the blowholes of bottlenose dolphins and other cetaceans were described and named *Kyaroikeus cetarius*. The size, shape and nuclear configuration are consistent with those described in this abstract. Although some fish develop a somewhat similar condition ("white spot disease" by *Ichthyophthirius multifiliis*), cetaceans appear to be the only other animals subject to dermatitis with invasive ciliates. When observed, the possibility of underlying immunosupression, such as that caused by morbilliviral infection or chronic exposition to toxins (DDT, PCBs, and so on), should be considered. In this case, we are performing virological and toxicological studies trying to know which is the cause of this immunosupression.

AN IMMUNOHISTOCHEMICAL STUDY OF *Tenacibaculum (Flexibacter) maritimus* IN NATURALLY INFECTED SOLE (*Solea senegalensis,* Kaup*)* CULTURED IN THE SOUTH OF SPAIN.

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Sole is a flat fish well adapted to water temperature and with a high economic value. Both *Solea solea* and *Solea senegalensis* (Kaup) are being cultured in the southwestern and Mediterranean areas of Spain and Portugal. However, as well as other new cultured fishes, the sole cultures have been affected by different pathologies (pasteurellosis, flexibacteriosis and vibriosis) in which bacteria are found.

From 2000, several outbreak associated with moderate mortalities (20-25%) in populations of sole cultured in farms in the south of Spain was observed. The main external signs of the disease being superficial and/or depth ulcers, sometimes haemorrhagic, affecting skin and/or fins. Occasional congestive images were observed at internal organs.

Both histopahology and microbiology studies were carried out to diagnose the pathology and the main bacteria isolated from ulcers. Severe cases showed haemorrhagic areas surrounding ulcers and muscle was also affected; *Vibrio harveyi* was present in ulcers together with *Tenacibaculum maritimum*. However *Vibrio harveyi* was the only microorganism isolated from intern's organs (liver, kidney and spleen).

Histopathology showed dermal changes including superficial or deep ulcers extending to the subcutaneous tissue or the musculature in severe cases, accompanied by inflammatory cell infiltrates. Liver, spleen and kidney were observed congestive.

We described an immunohistochemical method as an alternative to microbiological cultures because it allows observing pathogens implicated in pathology when microbiology results were negative. The immunohistochemical protocol is described for the detection of *Tenacibaculum (Flexibacter) maritimus* in formalin-fixed, paraffin-wax-embedded sole tissues. Rabbit anti-*Flexibacter maritimus* antisera (Microtek) used in this study.

Results showed a direct implication of *Tenacibaculum (Flexibacter) maritimus* in ulcers lesions affecting sole. However immunohistochemical studies did not show this microorganism associated to any lesions in organs. So, we think that *Tenacibaculum (Flexibacter) maritimus* skin infection is an "open door" for the entrance and establishment of secondary infections with pathogens like *Vibrio harveyi*.

LUNG FAT EMBOLISM IN CETACEANS STRANDED IN CANARY ISLANDS

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The objective of this study was to evaluate the presence of fat emboli in lung tissues obtained from cetaceans stranded in Canary Islands.
Lung samples from 84 cetaceans of 15 different species were studied. The animals stranded in Canary Islands coast between 1995 to 2003. The tissues had been fixed in 10% neutral buffered formaline solution. Tissues samples were prefijated with osmium tetroxide. Lately routin laboratorial technics for section and staining (hematoxilin-eosin) were made.
14 of 84 cetaceans of six different species, presented diverse lung fat embolism grades characterized by clear drops (H/E), black-stained with OsO_4 in the lumen of small and medium size of pulmonary vessels: *Kogia breviceps(4), Kogia simus(1), Mesoplodon densirostris (1), Physeter macrocephalus (3), Tursiops truncatus(1) y Ziphius cavirostris(4).* All these animals belong to deep and long time diving species*(13/14)* excepting the one *Tursiops truncatus*.
The cause of stranding and/or death of the 14 positive animals were relationed to: Antropogenic interactions (ship colision): 5/14; Massive strandings: 4/14; Unknown or natural causes: 5/14.
According to these results, a clear association between lung fat emboli with violent trauma (ship colision) and with deep and long time diving species is observed.

KUDOA SP. INFECTIONS IN FISH SPECIES WITH ECONOMICAL VALUE

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Most of *Kudoa* species infect the muscle of fish establishing cysts, which contain a large number of spores. This parasitic disease can result in *post-mortem* myoliquifaction and a loss of product quality. To detect infected fish it is necessary to destroy them. So, it is possible that cysts reach the consumer.

In this work the authors describe *Kudoa* sp. infections in fish species with economical value. Parasitic infections were found in the following species: large eyed dentex (*Dentex macrophthalmus*), maroquin dentex (*Dentex maroccanus*), bronze bream (*Pagellus acarne*) and horse mackerel (*Trachurus trachurus*).

CARACTERIZACIÓN ENZIMOCITOQUÍMICA DE LEUCOCITOS EN RODABALLOS (*Scophthalmus maximus*)

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El conocimiento de las características morfológicas y funcionales de los leucocitos resulta fundamental para valorar parámetros de biología y patología. En los peces, la morfología de estas células varía ampliamente entre especies y su correcta identificación resulta más complicada que en mamíferos. Por otra parte, los métodos inmunocitoquímicos, de gran validez en mamíferos, están menos desarrollados en los teleósteos debido a la escasez de anticuerpos comerciales que marquen específicamente los distintos tipos celulares. Por ello, la detección de enzimas presentes en células de la serie blanca es uno de los métodos de elección en la hematología piscícola. Estas técnicas enzimocitoquímicas se aplican normalmente sobre extensiones sanguíneas, improntas de tejidos y secciones de criostato obtenidas de muestras frescas, siendo esto un factor limitante para su empleo habitual. En este trabajo se ha determinado la actividad enzimática de fosfatasa ácida y alcalina, esterasas específica y no específica y peroxidasa, sobre extensiones sanguíneas, improntas y en muestras de tejidos empleando diferentes fijadores y métodos de inclusión en parafina. Los resultados obtenidos han permitido la estandarización de técnicas para su utilización sobre cortes convencionales y la caracterización enzimática de linfocitos, granulocitos, monocitos y macrófagos.

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TECNICA ENZIMÁTICA	ACETONA	ETANOL	TIEMPO
Fosfatasa ácida A	++	+++	120
Fosfatasa ácida B	++	+++	60-120
Fosfatasa alcalina	+	++	45
EE	-	-	-
ENE	-/+	++	10
Sudán Black B			
Peroxidasa	++	+++	30

INTRACYTOPLASMIC EOSINOPHILIC GLOBULES IN HEPATOCYTES OF STRANDED CETACEANS IN THE CANARY ISLANDS

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The presence in hepatocytes of intracytoplasmic inclusions has been described both in human and in veterinary medicine, being associated to different agents and pathogenic mechanisms. In cetaceans, the presence of this type of globules has been frequently described in animals stranded individual or massively.
For the accomplishment of this work, samples of liver, corresponding to 108 cetaceans of 17 different species, stranded in the Canary Islands had been studied. In the cytoplasm of hepatocytes of 58 animals of 12 species, hyalines eosinophilic globules were observed, with a size between 4 and 20 μm. In 49 out of the 58 livers showing those inclusions, histochemical (Pas-diastase) and immunocytochemical (detection of alpha-1-antitrypsine) techniques were perfomed on formaline-fixed, paraffin-embedded sections.
The results showed 26 positive livers to both techniques, 22 of which were associated with hepatic congestion, 10 were just PAS diastase positive, 6 only α-1-AT positive and 7 negative to both staining procedures. In the present study, the acute hepatic congestion was observed in 53% of the α-1-AT positive animals. The origin of these intracellular changes is probably related to hemodynamic phenomena suffered by the cetaceans stranded alive, in addition to hyperthermia and/or other factors wich may induce the production and storage of α-1-AT and as other acute phase proteins (under current study) in the hepatocytes.

POLYCYSTIC KIDNEY AND MYCOTIC PNEUMONIA IN A FREE-LIVING SPANISH IBEX (*Capra pyrenaica*)

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At present little is known about the diseases of wild artiodactyles from the Iberian Peninsula. In the course of a study on the distribution of Spanish ibex in South Central Spain, a 14 year-old female was found showing unspecific clinical signs that included postration, general decay and incordination.
At necropsy, only small kidneys and multiple renal cysts were observed. Microscopically these kidneys showed a non-uniform distention of the distal convoluted and collecting ducts, especially affecting the renal cortex, with occasional interstitial inflammatory infiltration and fibrosis and membranoproliferative glomerulonephritis. In the lungs, we observed scattered necrotic areas with a heavy linfocitic infiltrate and fungal hyphae, and a PAS positive reaction in the centre, isolated from the pulmonary parenchyma by a wide connective tissue capsule. The fungal hyphae were also found in small numbers in the kidney, without any associated inflammatory response.

DIGESTIVE LESIONS IN THREE SPECIES OF CETACEANS STRANDED IN THE CANARY ISLANDS

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Since 1992, several cetacean strandings have ocurred on the shores of these islands, involving both single and multiple strandings of adults as well juvenile cetaceans. In the study of the causes of death in stranded cetaceans, a variety of gastrointestinal lesions were found. In this work, gastrointestinal lesions observed in 6 common dolphins (*Delphinus delphis*), 11 striped dolphins (*Stenella coeruleoalba*) and 6 spotted dolphins (*Stenella frontalis*) found stranded along the coasts of the Canary Islands were analyzed. Postmortem examinations were undertaken and tissue samples of forestomach, fundic stomach, pyloric stomach and small and large intestine were collected. Selected sections were stained with haematoxylin and eosin for hitological examination.

