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CUTTING EDGE

PATHOLOGY

Abstracts



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Keynote lectures

KN1: CUTTING THROUGH TO THE TRUTH: LASER CAPTURE MICRODISSECTION AND TOXIC INJURY OF THE BILIARY TREE

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Laser capture microdissection allows an analysis of selected cell populations in complex organs composed of many cell types. Whole organ gene expression analysis often creates a “Tower of Babel” environment in which the genes expressed by multiple cell types creates a matrix of confusion that prevents a clear understanding of gene expression (or other variables) in a selected cell population. To better understand gene expression changes in biliary epithelium, in situ acute toxic responses of rat biliary epithelium to a model biliary toxicant, 1-naphthylisothiocyanate administered orally, were evaluated by microarray analysis of laser capture-microdissected bile duct epithelium or hepatic parenchyma at 2 and 6 hours post-dose, prior to any histologic changes. Distinct gene expression patterns between biliary epithelium and hepatic parenchyma were noted at the 2 hr post-dose time point where 375 genes, particularly those involved in endoplasmic stress, were altered in biliary epithelium but only 38 genes were altered in hepatic parenchyma. By 6 hours post dose, 620 genes were altered in biliary epithelium, but only 32 genes were altered in hepatic parenchyma. Expression of genes involved in endoplasmic stress had decreased compared with the 2 hour time point, while expression of genes involved in protein degradation such as proteasome-ubiquination pathways, and cell death pathways had increased. At this same time point, hepatic parenchymal gene expression changed little. A unique approach allowed evaluation of bile duct epithelium in its normal microenvironment revealed specific biliary epithelial gene expression changes within 6 hours of oral exposure indicative of a vigorous endoplasmic stress response and a subsequent activation of protein destruction and of cell death pathways, in contrast to minor changes in the hepatic parenchyma. At 24 hours post dose the majority of biliary epithelial cells were necrotic and unsuitable for evaluation. By 48 hours post dose gene expression changes were primarily related to replication of damaged ducts in the biliary tree and the hepatic parenchyma had increased gene expression, likely in response to cholestasis.

Reference

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Keynote lectures

KN2: COGNITIVE ERRORS IN VETERINARY DIAGNOSTIC PATHOLOGY

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Recent studies in human medicine show that ~ 80% of the errors made by doctors are caused by a cascade of cognitive errors, not ignorance of the clinical facts. As many as 15% of all diagnoses in human medicine are inaccurate. Because of the nature of what veterinary pathologists do, we are “immune” to some of these errors. 1. We are separated from our “patients” 2. We have little emotional involvement 3. We neither like or dislike them. 4. We do not interact with or even elicit a history for the owners. 5. We usually have little or no clinical data. The errors veterinary pathologists make are related to Perception and Analysis of gross and microscopic visual patterns. Good research on errors in human radiology is highly relevant to veterinary pathologists reading biopsies. Currently the average diagnostic error rate in interpreting medical images is in the 20-30% range. No studies have been done in veterinary pathology but there is every reason to believe the error rate is far higher than any of us want to admit. The practice of veterinary pathology has two components which are liable to error; **Perception** – we make an observation.; **Cognition** – we analyze what we see, what it may mean and the possible explanations for it.

These processes are repeated over and over. It is an intuitive assessment based on visual data that does not always occur in a linear, step by step combination of clues. Yet that is how we teach our students to approach diagnosis. Pattern recognition is a very “soft” subconscious thing that psychologists call “**Gestalt**”. It is affected by the innate variability of the image or pathologic process as well as technical aspects of the slide. It is also impacted by our mental and physical state, our emotions and fatigue.

Pathologic lesions or processes have a range of expression. Residents and graduate students learn the “**Classic**” or “**Prototype**” appearance and then spend the rest of their lives learning the variation around the classic. With time, they become comfortable with the fuller range of expression. The patterns of different entities may overlap at the margins of their expression and this is where experience pays dividends in sorting out the diagnosis. This is also where the variability in diagnosis among pathologists originates and is the area where errors in cognitive thinking originate as we sort through the list of differential diagnoses and try to settle on one or another diagnosis. It is the area of data collection that reinforces our bias for competing overlapping diagnostic entities. The information found here by one pathologist may stimulate a diagnosis of “histiocytoma” and another pathologist to say histiocytic sarcoma”