Chronic parasitic gastritis produced by *Pholeter gastrophilus* was the main lesion observed in most of the animals. The macroscopic evidence was the presence of large numbers of granulomas containing parasites or parasite remains in the wall of the two last gastric compartments. Histological analysis revealed multiple foci in the submucosa, contained parasites and parasitic ova surrounded by inflammatory cells, predominantly eosinophils, lymphocytes, macrophages and neutrophils. Surrounding these foci there were abundant fibrosis, which was accompanied with parasite ova and parasite remains, and mixed inflammatory infiltrate composed of multinucleated giant cells, numerous lymphocytes, and plasma cells.

Ulceration of the cardiac portion of the stomach, in association with *Anisakis simplex* infection was observed in two animals. These lesions were present in the ventral mucosal walls of the organ and most were about 0.5 cm in diameter. They were deep, with a punched-out appearance. Histological examination revealed cast cuticles of nematodes surrounded by an intense inflammatory reaction composed of mononuclear cells, polymorph and multinucleated giant cells. There was fibrosis associated with the lesion replacing the muscular layers.

Enteritis of unknown aetiology was found in some of the animals that was characterised by an lymphoplasmacytic infiltrate in the lamina propria. One common dolphin had a severe fibrinous peritonitis with abundant presence of fibrin surrounded by a marked

infiltrate of neutrophils, lymphocytes and plasma cells that in some areas were spreading into the muscular layer.

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INMUNOHISTOCHEMICAL STUDY OF DIGESTIVE LESIONS OF CETACEANS STRANDED IN THE CANARY ISLANDS

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The nature of the cellular infiltrate (lymphocyte populations, macrophages and immunoglobulin-bearing plasma cells) observed in natural cetacean diseases only have been studied in common dolphins (*Delphinus delphis*) and striped dolphins (*Stenella* coeruleoalba) with hepatic disorders. The aim of this study was to examine the distribution and cross-reactivity of a panel of commercially available antibodies (CD3, IgG, IgM, IgA, S-100 protein, MHC class II and lysozyme) in lymph nodes and digestive lesions observed in common dolphins (*Delphinus delphis*), striped dolphins (*Stenella coeruleoalba*) and spotted dolphins (*Stenella frontalis*) found stranded along the coasts of the Canary Islands. The avidin-biotin-peroxidase (ABC) method was used. Dolphin mesenteric lymph node and healthy digestive tissue sections were used as positive controls.

Chronic parasitic gastritis and lymphoplasmacytic enteritis were diagnosed. Chronic parasitic gastritis was granulomatous type, affecting mucosa, submucosa and muscular layer, with variable amount of lymphoplasmacytic infiltrate and fibrosis. The inflammatory infiltrates was composed mainly by $CD3^+$ T lymphocytes and IgG^+ plasma cells, being moderate to sparse the presence of IgM^+ and IgA^+ plasma cells sited in lamina propria of glandular areas of the stomach. The immunoreactivity with lysozyme and MHC class II was variable in macrophages observed in granulomata lesions and fibrotic areas. The anti-S100 protein pAb yielded a diffuse nuclear and cytoplasmic immunoreactivity in stellate cells, similar to follicular dendritic cells, located in these areas, suggesting that these infiltrates were highly organised to enhance antigen presentation to B and T cells.

Lymphoplasmacytic enteritis was characterised by an inflammatory infiltrate composed of $CD3^+$ T lymphocytes and IgG^+, IgM^+ and IgA^+ plasma cells in lamina propria and submucosa. The anti-lysozyme reacted with sparse number of macrophages and monocytes in lamina propria, and ocasionally, in submucosa. The anti-MHC class II labelled moderate to sparse number of macrophages located in lamina propria, but no lymphocytes were labelled with this antibody.

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INTRAFIBER LIPID DROPLETS IN SWIMMING SKELETAL MUSCLE OF STRANDED CETACEANS IN CANARY ISLANDS

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The present study is focused on the analysis of swimming skeletal muscle samples (*Longissimus dorsi*) from 15 different species of 86 stranded cetaceans in Canary Islands from 1996 to April 2004.
The purpose of this study was to evaluate the presence of spherical intrafiber lipid droplets to explain the aerobic capacity of skeletal muscles under the hipoxic conditions of diving.
The previously fixed tissue in 10% neutral buffered formalin solution was post fixed in 1% osmium tetroxide, dehydrated in graded ethanol series and embedded in paraffin. Sections (5 mm in thickness) were cut, treated with picric acid for 24 hours and stained with hematoxilyn-eosin.
As result 19 of the 86 animals (22%) belonged to 7 of the 15 species of cetaceans presented different degree of intrafibrilar doplets with the osmium tetroxide method.
The percentages exhibited for each specie were the following: *Globicephala macrorhynchus* (n=8)38%; *Kogia breviceps* (n=5)20%; *Kogia simus* (n=2) 50%; *Mesoplodon densirostris* (n=2) 100%; *Physeter macrocephalus* (n=7) *43%*; *Stenella frontalis* (n=12) 8% y *Ziphius cavirostris* (n=10)70%.
According to the results described above we may conclude that swimming skeletal muscle of deep, long-duration divers species showed a greater amount of lipid intrafiber droplets that swimming skeletal muscle of short-duration divers species.

DISSEMINATED HISTOPLASMOSIS IN GAZELLE DORCA (*Gazella dorcas*) KEPT IN CAPTIVITY CONDITIONS

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A female gazelle dorca *(Gazella dorcas)*, 17 months old, showed several illness evidences such as lethargy, abnormal attitudes, fur stood on end, and bent down position. Different diagnostic tests were performed. X-ray revealed different foreign bodies into the rumen and the gazelle was surgically operated. After this surgical operation, the animal died.

Necropsy (Pathology)

The affected animal looked very thin and its mucous membranes were very cleared. Both, subcutaneous tissue and peritoneum appeared with white spots. The lung was congested and oedematous. The trachea was filled up with whitish foam. The mesenteric lymph nodes were hypertrophied and splenomegaly was observed. Omasitis and haemorrhagic enteritis could be observed. Moreover a lot of foreign bodies appeared into the rumen. The uterine horns appeared filled up with a chocolate coloured liquid.

Microscopic studies revealed a fibrinous pleuropneumonia accompanied by infiltrations mainly composed of histiocytes and numerous yeast shaped elements with a small eccentric nucleus and many peripheral cytoplasmatic retraction halos. Those fungal elements often appear forming typical "yeast lakes" and others which invade the vascular lumen of small lung vessels.

A specific fungus stain was performed using methenamine silver nitrate according to Groccot method. This stain revealed the yeast cellular walls strongly stained. The typical cellular walls lay out and stain characteristics made easy to identificate the organism as *Histoplasma capsulatum* and to differentiate it from other similar organisms. The intestine showed haemorrhagic necrotic enteritis accompanied by haemorrhagic ulcers on the mucous membranes and lymphohistiocitic infiltrations. It also could be observed on different mucous membrane areas the same fungal elements observed inside the lungs. At the spleen level it's noticeable the incipient proliferation of macrophages, some of them with its cytoplasm full of yeast cells like described before. The liver is congested and multifocal hepatitis with focus composed of macrophages and lymphocytes associated with necrotic hepatocytes and biliar hiperplasia were observed.

The kidney showed interstitial inflammation together with the same leucocitary components described before, moreover vascular congestion and intertubular haemorrhages. Tubular epithelia appeared degenerated and some tubular lumens were observed with scarce acidophilic fibrilar remains inside or filled up with homogeneous proteinic exudate. Homogeneous eosinophilic and fragmented cytoplasmatic fibres appeared inside the heart and slight myocarditis could be observed. At uterine levels it's noticeable a chronic endometritis accompanied by abundant lymphohistiocitic infiltrations. Brain showed vascular changes with congestion, oedema and small haemorrhages as well as neuronal satellitism phenomena. All these lesions are diagnostic of histoplasmosis.

In our case the histomorphological response pattern with the presence of abundant histiocitic components accompanied with lots of intracitoplasmatic yeasts, extracellular "yeast lakes", granulomatous response absence and secondary diffusion of the pathogen are evidences of a previous immunodepressive state. These injuries are similar to those observed by other authors for histoplasmosis in cetaceans.

According to the gazelle captivity situation, the main immunodepression causes could be:

Immunodepression related to the animal feeding state.

Immunodepression related to captivity stress situations.

Secondary immunodepression related to surgical interventions.

It's not surprising that one or all of the immunodepression causes named before could exert a similar effect, causing a disseminated histoplasmosis developing with a very similar histological response pattern showed by people who have a severe immunity damage.

SEPTICAEMIA IN A *PYTHON MOLURUS* ASSOCIATED WITH POOR MANAGEMENT

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A *Python molurus* snake of 35 Kg and 7 years old was euthanasied before suffering several clinical episodes of stomatitis, pneumonia, ophthalmitis and dystocia during the last four years. The animal lived in terrarium inside a circus truck with the adequate temperature, but during the circus show the snake was under stress conditions for hours (low temperatures, noises, brilliant lights…).