Pattern Recognition is real and important and often right. It’s the mark of an experienced pathologist that becomes refined over the years of practice aided by remembering when you were wrong. Doctors (including pathologists) achieve competence by recognizing their mistakes and incorporating them into their memory. The problem in veterinary surgical pathology is that we get relatively little feedback about our diagnoses. Pathologists working in academic veterinary medical centers have more opportunity for this than those in the commercial or government diagnostic labs. Labs with a single or small cadre of pathologists are insular and may have insufficient diversity of opinion necessary to keep pathologists thinking about their diagnoses. The pathology community needs to solve this problem and promote more clinician-pathologist interaction. Pattern recognition while extremely useful can also be dangerous. Research shows that most medical judgment is made within seconds after perception. Experts form an opinion on average in 20 seconds. The more seasoned and experienced you are the greater is the temptation to rely on “Gestalt” alone. Cogent pathologic evaluation combines the 1st impression in pattern recognition with deliberate analysis.

Keynote lectures

KN2: COGNITIVE ERRORS IN VETERINARY DIAGNOSTIC PATHOLOGY

ANCHORING: One of the dangers of “Gestalt”. The observer does not consider the multiple possibilities but *quickly and firmly latches on to his or her “First Impression”* and ignores discrepancies that would argue to reject it. We see only the landmarks we want to see and so become “anchored” in our opinion.

CONFIRMATION BIAS: The *tendency to search for or interpret new information in a way that confirms or reinforces your diagnosis* and avoid or ignore information that contradicts or would lead you away from prior belief. “*Cognitive Cherry Picking*”. Usually follows “anchoring” in a “Gestalt” diagnosis.

SEARCH SATISFACTION: The natural cognitive tendency *to stop thinking when we make a major finding*. The detection of one finding interferes with that of others. This is a well known error among human radiologists and is a major factor in false negatives. (We test this on the ACVP and ECVF exam w/slides containing multiple diagnoses).

FALSE NEGATIVES: Our minds favor the perception of “positive” data over “negative”. We are more likely to see lesions that are present than lesions that result in the absence of something. Especially if the lesion is diffuse. The Paradox of Anatomic Pathology = “Sometimes the most extensive, widespread or diffuse lesion is the easiest to overlook because there is no normal for comparison”.

FRAMING: *Focusing on what is wrong* and the cause of the problem. Often improper or lack of framing leads to errors in thinking. Mostly for surgical pathologists the clinician or surgeon “Frames” the case. No or **inadequate framing is a serious problem for veterinary surgical pathologists**. It is likely that concern about improper framing or leading the pathologist astray is what motivates some clinicians to say, “*Don’t tell the pathologist anything, you will bias him/her*”. The reality is that without some clinical clues, perception and cognition are significantly hampered. We may be able to decrease errors in surgical pathology by at least some framing of the case by clinicians. This is one of the values of working with clinicians and providing a proper submission form that indicates what information is needed or desired.

AVAILABILITY: This is the tendency *to judge the likelihood of an event (diagnosis) by the ease with which relevant and recent examples come to mind*. We teach our students to make the “most likely diagnosis” given the image or facts. Indeed, we test for this on the certification examinations also. “**When you hear hoof beats, think horses not zebras**” Is a good rule most of the time because common things occur commonly. But if you get “anchored” to the idea, you will miss some unusual diagnoses.

ZEBRA RETREAT: *This is the shying away from a rare diagnosis*. Powerful forces discourage “zebra hunting”. Often “zebra hunters” are considered to be “show boats” or arrogant. To verify the occurrence of a Zebra diagnosis can cost money and time and cost containment issues blunt this activity. Mostly the lack of experience with the rare diagnosis fosters a lack of confidence so the diagnosis is not pursued aggressively.

DIAGNOSIS MOMENTUM: *A ripple effect through a group of pathologists*. A pathologist makes an initial diagnosis that is accepted by peers and subordinates without challenge. Subsequent opinions agree and soon the diagnosis is universally agreed upon. This occurs especially when the first opinion is made by an expert or senior experienced pathologist. I have seen this many times in seminars when a senior resident gives a diagnosis and all of the other residents follow suit even when the first diagnosis is wrong. Soon the diagnosis gains enough force to crush all other opinions.

Keynote lectures

KN2: THE MANAGEMENT OF COGNITIVE ERRORS

1. **Be aware of the cognitive traps** Other experienced pathologists have similar mechanisms. “*Man up!*” You make errors. We all do. Managing your cognitive errors begins with “accepting your story”

Slow the perception and analysis process. Time opens the mind. However, *time is the most precious commodity in medicine*. None of us has the luxury of making diagnoses with unlimited time. Most difficult to do in surgical biopsy and on the certification examination. Consultation with colleagues when possible. Set the case aside and come back to it later. Often you see the lesions with a more open mind.