At necropsy, multiple lesions were observed in some organs. In the oral cavity, a severe purulent stomatitis complicated with osteomyelitis was seen in the upper jaw. The right lung showed a severe pneumonia with copious yellowish exudates. As well, abscesses in spleen, stomach and ovaries were described.

In the microscopic study, purulent lesions consisted of a heavy accumulation of gram-positive rods with a lot of heterophils and necrotic material. In some cases, connective tissue with calcifications was as well observed.

The microbiologic culture reported the presence of *Corynebacterium macginleyi* and *Stenotrophomonas maltophilia* in the different purulent lesions.

The clinical disease of the snake in combination with the pathological lesions suggested a septicemic process caused by *Corynebacterium macginleyi*, probably initiated from the stomatitis in the oral cavity. *Stenotrophomona maltophilia* might have acted as a secondary agent.

Finally, it must be considered that the factor that sparked off the first infectious process and the subsequent septicaemic process could be a stressful state caused

INFECÇÃO POR *CAPILLARIA HEPATICA* EM PEQUENOS PRIMATAS

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A infecção por *Capillaria hepatica* foi diagnosticada em vários pequenos primatas das famílias *Cebidae* e *Callithricidae* da colecção do Zoo de Lisboa.
A extensão das lesões era variável, constituindo, na maioria dos casos, um achado de necrópsia, sendo a causa da morte devida a outros agentes (*Yersinia pseudotuberculosis*, *Leptospira* sp. e outros).
Histológicamente as lesões hepáticas correspondiam a acumulações de ovos bioperculados, envolvidos por uma discreta reacção inflamatória e fibrose.
A observação destes casos associada às particularidades do ciclo biológico do parasita levou a que o Zoo procedesse a uma remodelação das instalações de pequenos primatas do Novo Mundo e à alteração de algumas normas do maneio alimentar.

SUDDEN DEATH IN LIONS (*Panthera leo*) ASSOCIATED WITH *Clostridium sordellii*

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During spring of 2003, five lions (*Panthera Leo*) from the Tabernas Desert Zoological Reserve placed in Almería (Spain) died in the course of 48 hours showing depression, inapetence and postration. Serological tests for viral detection were negative, no evidence of parasites was detected and haematology data were within normal limits in all lions. At necropsy, multifocal to locally extensive haemorrhages were observed in the external muscular layer and serosa of duodenum and jejunum. Pericardial and mesenteric fat tissue was soft and edematous. Histologically, lesions consisted with acute hemorrhagic enteritis, acute small intestine and epicardial myositis, and mesenteric and pericardial cellulitis. Aerobic cultures failed to yield any significant bacterial agents. After 72 hours, several black colonies were observed in TBS cultures obtained from samples of stomach, small and large intestine, liver and kidney. The colonies were identified using the enzymatic identification system for anaerobes rapid ID 32 A (Bio Mérieux, France) as *Clostridium sordelli* (urease, indole production and proline arylamidase positive).
On the basis of gross and histological features and the isolation and identification of *Clostridium sordellii*, a diagnosis of intestinal clostridiosis with profound toxemia and sudden death was made. *Clostridium sordellii* is a gram-positive, anaerobic bacillus that has been reported as one of the rarer causes of clostridial myositis in humans and animals. Ruminants, horses, and swine are highly susceptible to these infections, whereas carnivores are rarely affected. No previous reports of this disease have been done in wild felines. gramirez@becarios.ulpgc.es

NODULAR OEDEMA OF PREPUCE IN A BOAR

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Accumulation of subcutaneous fluid in the inguinal region can be originated by regressive changes of inguinal lymph nodes that obstruct the correct drainage of the zone. It can be caused by infectious agents, traumatism, toxins, tumors or nutritional alterations.

We described a subcutaneous swelling in a Pietrain boar, located in both sides of the prepuce region. Cut surface was 15-20 cm in diameter and exuded abundant serous fluid. Superficial inguinal lymph nodes were decreased in size and increased in consistence.

Samples were collected in 10 % neutral buffered formalin, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin-eosin, Masson tricrome and Congo red. Microscopically, the swelling corresponded with an oedema affecting the deep dermis. Oedema areas were separated by bundles of collagenous fibres alternating with areas of diffuse oedema. Dilated lymphatic vessels were found in both areas. Dilated lymphatic vessels corresponding to afferent lymphatic vessels together with efferent lymphatic vessels of lesser diameter appeared in the cortical region of the lymph nodes. In cortical areas and around the nodular units a proliferation of connective tissue intermingled with small cellular infiltrates composed mainly by lymphocytes and plasma cells were seen. An acidophil material, green in color with Masson tricrome stain and slightly positive with Congo rouge stain appeared in the dense nodular lymphoid tissue around the follicles. Samples stained with Congo rouge and observed with polarized light microscopy showed a filamentous material green in color distributed by the whole parenchyma and more abundant in the perifollicular areas. Focal atrophy images in nodular units were also seen.

These observations point out that the animal presented a chronic lymphadenitis that together with the amyloid deposit and focal atrophy caused a decrease of correct drainage of the zone and an accumulation of liquid with enlargement of lymph vessels previous to the nodular lesion and a proliferation of connective tissue along the time.

Nodular oedema caused by regressive alterations of the lymph nodes in a chronic lymphadenitis must be taken into account in the differential diagnosis with acute inflammations or tumors in the prepuce zone.

SILICATE PNEUMOCONIOSIS IN HENS: A POSSIBLE RELATION WITH SILICATE-RICH ATMOSPHERE

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Pneumoconiosis is an interstitial pulmonary disease associated with inorganic dust inhalation. This pathology is uncommon in animals because they lack occupational exposures to dust. Although silicosis has been described both in avian and mammalian pathology, the amount of dust retention appears to be greater in birds.

We report 18 cases of silicate pneumoconiosis in 3-4 year-old hens in the Canary Islands. The hens were raised in an outdoor system in a rural environment. Lung samples were fixed in 10% buffered formalin, embedded in paraffin-wax and stained with hematoxylin-eosin for light microscopy. For electron microscopy study, samples were fixed in glutaraldehyde.

Pulmonary changes consisted with multifocal clusters of macrophages containing intracytoplasmic foreign particles with a crystalloid appearance, admixed with lymphocytic aggregates and located in the infundibula and interatrial septa of parabronchi and in the lamina propria of the bronchial mucosa.

Inert particles appeared as bluish-white crystals under polarized light.

Energy dispersive x-ray microanalysis and scanning electron microscopy revealed that dust particles were mainly composed of silicon. Other elements such as calcium, barium, sodium, potassium, magnesium and copper were also detected.

Canary Islands is an archipelago placed near to the north-west of Africa. Few times during the year, mainly from January to March, Canary Islands receive breezes charged with large amounts of sand coming from The Sahara desert. Silicon is the main component of sand. The relation between silicon particles in the lung of numerous hens and the climate conditions they have been exposed is discussed.

INCLUSION BODY DISEASE OF FALCON IN SPAIN: DESCRIPTION OF FOUR CASES

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Herpesvirus infections were first described in raptors from Austria in the early1900s and have since been documented in free-ranging and domestic raptors in theUnited States, Europe and Asia. Frequently referred to as "inclusion body hepatitis"of falcons, the herpesvirus that causes this disease has serologic similarities tothe herpesvirus that are found naturally in pigeons and owls.

Infected falcons usually die acutely with no premonitory signs, but may exhibitdepression, lethargy and anorexia for 24 to 72 hours before death. Mortality ratesin affected birds approach 100% with death usually occurring one to two days afterclinical signs develop. Both young and adult falcons have been found to besusceptible to falcon herpesvirus infections. Gyrfalcons and Prairie Falcon areconsidered highly susceptible while Peregrine Falcons appear to be relatively resistant.

The genomes of pigeon and falcon herpesviruses are similar to each other, while thegenome of Pacheco´s disease virus is different from both pigeon and falcon herpesviruses.

This finding supports the field observations that infection is thought to occur byingestion of infected prey species, in particular pigeons. Lierz has shown that 35%of wild injured raptors tested in his survey in Germany were seropositive for falconherpesvirus.

Falcon herpes virus is only considered to occur occasionally in Spain with very fewreported cases. (in Spain is eliminated) During January and March 2004 we havereceived in the laboratory four cases of herpesvirus infections in falcons belongingto distinct free-ranging species (1 Merlin, 2 White Gyrfalcons and 1 PrairieFalcon), all of them from Andalusian Region (Spain).

Clinical history reported by the veterinarian clinician only describe a picturecaracterized by depression and lethargy 48 hours before death of animals.

Histological examination shows acute multifocal coagulative necrosis in the liverand spleen. Small mainly eosinophilic intranuclear inclusion bodies (Cowdry type B)can usually be observed in degenerate hepatocytes at the periphery of these necrotic lesions.

Other findings were captive or dietetic myopathy. The aortic vascular wall presentmyxoid degenerative lesions affecting to intimal and media. Kidneys were verycongested with degenerative changes of tubular epithelial cells.
The reported cases in free-ranging falcons must be a signal for take in care thecontrol of this disease in our raptors populations.