2. **Deconstruct the pattern recognition image** mentally or in writing just as we teach our students to do. Use your Pattern Recognition skill (*Gestalt*), its valuable and often correct but check it with a cogent analysis of all the facts if possible (“*Corroborative testimony*”). I always ask myself before I commit to a diagnosis “*Does it all add up*” or “*What else could this be*”?

3. “**Describe uncertainty**” because it forces you to slow down and evaluate the separate parts of the “*Gestalt Image*”. But employ a style that fits the purpose of the task. Most critical in biopsy. Control fatigue by work flow management and an efficient style that suits the biopsy reports purpose. “*The amount written is inversely proportional to the certainty of the diagnosis.*” It’s different for everybody.

4. **Make a mental list of DDx’s** and work from that. Again, the ACVP and ECVP certification examinations test this skill for a good reason.

5. **Use the Total Patient Evaluation Concept.** Properly framed cases often provide a lead or information that may set off a DDx list or even stimulate a thought or idea that you were not considering. Valuable but in some tasks either purposely denied, as on the certification examinations, or omitted by clinicians for whom you are working. Always interpret framing cautiously because if not accurate, it can lead you astray. “*To examine for yourself*”

Keynote lectures

KN₃: OF MICE AND MEN: AN ANATOMICAL, FUNCTIONAL AND MOLECULAR IMAGING PERSPECTIVE

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Along with approaches like immunologic assays, genomics and proteomics, imaging is part of the biomarker concept, which plays an increasing role in biomedical and in pharmacological research. A biomarker is a characteristic that is objectively measured and evaluated as an indicator of a normal biological process, a pathological process, or a response to a therapeutic intervention. A biomarker strategy aims to improve early decision making on compound safety and efficacy, by providing knowledge that validates a therapeutic concept, endorses a candidate molecule and facilitates dose selection (1,2). The time to bring new medicines to patients can thus be potentially reduced. Also, availability of early indicators may avoid large numbers of subjects being exposed to experimental compounds with little or no therapeutic potential. What differentiates imaging biomarkers from e.g. analytes in blood serum and urine, used for decades in medicine and in drug development, or more recently proposed proteomics biomarkers, is the fact that imaging readouts tend to be much more closely related to the disease phenotype. Thus, the use of imaging biomarkers facilitates direct associations between therapy and effect.

The non-invasive character of imaging enables the evaluation of treatment efficacy over extended periods of time, allowing analysis of morphological and physiological changes with respect to a pretreatment reference state. Intra-individual variability is thereby reduced and thus statistical significance may be obtained with smaller groups. Imaging can also be applied for stratification of treatment groups: Prior to therapy administration, individuals can be classified into 'homogenous' treatment groups which should translate into data with improved statistical relevance. In the case of animal studies involving imaging, depending on the protocol, the number of subjects can be reduced by up to 90% as compared to conventional, invasive readouts.

An additional important advantage of imaging is the fact that the tissue is analyzed in its host environment. Possible artifacts generated during tissue collection, fixation or processing are thereby largely reduced. Tissue collection is invariably linked to a period of global ischemia for a specimen, which affects the levels of energy metabolites. Similarly, histological processing may lead to morphological distortions that can affect morphometric measurements.

A final main argument in favor of using imaging methods to characterize animal models of human diseases is that they facilitate the translation between preclinical and clinical activities (3). Once potential biomarkers are identified and validated, similar study designs can be applied to preclinical and clinical studies. Moreover, investigations in animals can serve as basis to rationalize experimental findings in humans using analogous biomedical readouts.

Imaging modalities such as micro-computed tomography (micro-CT), micro-positron emission tomography (micro-PET), high-resolution magnetic resonance imaging (MRI), and optical imaging have become invaluable tools in preclinical pharmaceutical research (4-6). They can be used to non-invasively investigate, under in vivo conditions, rodent biology and metabolism, disease models, and pharmacokinetics and pharmacodynamics of compounds. These imaging modalities have their counterpart in clinical centers, where they are adopted as diagnosis and/or research tools in patients. We aim to illustrate how these techniques can be used to study anatomical, functional and molecular changes associated to pathological processes in animal models of disease and in humans, and thereby provide support to pharmacological research.

Keynote lectures

KN₃: OF MICE AND MEN: AN ANATOMICAL, FUNCTIONAL AND MOLECULAR IMAGING PERSPECTIVE

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Keynote lectures

KN4: OVINE RETROVIRUSES... IN-BETWEEN PATHOLOGY AND PHYSIOLOGY

Journal of Comparative Pathology Plenary Lecture 2011

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The domestic sheep and ovine betaretroviruses provide a fascinating model for studying the co-evolution between retroviruses and their host.