A POSTMORTEM STUDY IN WILDLIFE IN THE NORTHWESTERN SPAIN

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A postmortem study was conduced on 288 birds from 52 species died in the Wildlife Animal Recovery Centre of Cotorredondo (Pontevedra, Spain), during 1999-2002- The objective of this study was to determine the most common causes of mortality of birds in Galicia and to compare results with those obtained in other geographic regions. For this review, orderly systematic necropsy was achieved to birds and we take samples from intern organs. The results indicate that human related acticity plays an important role in bird's mortality. The most frequent causes of death were trauma related (115), aspergillosis (34), infectious pneumonia (24), parasitic diseases (199, sepsis (17), problems related with spill's oil (14) and electrocutions (8)

HISTOPATHOLOGICAL STUDY OF THE SKIN IN FAST-GROWING BROILER CHIKENS WITH OILY BIRD SYNDROME

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Problems such as oily or greasy birds, water pockets under loose skin, and broken skin have been occurring with some frequency at slaughterhouse. These sometimes unrelated appearing problems have been collectively referred to as the "oily bird syndrome" (OBS) (GARRETT, 1975). Some factors postulated to cause OBS are high temperature, fat deposition due to diet and processing plant stress factors (FLETCHER & THOMASON, 1980; JENSEN et al., 1980).

Here we present the histopathological study of the skin in fast growing broiler chikens with Oily Bird Syndrome (OBS).

At the slaughterhouse we sampled 8 animals with normal aspect of the skin for the naked eye that were considered as controls, and 24 broilers with gross appearance of OBS. OBS broilers grossly showed a very oily condition of the skin with the common presence of subcutaneous pockets filled with an oily mass that can be easily dissociated by its lack of junction with the connective subcutaneous tissue. Samples taken from the dorsal skin in controls and OBS broilers were fixed in Bouin for conventional histopathology or freeze for Sudan IV staining of fats.

Histopathology showed that all the fast-growing broilers considered as controls had an skin with inicial fatty degeneration of adipocytes in the hypodermis.

Histopathology of the skin in OBS broilers showed middle to severe fatty degeneration of adipocytes associated with esteatitis or inflammation in the hypodermis that, in severe cases, also affected the dermis.

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ESTUDIO COMPARADO DE LA NEUROPATÍA DIABÉTICA EN RATONES CD-1 Y EN RATONES CD-1 TRANSGÉNICOS RIP1/IFN-β

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La neuropatía diabética es una de las complicaciones más comunes asociadas a la *Diabetes Mellitus* tipo 1. Se caracteriza por una afectación tanto sensorial como autónoma y motora del sistema nervioso periférico. Las lesiones que se observan son una axonopatía distal y una desmielinización segmental.
Nuestro objetivo es estudiar el cuadro neuropatológico asociado a la diabetes tipo 1 en ratones CD-1 y en ratones CD-1 RIP1/IFN-β, a fin de caracterizar su potencial utilidad como modelo de estudio de la neuropatía diabética humana.
Una vez inducida la diabetes, mediante administración de estreptozotocina, se evaluaron las vías sensitivas y motoras mediante estudios electrofisiológicos. Posteriormente, se realizó el estudio histopatológico, incluyendo técnicas de inmunomarcaje, para evaluar los distintos componentes del sistema nervioso periférico. Asimismo, se procesaron los nervios ciáticos y tibial para el estudio ultraestructural, así como para el estudio de fibras nerviosas aisladas (*teased fiber*).
Se ha observado una disminución de las terminaciones nerviosas, tanto sensitivas como autónomas, a nivel de los cojinetes plantares, en ambos tipos de animales. Este cuadro neuropatológico observado en los nervios periféricos de los ratones, así como otras lesiones observadas en otros órganos, es similar al descrito en la neuropatía diabética humana.
La neuropatía diabética murina se caracteriza por una pérdida axonal distal con desmielinización focal o segmental, semejante a la descrita en la especie humana. En nuestro modelo de animales transgénicos RIP1/IFN-β hemos observado estos mismos parámetros, por lo que estos ratones pueden ser un buen modelo animal para el estudio de la neuropatía diabética humana.

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ROECKL´S GRANULOMA IN A SEIZED BOVINE IN THE SLAUGHTERHOUSE

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During the last year, our laboratory has received some pieces from the Leganes slaughterhouse for their diagnosis. These large skeletal muscle of thoracic wall and hind limbs came from an adult male bovine which was totally seized. We observed the presence of numerous nodules (0.5-1 cm diameter) rosary-like distributed, mild consistency and caseous aspect with concentric layers when cut it, with a small central cavity. They looked like typical pseudotuberculosis lesions.

With ordinary techniques we appreciated the central zone presented a caseous necrosis distributed in concentric layers and surrounded by granulation tissue and skeletal muscular cells where we were able to see parasites of the *Sarcocystis genre.* Microbiological isolation revealed absence of growth and was not able to identify any significative pathogen. Macroscopical and histological images could correspond with the denominated by Jubb and Kennedy as "Roeckl`s granuloma". They consider that they are nodular lesions of skeletal muscle, specific for cattle and associated with similar lesions in internal organs.

The lesion is also known as "nodular necrosis" and the list of suggested causes is quite varied: tuberculosis, pseudotuberculosis, sarcocystosis, blastomycosis and /or larvae of *Hypoderma bovis.*

Other authors as Saito et al. described a chronic infection by *Sarcocystis spp* in salughtered cattle from different abattoires in Japan, but in those cases nodules were smaller. Most authors coincide that an eosinophilic myositis associated to the existence of *Sarcocystis spp* is the definitive diagnosis.

Our case does not show any kind of infiltrate in muscular cells next to granulomas. We also took samples for electronic microscopy and inmunohistochemical examinations.

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"SOBRE LAS INSPECCIONES CADAVERICAS" DESCRITAS EN 1863.

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El objetivo de la presentación de este documento es concienciar a las nuevas generaciones de patólogos veterinarios, sobre el interés de los diversos conocimientos históricos de nuestra ciencia con el fin de conocer mejor el momento actual de la misma
En el código de Hammurabi, 2.000 años a. de C. reseñaron algunas lesiones en cadáveres, tal es el caso, por ejemplo, de las alteraciones producidas en la base de la cola en el curso de la, hoy denominada enfermedad melanósica del caballo tordo.
Por tanto, nuestra ciencia anatomopatológica macroscópica, la cual no estuvo sometida al descubrimiento de otros materiales, tales como lupas o microscópios, evolucionó al ritmo del hombre, entendido como "homo sapiens".
Los métodos de trabajo utilizados son semejantes a los primitivos. Han cambiado los conocimientos adquiridos por el hombre a través de los siglos y en este contexto, exponemos un texto de 1863, escrito por un veterinario, de probable origen catalán, D.Juan Mayot Malondre, en el que se otorga un enorme valor a las inspecciones cadavéricas por parte de los "*profesores veterinarios"** de la época y en el mismo, explica como debe realizarse una necropsia.

*A los licenciados en veterinaria de aquellos años, se les denominaba: profesores veterinarios

RELATION BETWEEN SEROLOGICAL LEVELS OF OESTRADIOL AND PROGESTERONE AND THEIR RECEPTORS IN THE PREIMPLANTATIONAL UTERUS IN EWES UNDER DIFFERENT HORMONAL TREATMENTS

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The steroid hormones are the prime regulators of uterine function, but it is becoming increasingly apparent that their influence varies both between uterine compartments. The physiological actions of ovarian steroid hormones are mediated in the target cells by the expression of their receptors (Carson *et al*, 2000). Oestradiol can increase the concentration of both oestradiol and progesterone receptors (Bergman *et al*, 1992). There is also evidence that progesterone can down regulate the oestradiol receptor, as oestradiol receptor concentrations measured in tissue homogenates in overiectomized ewes given a constant oestradiol infusion increased within a few hours of progesterone withdrawal (Leavitt *et al*, 1985).

In this study, 30 adult Manchega breed ewes were divided into two even groups. In one group natural estrus was induced using prostaglandins (control group) and in the other estrus was synchronized with progestins and PMSG. Animals were sacrificed on days 4 and 7 post coitum to collect embryos in oviduct and uterus. Immunohistochemical methods were used to quantify estrogen receptor alpha (ER alpha) and progesterone receptor (PR) for luminal and deep regions of the endometrium and of the myometrium. The evaluation of serological levels of oestradiol and progesterone previous mating and during the first days of pregnancy was done by RIA.

Levels of oestradiol decrease significantly in animals treated with prostaglandins, this could be due to a bad quality of the preovulatory follicles. The levels of progesterone also present a marked decrease in this group, which could be related to the precariousness of the developed corpus luteus.

Comparing the two experiences, the animals treated with progestins present a notable decrease in the expression of ER in the luminal compartment of the uterus, while the PR expression only decreases on day 4 in the endometrium. These incidents coincide with lower serological levels of both hormones detected in this group and could be related to the lower embryo viability that is observed in females treated with progestins since the maternal environment and the embryo development suffer an asynchrony (Barnes, 2000).

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IMMUNOHISTOCHEMICAL LOCALIZATION OF EPIDERMAL GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR ALPHA IN THE OVINE GENITAL TRACT DURING THE PREIMPLANTATION PERIOD

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Implantation process requires the development of an optimal enviroment and an embryo-maternal cross-talk, mediated by the coordinated actions of steroid hormones. Several growth factors, among them the Epidermal Growth Factor (EGF) family are considered as local mediators of the steroid hormone actions (Das *et al*, 1994).
This study involved immunohistochemical detection of Epidermal Growth Factor (EGF) and Transforming Growth Factor alpha (TGF-alpha), members of the EGF family, to determine their role in the ovine pre-implantation period.
In this study, 20 Manchega breed ewes, with synchronized estrus and controlled mating were sacrificed on days 4 and 7 post coitum, obtaining samples from different portions of the genital aparatus (oviduct and uterus). These samples were fixed in 4% paraformaldehide and routinely processed. The streptavidine-biotin-peroxidase method was used for immunohistochemical staining of EGF and TGF-alpha using established procedures.
Expression of EGF and TGF- alpha has been found in the different portions object of this study, with slight differences between the days they were analyzed. Nevertheless, a different immunoreaction has been observed between the two factors studied. EGF is expressed in the luminal epithelium of the oviduct and uterus with a marked positivity, that is maintained but less intensely in the luminal and deep glands of the endometrium. The lamina propria is negative, while the muscle presents low and constant levels of this growth factor. On the contrary, TGF-alpha presents a higher expression in deep portions, in the epithelium of the oviduct as well as in the uterus, in which the deep glands present the highest expression.
These results coincide with the ones obtained by Tamada *et* ***al*** **(2002) in ovine** uterus during days 14 and 20 of pregnancy. Even though the physiological significance of the higher expression of TGF-alpha in the deep gland portion is not established, these findings suggest that this growth factor is synthesized and secreted to the light, favoring the development of the embryo. The presence and distinct expression pattern of members of the EGF family indicates important and overlapping functional roles for these growth factors during the preimplantation period.

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UTERINE CELL PROLIFERATION IN THE PREIMPLANTATIONAL PERIOD IN EWES UNDER DIFFERENT HORMONAL TREATMENTS

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Ovarian steroid hormones are considered the primary regulators of early pregnancy as shown by dynamic changes in various cell types of the uterus (Flores *et al* 2001). Important events that ensure a uterine luminal environment that will support the rapid development of the conceptus must occur, such as dramatic uterine growth, extensive vascularization of endometrial tissues, and marked increases in uterine blood flow (Reynolds and Redmer, 1992). The nuclear antigen Ki-67 is one of the cell proliferation markers seen throughout the entire cell cycle (Guillomont *et al*, 1998).

The aim of this study is to determine the relatios between steroid hormones and the uterine cell proliferation during early pregnancy in sheep.

In this study, 30 adult Manchega breed ewes were divided into two even groups. In one group natural estrus was induced using prostaglandins (control group) and in the other estrus was synchronized with progestins and PMSG. Animals were sacrificed on days 4 and 7 post coitum to collect embryos in oviduct and uterus. Immunohistochemical methods were used to detect the nuclear antigen Ki-67. The evaluation of serological levels of oestradiol and progesterone before mating and during the first days of pregnancy was done with RIA.

Animals treated with progestins show an increase in cell proliferation, more abundant in luminal glands and epithelium, even though levels of oestradiol are inferior in this group (oestradiol usually has the effect of inducing cell proliferation).

These results could be due to the effects of progesterone over hormone receptors in the endometrium. Therefore, even though inicially the levels of estrogen receptors in the endometrium are lower because of the progesterone effect, a prolonged exposition to this hormone reduces the number of progesterone receptors (PR) and unblocks the estrogen receptors inducing cell proliferation (Spencer and Bazer, 1995)

The results obtained could indicate that cell proliferation in the ewe´s endometrium during de preimplantation period is related to steroid hormones but other paracrine/autocrine factors such as growth factors could also be involved.

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ECTOPIC OSSIFICATION IN NECK MUSCULATURE IN A P.R.E. HORSE

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The ectopic ossification is due to the bone production in other organs different to this, being able to correspond to a metaplasia process, in which the formed bone is directly developed from another connective tissue by re-differentiation of its mesenchymal cells. When this pathology affects to the muscle, the applied denomination is myositis ossificans.

A 4 year-old Spanish Pure Race horse, not castrated is referred to the Veterinary Hospital of the UEX showing an apparent deformation in its neck, in the right ventrolateral region, mediodorsal to the trachea and ventral to the vertebras, and also displaces laterally to the jugular.

The radiological study evidences a radiodense mass without bone changes in the vertebral bodies and a normal thoracic radiological exploration. The morphologic analysis, carried out by cytology and biopsy samples, demonstrates the absence of a malignant tumor process, what encourages to the surgical extirpation of the mass.

However and in the course of the extirpation, the euthanasia is carried out due to the size of the tumour which involves vital structures and disables its resection. The later postmortem study reveals that the process only affects to cervical musculature invading the muscles longus colli and longus capitis. The analysis of the neoplastic mass establishes the existence of a fibro-osseous process that invades and destroys the fibres of the affected muscles and shows a marked central blood clot. The musculature nearest the lesion also appears affected showing haemorrhages, inflammatory infiltrate, fibrous proliferation and changes of the muscle fibres.

EXPRESSION OF EPITHELIAL DIFFERENTIATION MARKERS IN CUTANEOUS ADNEXAE OF FOOD-PRODUCING ANIMALS

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One striking feature of terminal differentiation in mammalian epidermis is the deposition of a 20 nm thick, insoluble layer of protein on the cell's inner surface, named cornified cell envelope (CE). The dermal adnexae show a different and more complex differentiation pattern than other squamous stratified epithelia.

The main objective of this work was to evaluate, by means of immunohistochemical techniques, the expression and distribution of keratin filaments and cornified cell envelope components in the adnexae of different cutaneous regions from food-producing animals. Samples from eight cutaneous regions were collected from goat, sheep, cow and pig.

For characterization of keratins, two monoclonal antibodies (DAKO) were used. MNF116, labeling human keratins 5, 6, 8, 17 and 19 in non differentiated keratinocytes and simple epithelia and LP34, labelling human keratins 5, 6 and 18 in well-differentiated keratinocytes. The distribution of corneum envelope precursors was studied using two antibodies: monoclonal antibody anti-human involucrin (NOVOCASTRA), labelling keratinocytes of the upper *stratum spinosum* and *stratum granulosum*, and polyclonal antibody anti-human profilaggrin (ZYMED), labelling keratinocytes of the *stratum granulosum*.

With the MNF 116 antibody, the follicular epithelium was positive in all species, always in the outer root sheath and rarely in the inner root sheath. The cutaneous glands and ducts were also positive. With the LP34 antibody, was positive the outer root sheath from follicular infundibulum. The sebaceous ducts were also positive in some sheep and pig regions.

With the anti-involucrin antibody, the follicular outer root sheath was positive in most studied regions and the inner root sheath was positive in the sheep and cow. Only small ruminants species revealed positive reaction in the glandular epithelium from some cutaneous regions. The follicular outer root sheath was positive with the anti-profilagrin antibody in the infundibula from all studied species. The epithelial components of nasal and labial glands revealed an irregular reaction.

It was possible to conclude that cutaneous adnexae expresses keratins characteristics from simple epithelia with major intensity than epidermal layers and that the follicular epithelium expresses keratins characteristics from well-differentiated keratinocytes when it's differentiation and keratinisation are similar with those from epidermis.

It was also possible to conclude that the follicular outer root sheath of the studied species show involucrin and profilaggrin expression and that the inner root sheath expresses involucrin in sheep and cow. Both corneum envelope precursors were expressed in glandular components, with a variable expression between the species.

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EPITHELIAL DIFFERENTIATION MARKERS IN MUCOCUTANEOUS JUNCTIONS OF DIFFERENT FOOD--PRODUCING ANIMALS

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The distribution of keratins and the expression of cornified cell envelope proteins are well studied in epidermis and mucous membranes of human specie, laboratory species and, scarcely, in the dog. The aim of this study was to evaluate, by means of immunohistochemical techniques, the expression of these components in the nasal and labial mucocutaneous junctions from food-producing animals.
Samples from two mucocutaneous junctions were collected from goat, sheep, cow and pig, fixed in formalin 10% and embedded in paraffin.
For chacterization of keratins, two monoclonal antibodies (DAKO) were used. MNF116, labelling human keratins 5, 6, 8, 17 and 19 in non differentiated keratinocytes and simple epithelia and LP34, labelling human keratins 5, 6 and 18 in well-differentiated keratinocytes. The distribution of corneum envelope precursors was studied using two antibodies: monoclonal antibody anti-human involucrin (NOVOCASTRA), labelling keratinocytes of the upper *stratum spinosum* and *stratum granulosum*, and polyclonal antibody anti-human profilaggrin (ZYMED), labelling keratinocytes of the *stratum granulosum*.
With the MNF 116 antibody, the labial and nasal mucocutaneous junctions from goat, as well as labial from sheep and nasal from cow, revealed an intense reaction located at the *suprabasale stratum*. The labial mucocutaneous junction from pig was negative. With the LP34 antibody, were positive all *suprabasale strata* from both mucocutaneous junctions in ruminant species. In the pig, both mucous membranes were negatives.
With the anti-involucrin antibody, the nasal mucous membrane was positive in ruminants species, at the upper part of the *stratum spinosum* and in the *stratum granulosum*, but was negative in the pig. The labial mucous membrane was positive at the same layers in most species, except in the sheep. Both mucocutaneous junctions in all studied species were negative with the anti-profilagrin antibody.
It was possible to conclude that keratins were detected in nasal and labial mucous membranes from all studied species at *supra-basale* layers. In both mucous membranes from goat and cow, as well as in nasal mucous membrane from sheep and labial mucous membrane from pig, was also detected involucrin, at the upper part of the *stratum*

spinosum. Both mucocutaneous junctions from all species didn't revealed profilaggrin expression.

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DETECCIÓN DE LA PROTEÍNA TAU HIPERFOSFORILADA EN RATONES TRANSGÉNICOS (bo-PrP) CON EEB EXPERIMENTAL

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La denominación " taupatías " hacen referencia al grupo de enfermedades neurodegenerativas caracterizadas histopatológicamente, por presentar inclusiones filamentosas en neuronas (neurofilamentos) y en la neuroglía, siendo resistentes a la proteólisis. Estos neurofilamentos o filamentos helicoidales pares (PHF) son constituidos, principalmente, por formas hiperfosforiladas de la proteína tau, y de las proteínas asociadas a los microtúbulos, caracterizados ultraestructuralmente por formar dos hebras entrelazadas localizándose intracelularmente y eventualmente, en el espacio extracelular. En la actualidad, se ha señalado que la hiperfosforilación de la proteína tau forma parte de la patogenia de las enfermedades priónicas, incluyendo a la encefalopatía espongiforme bovina (EEB).

El objetivo de nuestro trabajo fue la realización de un estudio inmunohistoquímico que pusiera de manifiesto a la proteína tau hiperfosforilada en el SNC de ratones transgénicos infectados con la EEB, intentando relacionar observaciones en estudios ultraestructurales previos, a los 390 y 480 dpi en corteza cerebral la presencia encontrada de una estructura proteica fibrilar electrodensa formando grupos en disposición radial dentro del citoplasma de algunas neuronas, identificadas como alteraciones de los microtúbulos del citoesqueleto neuronal. Para ello, se utilizaron 210 ratones transgénicos (bo-PrP): 200 de ellos se inocularon intracranealmente con un macerado de cerebros de bovinos infectados con la EEB y los 10 ratones restantes se utilizaron como control. Los ratones fueron eutanasiados secuencialmente entre el día 30 y los 480 dpi. El estudio IHQ se realizó utilizando al anticuerpo policlonal anti-tau hiperfosforilada [pS214] (Biosource) y al anticuerpo anti-prión 2A11 (CISA-INIA).

La detección de la proteína tau hiperfosforilada empleando el anticuerpo anti-tau se presentó en cerebros de animales infectados, marcando al citoplasma neuronal en forma de un punteado fino granular y formando acúmulos intracitoplasmáticos pudiendo ser una alteración del citoesqueleto indicativa de degeneración neuronal durante la EEB.

Este trabajo ha sido financiado por la Consejería de Agricultura, Pesca y Alimentación de la Junta de Andalucía y por el Ministerio de Ciencia y Tecnología (EET-2002-05150).

METYRAPONE PARTIALLY RESCUE THE PHENOTYPE ON TOOTH DEVELOPMENT OF TRANSGENIC MICE OVEREXPRESSING THE GLUCOCORTICOID RECEPTOR UNDER THE CONTROL OF KERATIN K5 PROMOTER

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We have generated a transgenic mouse model in which the glucocorticoid receptor (GR) is targeted to epithelial cells by means of the keratin K5 promoter (K5-GR mice). K5-GR mice exhibit multiple defects that collectively resemble the clinical features in the human Ectodermal Dysplasia Syndrome (ED) (Perez P, et al., 2001; Cascallana et al., 2003).

We performed histopathological and Immunohistochemical studies on 18.5 dpc embryos and newborn K5-GR mice to demonstrate the effects on odontogenesis elicited by GR overexpression as well as the consequences of Dex and Metyrapone treatment on the oral phenotype.

In 18.5 dpc embryos and newborn K5-GR mice, we observed a marked delay in tooth formation as well as abnormal differentiation of the epithelial cells in tooth primordial. Altered differentiation of the teeth correlated with a strong expression of the GR transgene in the nuclei of the internal and external dental epithelial cells. In 18.5 dpc and newborn wild type mice intrauterinely exposed to Dex, a marked delay in the differentiation of internal epithelial cells and dental papilla cells were observed. In addition, when K5-GR mice were treated *in utero* with the GC synthesis inhibitor Metyrapone, we observed reversion of the tooth phenotype, as demonstrated by an almost normal differentiation and organization of ameloblasts and odontoblasts.

The reported alterations in tooth formation of transgenic embryos and newborn mice were also found in adult K5-GR mice, which showed oligodontia and/or microdontia of the molar teeth, being the absence of the third and, sometimes, second molar, the commonest situation.

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LINGUAL *CALCINOSIS CIRCUMSCRIPTA*: CASE REPORT IN A PORTUGUESE DOG BREED

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Lingual *calcinosis circumscripta* (LCC) was diagnosed in a 9-month-old "Serra da Estrela" female dog, that presented two lesions in the lateral surface of the tongue. The lesions were non-ulcerated, nodular, hard and well circumscribed, measuring approximately 1.5 x 1 x 1 cm and 2 x 2 x 1.5 cm. General physical examination, complete blood count and biochemistry panel was unremarkable. A fine needle biopsy was performed, revealing a granular basophilic material in the background, and a granulomatous inflammatory response consisting of macrophages and giant cells, some of which contained clear, crystal-like structures. We used an adaptation of Von Kossa's staining for cytology, corroborating the clinical diagnosis. Surgical biopsy was also performed, further confirming the previous observations.
To better elucidate the diagnosis of *calcinosis circumscripta* in cytology and histopathology, different staining methods were tested, based in variations of the Von Kossa and Alizarin Red S methods. In cytology, it was concluded that Von Kossa, counterstained with Methylene Blue 0.05%, provided the best results as to general contrast and easiness of interpretation. Although LCC can be diagnosed using routine Haematoxylin-Eosin, calcium deposits are usually confirmed in histopathology. We found that Alizarin Red S was faster and less laborious than Von Kossa staining. In this vein, we judged Alizarin Red S 2% (pH = 4.2), counterstained with Methylene Blue 0.05%, as the best method to demonstrate calcium at the histopathology level.
Calcinosis circumscripta has been reported in the skin, spine, aorta, salivary gland and tongue. Most of the cases have been reported in German Shepards or in other rapid growing breeds, less than two years of age. The present case is particular, as it is one of the first detailed reports in a portuguese breed. The pathogenesis of *calcinosis circumscripta* has not been established, but metastatic, dystrophic and idiopathic forms of calcification have been proposed. In this case the normal calcium levels excluded the metastatic form. Although the dystrophic form associated with local trauma is possible, the present case suggests an idiopathic form of calcification. This is so because no previous lesions were seen in the tongue, the lesions naturally regressed two months after diagnosis, and, finally, they did not recur after a seven months period of follow-up.

We thank Prof. Fátima Gärtner and Ms. Célia Lopes for providing some sections for staining assays.

IMMUNOHISTOLOGICAL STUDY OF CYTOKINES EXPRESSION ON PARAFFIN-EMBEDDED TISSUES FROM CONVENTIONAL CALVES

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The aim of our study was to report on immunohistochemical techniques developed for the detection of proinflamatory cytokines (TNFα, IL-1α and IL-6) in fixed and paraffin-embedded calve tissues from five healthy animals of 4-6 months old.
Samples of spleen, thymus, retropharyngeal and mesenteric lymph nodes, tonsil, ileum, lung, liver and kidney were fixed in 10% buffered formalin solution (24 hours) and Bouin´s solution (8 hours) for structural and immunohistochemical studies. The avidin-biotin peroxidase (ABC) method in combination with different antigen unmasking techniques was used for the identification of cells expressing cytokines.
Bouin´s solution was the best fixative for immunohistochemical detection of these chemical mediators, and the permeabilisation with Tween 20 the most suitable unmasking antigen method. Monocytes-macrophages (m-M∅) were identified as the main cytokine-producing cells, although other cell types (mainly neutrophils) also stained positively. TNFα and IL-1α expression was located in the medulla and the mantle zone of follicles of lymph nodes, in the splenic red pulp, cortex and medulla of thymus and in the lamina propria of ileum. Occasionally, intraepithelial immunopositive-m-M∅ were observed in the crypts of Lieberkühn, as well as in lymphoid follicles of spleen and Peyer's patches of ileum. A few IL-1α stained-cells appeared in the diffuse lymphoid tissue of tonsil, while occasional presence of intravascular and interstitial m-M∅ immunolabelled against TNFα was observed in lung. On the other hand, lymphocytes and m-M∅ were the main IL-6 producing cells, as well as some fibroblasts and endothelial cells. Focal presence of IL-6 positive cells was observed in the interfolicular area of lymph node cortex and thymic cortex, appearing occasionally immunostained cells in the splenic white pulp, tonsil and gut-associated lymphoid tissue. Additionally, some pulmonary m-M∅ were stained against IL-6. Specific immunoreaction against the studied cytokines was not observed in the rest of organs and tissues.

This work was supported financially by grants from MCYT (AGL 2003-252)

DOS CASOS DE TORSIÓN PULMONAR CANINA, UNO ASOCIADO A MOQUILLO Y OTRO DE ETIOLOGÍA INDETERMINADA

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Áreas de fibrosis. Se describen dos casos de torsión pulmonar canina, que fueron remitidos a la Sala de Necropsias del Hospital Clínico Veterinario de la Facultad de Veterinaria de Madrid.

Caso 1: Rottweiler, hembra, 4 años, epiléptica. Antes de la muerte presenta vómitos y dificultad respiratoria, muere en 24 horas.

Caso 2: Pequinés, 2 meses, macho. Antecedentes de cuadro neumónico de 6 días de evolución.

En ambos casos se realizó la necropsia completa, ordenada y sistemática. Las lesiones más destacadas se encontraron en la cavidad torácica:

Caso 1: hemotórax y torsión del lóbulo pulmonar caudal izquierdo con infartación hemorrágica. El resto del pulmón presentaba atelectasia y enfisema. No se tomaron muestras para estudio histopatológico debido al avanzado estado de autolisis.

Caso 2: presencia de líquido serohemorrágico y rotación sobre su eje del lóbulo pulmonar craneal izquierdo con infartación hemorrágica. Lóbulo craneal derecho consolidado y de coloración grisácea. Se tomaron muestras en formol al 10% para estudio histopatológico e inmunohistoquímico (anti-virus moquillo canino) que se procesaron siguiendo el protocolo de rutina. Se obtuvieron los siguientes resultados histopatológicos:

- Lóbulo craneal izquierdo: congestión vascular intensa, hemorragias masivas y necrosis (infartación hemorrágica).
- Lóbulo craneal derecho: neumonía bronquiolo –intersticial.
    - Presencia de PMNN en luces bronquiolares y metaplasia escamosa ocasionalmente.
    - Extensas zonas de reepitelización.
    - Presencia de células gigantes, a menudo multinucleadas.
    - Macrófagos en luces alveolares.

- Ganglios mediastínicos: marcada depleción linfoide.
- Timo: marcada atrofia y depleción linfoide.

Las lesiones descritas en el pulmón derecho y en ganglios mediastínicos y timo son características de un cuadro de moquillo canino.

En este caso opinamos que la torsión del lóbulo craneal izquierdo puede ser consecuencia de la efusión pleural y de las zonas de consolidación neumónicas. Este proceso patológico es poco frecuente en la especie canina por lo que existen escasas referencias bibliográficas.

APOPTOSIS DETECTION IN PARAFFIN EMBEDDED LYMPHOID ORGANS FROM HEALTHY PIGS BY TUNEL AND CLEAVED CASPASE-3 IMMUNOHISTOCHEMISTRY

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Apoptosis is a programmed form of cell death involved in morphogenesis and homeostasis of organs and tissues (Rathmell et al., 2002). The frequency and the distribution of apoptotic cells were investigated in formalin-fixed paraffin-embedded lymphoid tissues from healthy conventional pigs at four different ages (6 days, 2 months, 3.5 months and 5 months). Samples of tonsil, mesenteric lymph node, spleen, thymus and Peyer's patches were histologically processed and apoptosis evaluated with the TUNEL reaction and cleaved caspase-3 immunohistochemistry. In each technique, quantification of positive labelling was done for each particular lymphoid tissue area. The labelling pattern and distribution were similar for TUNEL and cleaved caspase-3. TUNEL stained mainly apoptotic bodies inside macrophages, but signal was also seen in free apoptotic bodies and in the nuclei of lymphocyte-like cells. The anti-cleaved caspase-3 antibody labelled mainly nuclei of lymphocyte-like cells. All tissues presented a similar distribution pattern of apoptosis, except for the 6 day-old group. In this group, a scattered distribution of positive cells was detected in tonsil, lymph node and spleen. In the tonsil and mesenteric lymph nodes from the older pigs, follicular areas presented higher amounts of positive cells than interfollicular areas. Moreover, the splenic white pulp showed more positive reaction than the red pulp, especially when they included germinal centres. In all groups, the follicular areas of ileal Peyer's patches presented more labelled cells than the dome and the lamina propria. In the thymus, the higher apoptotic rates were found in the cortex. In general, TUNEL yielded higher rates of positive cells than cleaved caspase-3 immunolabelling. A good correlation between the two techniques was found for thymus, tonsil and mesenteric lymph node, but not for Peyer's patches and spleen. This study describes a detailed histochemical characterization of apoptosis in pig lymphoid tissues using TUNEL and a cleaved caspase-3 immunolabelling at different ages. Moreover, our results indicate that TUNEL and cleaved caspase-3 techniques can be equivalent only when tissues have a high or low levels of apoptosis, since a considerable discrepancy was found in intermediate situations. Data from this study should be useful for future comparative studies under disease conditions.

STUDIES IN FETAL AND POSTNATAL CANINE MERKEL CELLS: APPEARANCE, DENSITY, AND DISTRIBUTION

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Merkel cells (MCs) are supposed to originate from the neural crest. They migrate to their final epithelial location in fetal and postnatal stages. The density and distribution of MCs in canine fetal and postnatal skin and mucosa were studied using an immunolabeling technique on skin and mucosa sections. Samples from different corporal areas were obtained from fetuses and newborn animals for histology and immunohistochemical (avidin-biotin immunoperoxidase technique) studies. MCs were identified and immunohistochemically characterized by the known cytokeratin markers CK8, CK18 and CK20 and by neuronal and endocrine markers.

Changes in shape and size of individual MCs were evident in the different stages of canine development. In fetal stages, MCs have an oval-long shape, small size, and they are only distinguishable of the other epithelial cells by immunohistochemistry. Postnatal and final fetal canine MCs are larger, clear in appearance, round to oval shaped with typical lobulated clear nucleus, and they can even be distinguished with H&E. Number, location and distribution of canine MCs in the anatomical locations studied were also different, and orientation and location within the epithelium differed between fetal and postnatal MCs. These results are in concordance with observed in human fetal and postnatal skin. gramirez@becarios.ulpgc.es

FOLÍCULOS COM MÚLTIPLOS OÓCITOS NA CADELA

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Está referido na literatura a existência de folículos com múltiplos oócitos (multioocyte follicles – MOFs) em murganhos, como consequência da exposição neonatal destes animais à genisteína, uma substância com acção estrogénica.
A avaliação sistémica de ovários obtidos através das ovário-histeriectomias em cadelas revelou que, em alguns animais, se encontram alguns folículos contendo múltiplos oócitos, em número que pode variar de 2 a 5 por folículo.
O significado deste achado não está esclarecido. A possível associação à ingestão de fito-estrogénios ou de outras substâncias com estas características não é clara: alguns animais estão sujeitos a uma dieta comercial, enquanto outros a dieta caseira, variando também as suas idades.
Neste trabalho as autoras avaliam os aspectos morfológicos e histoquímicos deste achado.

ESTUDIO DE LA DISTRIBUCIÓN DE PROTEÍNA ICA RESISTENTE EN LA CABEZA DE VACAS DIAGNOSTICADAS DE EEB EN CATALUNYA

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En el marco del plan de Vigilancia de las Eets en catalunya, el laboratorio PRIOCAT, centro de referencia en EETs animales de catalunya se desplaza, cada vez que um caso de EEB le es referido, al matadero dónd dicha res ha sido sacrificada con el fin de obtener el mayor número de muestras posible. En general, únicamente la cabeza del animal puede ser inspeccionado.

Las muestras que se obtienen del cráneo son: el encéfalo entero (que se diseca en 16 áreas significativas), ganglios trigéminos, hipófisis, tonsilas, ojos, nervio óptico, linfonodos parotídeos, parte craneal del esófago, glándula salivar, músculo esquelético (masetero normalmente)...

Una mitad de las muestras se fija en formol para su estudio immunohistoquimico mientras que la otra es congelada para su estudio bioquimico. En todas estas muestras se ha realizado el test de Western Blotting (Prionics®) para la detección de proteína priónica. Se han introducido pequeñas modificaciones en el protocolo para adaptarlo a tejidos más duros que el nervioso.

Con los resultados de dicho estudio se han clasificado los casos EEB en función de una hipótesis de evolución del prión a través del encéfalo; en los casos más tempranos sólo de detectar dicha proteína en todo el encéfalo e incluso en estructuras periféricas al encéfalo cómo la hipófisis o los ganglios trigéminos. La PrPres se iría expandiendo des de la medula oblonga por el tronco encefálico primero, subiendo a estructuras cerebelares y en el cuerpo estriado luego, siguiendo por el rinencéfalo y, finalmente, ascendiendo al neocortex. Estos resultados se respaldan con un examen immunohistoquímico.

Este estudio ha sido posible gracias al programa de Vigilancia y erradicación de las EET llevado a cabo por los Departamentos de Sanidad y Seguridad Social (DSSS) y Agricultura Ganaderia y Pesca (CARP) de la Generalitat de Catalunya.

PERFIL MOLECULAR Y COMPARACIÓN DE LOS CASOS DE ENCEFALOPATÍA ESPONGIFORME TRANSMISIBLE NATURALES DIAGNOSTICADOS EN CATALUNYA; ESTUDIO DE LA UNIÓN DE LOS ANTICUERPOS MONOCLONALES 6H4 Y P4 AL PRIÓN DE ENCEFALOPATÍA ESPONGIFORME BOVINA Y SCRAPIE.

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El perfil molecular de la proteína priónica se viene aplicando últimamente para la caracterización de las cepas de Encefalopatías Espongiformes Transmisibles. La posibilidad de diferenciar entre una infección de EEB o scrapie en la oveja, hasta el momento sólo a nivel experimental (Stack el al. 2002, Thuring el al, 2004), o la descripción en Francia de casos atipicos de EEB (Biacabe et al. 2003) son ejemplos muy interessantes de esta aplicación.

Hemos comparado 26 casos de EEB y 2 de scrapie diagnosticados en nuestro laboratorio mediante la técnica del Western Blotting El perfil molecular se ha obtenido en base a:

- Comparación del patrón de glicosilación (es decir, de la proporción relativa de les tres glicoformas de PrPres en cada caso valorada por densitometria).
- Peso molecular de la forma de prión resistente a la digestión com proteinasa K (se ha realizado una deglicosilación com PGNasa para facilitar el reconocimiento de diferencias en el peso molecular).
- La unión de las formas celular y resistente de la PrP a los anticuerpos monoclonales P4 y 6H4.

La información sobre el perfil molecular está respaldada por el estudio histopatológico e immunohistoquímico realizado en todo el encéfalo de dichos animales.

Analizando los resultados obtenidos durante la caracterización de nuestros casos naturales de EET junto con los datos publicados en la literatura discutimos, en este póster la especificidad de la unión del anticuerpo monoclonal P4 el prión de scrapie (versus al de la EEB). Aunque creemos que la utilización del Anticuerpo P4 será de gran

utilidad para diferenciar entre fuentes de infección en casos naturales de EET ovina, algunos resultados deberían ser revisados cuidadosamente.

Además se discute la hipótesis de que un mismo caso se pudieran encontar simultáneamente (y pudieran ser diferenciados) ambos agentes.

Este estudio ha sido posible gracias al Programa de Vigilancia y erradicación de las EET llevado a cabo por los Departamentos de Sanidad y Seguridad Social (DSSS) y Agricultura Ganadería y Pesca (DARP) de la Generalitat de Catalunya.

ESTUDO DE LAS VÍAS OLFATORIAS Y ESTRUCTURAS RELACIONADAS EN 19 CASOS NATURALES DE ENCEFALOPATÍA ESPONGIFORME BOVINA

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Estudios de distribuición de la proteína priónica celular (PrPc) realizados en hámster (N.Salés et al 2002) han demonstrado la presencia de gran cantidad de PrPc en los nervios olfatorios en comparación a otras áreas de sustancia blanca, esto es debido al hecho que dichos axones crecen de forma continua a lo largo de la vida del animal. Recientemente Zanusso G et al. (2003) HAN DESCRITO LA PRESENCIA DE PROTEÍNA PRIÓNICA RESISTENTE (pRpRES) Creutzfelt Jakob (ECJ). Ambos autores hipotetizan sobrela implicación del las vías olfatorias en la patogénia de las EETs.

Cada vez que un caso de Encefalopatía Espongiforme Bovina (EEB) se diagnostica en catalunya, nuestro laboratório (PRIOCAT, Fundación CReSA) confirma el diagnóstico y recoge muestras del cráneo (incluyendo el encéfalo entero y otras muestras del cráneo). De 19 casos de EEB detectados en el programa de vigilancia activa de la EEB se ha realizadp un estudio bioquimico (Western blotting) e immunohistoquinico dela distribuición de la PrPres.

El estudio bioquimico indica que el mayor acumulo de PrPres se encuentra, n todos los casos en el tronco encefálico, sin embargo, en algunos casos otras estructuras se ven también afectadas. En aproximadamente la mitad de los animales estudiados (10/19) se detectó PrPress en los bulbos olfatorios, en todos estos casos también se podía detectar en el neocortex así cómo en el paleocortex (lóbulo piriforme) y el archicortés (hipocampo). En los casos en que el bulbo olfatorio era negativo (9/19) los diferentes lóbulos corticales eren, generalmente, negativos (8/9).

No se pudo detectar la presencia de PrPres en la mucosa olfatoria en ningún caso, ni mediante Western Blotting ni mediante immunohistoquímica.

En este póster se describen los resultados obtenidos en ambas técnicas y se discute el papel de las estructuras relacionadas con las vías olfatorias (mucosa olfatoriq, bulbos y

pedúnculos olfatorios, lóbulo prirforme, hipocampo y corteza frontal9 en la patogénia de la EEB.

Este estudio ha sido posible gracias al Programa de Vigilancia y erradicación de las EET llevado a cabo por los Departamentos de Sanidad y Seguridad Social (DSSS) y Agricultura Ganadería y Pesca (DARP) de la Generalitat de Catalunya.

GASTRIC EXTRAMEDULLARY PLASMACYTOMA ASSOCIATED WITH AMYLOID DEPOSITION IN DOG

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gastric extramedullary plasmacytomas are extremely rara tumors in dogs. Digestive plasmacytomas have been described affecting oral cavity, and less frequently colon and rectum. A 9-year-old male, Chihuahua dog was wxamined because a 2-month history of sporadic, bloody vomiting. On laparotomy examination, a 6,5x4,5 cm in diameter mass was observed affecting most of the anatomy of the stomach. Partial gastrectomy was performed and samples of the lesion was fixed in formalin and remitted for histopathological study. Histologically, the tumor, which was located in the gastric submucosa, had variable number of well-differentiated plasma cells and few multinucleated giant cells. Immunohistochemically, alll the neoplastic cells reacted for lambda-light chains of immunoglobulins. The neoplastic growth was admixed with abundante amyliod. Amyloid appears as large aggregates scattered in the stroma within and close to the tumor. Varying numbers of macrophages and foreign body type giant cells were associated with amyloid deposit. Amyloid deposition has been found in a small proportion of extramedullary plasmacytomas and its presence does not correlated with the behavior. Extramedullary plasmocytomas usually exhibit good behavior with no recidives nor metastases after surgical remove; however the small number of gastric plasmacytomas reported in the veterinary literature does not permit to assess this insight The dog in our case was euthanized 2 days after the surgery due to poor and deteriorating body condition, and although the necropsy was not permitted by the owner, the animal did not show regional lymphoadenomegaly or changes indicative of metastases on laparatomy examination.

DINÁMICA DE PROLIFERACIÓN DE CÉLULAS RODLET Y MUCOSAS EN EL TUBO DIGESTIVO DE RODABALLOS INFECTADOS EXPERIMENTALMENTE CON *Enteromyxum scophthalmi*

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Las células rodlet han sido descritas especialmente asociadsa a los epitelios de distintos órganos de peces teleósteos tanto de agua dulce como marinos y en algunos anfíbios. Si bien existen actualmente discusiones respecto a la función que desarrolla este tipo celular existen indicios de su participación en los mecanismos de defensa frente a patógenos. Por otra parte, se ha comprobado que las secreciones de las células mucosas contienen sustancias capaces de inhibir la colonización o invasión potencial de microorganismos infecciosos incluyendo parásitos metazoarios. Por ello, nos propusimos como objectivo del presente trabajo valorar la proliferación de células rodlet y mucosas en rodaballos (*Scophthalmus maximus*) juveniles infectados experimentalmente con *Enteromyxum scophthalmi*.

Utilizamos 20 ejemplares distribuidos en dos grupos: uno control (n=109 y otro de peces retados (n=10). Los animales fueron sacrificados a los 49 dias postinfección del grupo retado. Se extrajeron muestras de estómago, ciegos pilóricos e intestino anterior, medio y posterior que fueron fijadas en formol calcio al 10% e incuidas en parafina. Se realizaron cortes de 1um de espesor que fueron teñidos com azul de toluidina. Para cada animal y en cada región del tubo digestivo muestreado se fotografiaron al azar 10 campos de 75660 um2, en los cuales se determinó el número total de células rodlet y células mucosas. Posteriormente se evaluó si existían diferencias significativas ($p< 0.05$) en el número de células entre grupo control y retado para cada una de las regiones del tubo digestivo mediante el test de Mann Whitney.

Nuestros hallazgos demostraron diferencias significativas entre grupos en el número de células rodlet y células mucosas en ciegos pulóricos, intestino anterior y posterior, siendo siempre mayores en peces infectados. Paralelamente se observó la presencia de estadios de desarrollo de Enteromyxum scophthalmi en el epitelio de revestimento del intestino de estos rodaballos por lo que nuestros resultados podrían indicar la implicación de esos tipos celulares en la respuesta tisular frente al parásito.

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NEOPLASIAS DO OVÁRIO EM CADELAS – ASPECTOS IMUNOHISTOQUÍMICOS

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A apreciação rotineira do tracto genital feminino excisado por ovário-histeriectomia, permitiu aos autores comprovar a existência de vários casos de neoplasias do ovário, não diagnosticadas clinicamente, e que passam por vezes tabém despercebidas ao exame macroscópico.

Na maior parte dos casos, a ausência de sinais clínicos reduz a probabilidade de se tratar de uma neoplasia com capacidade hormonal significativa.

As neoplasias do ovário apreciadas pelos autores dispersam-se por várias categorias: epiteliais (adenoma papilar, cistoadenoma e adenocarcinoma), estromais (edenoma da *rete ovarii*) e germinativos (tumores de células da granulosa).

Neste trabalho discute-se a aplicabilidade de alguns marcadores de imuno-histoquímica na caracterização e diagnóstico destas neoplasias.

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