The ovine betaretroviruses include a group of exogenously (i.e. horizontally) and endogenously (i.e. vertically) transmitted retroviruses with very similar genetic characteristics but dramatically different biological properties. Jaagsiekte sheep retrovirus (JSRV) is an exogenous retrovirus and the causative agent of a contagious lung cancer in sheep known as ovine pulmonary adenocarcinoma (OPA). JSRV is a unique oncogenic retrovirus. It is the only virus known to induce a naturally occurring lung cancer and the only virus with a structural protein functioning as a dominant oncoprotein. Thus, productive virus infection and cell transformation are mutually dependent in OPA and this creates an “evolutionary dilemma” as abundant viral replication is entirely dependent on tumor development in the host. We have recently shown that JSRV and its host have reached an evolutionary equilibrium in which productive infection (and transformation) can occur only in cells that are scarce for most of the lifespan of the sheep.

Interestingly, the sheep genome harbours at least 27 copies of endogenous betaretroviruses (enJSRVs), highly related to the exogenous and pathogenic JSRV. enJSRVs have been integrating into the genome of their host throughout the evolution of the Caprinae and can be considered in “symbiosis” with their host. enJSRVs have become essential for the reproductive biology of sheep and interfere with the replication cycle of related exogenous retroviruses.

This lecture will provide insights on the interplay between retroviruses and their host during evolution. Particular emphasis will be placed on the cells originating OPA and the role of inflammation to the respiratory epithelium in lung adenocarcinoma development. The data accumulated in the JSRV/OPA model in the last few years have a broad significance in pulmonary biology, carcinogenesis and retroviral pathogenesis.

Keynote lectures

KN5: DISSECTING THE EVIDENCE: THE FORENSIC PATHOLOGIST'S ROLE

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The purpose of conducting a forensic examination is: (a) to discover and record any injury, disease or abnormality and (b) to interpret these findings in a manner that allows a Court of Law to understand the causes and significance of any changes.

Forensic veterinary pathology is a wide and extremely varied discipline covering, for example:

- animal welfare concerns such as neglect and non-accidental injury
- violations of regulations related to transportation of livestock
- wildlife offences including poaching, killing of protected species and out-of-season shooting
- Insurance claims for unexpected mortalities, disease incidents and disputes over veterinary procedures.

The multiplicity of species which is presented to veterinarians is a complicating factor and it is important that any veterinarian conducting a forensic examination should be knowledgeable and experienced in the type of animal presented for examination.

The forensic pathologist is not required to 'prove' anything but has the deep responsibility to ensure that the court understands what has happened to the animal. Great reliance is placed on the forensic pathologist's knowledge to provide information and answers that are unavailable through any other means. However, herein lies a danger because there are many gaps in forensic veterinary knowledge and veterinarians must constantly be aware of their limitations.

An intimate relationship exists between diagnostic and forensic pathology. In my view, when undertaking a forensic necropsy, it is a great advantage to have a thorough background in diagnostic work. Nevertheless, the interpretation of the necropsy findings in forensic cases can, on some occasions, be challenging. Consequently, in the course of this lecture we will consider the interpretation of post-mortem findings in cases of non-accidental injury, neglect and firearms injuries in companion animals, livestock and wildlife.



Oral presentations ESVP/ECVP

Session A – Infectious diseases

MYCOBACTERIAL DISEASE IN BRITISH CATS

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Introduction: Since the introduction of pasteurisation, mycobacterial infections have largely been considered of little importance in cats. This paper describes a survey of British cats with suspect mycobacteriosis.

Materials and Methods: Between 2004-2008, 339 samples from cats with suspect mycobacterial infection were investigated. Detailed semi-quantitative histopathology (acid fast bacilli (AFB), histiocyte, multinucleated cells and neutrophil infiltration; necrosis; mineralisation) of 45 samples from these cats infected with different mycobacteria was carried out.

Results: *M microti* was detected in 19% of cases, *M bovis* in 15% of cases, *M avium* in 7% of cases, other mycobacteria in 6% of cases and no growth observed in 53% cases. In all 45 cases, histopathology revealed multifocal to coalescent, histiocytic inflammation. At least one AFB was detected in all sections, but very difficult to find in 47% cats. Neutrophil infiltration was present in 71% of cats, necrosis in 66%, multinucleated cells in 7% and mineralisation in 2% independent of the aetiological agent.

Discussion and Conclusion: Significant levels of mycobacterial infections are present in British cats. Typical field cases show granulomatous or pyogranulomatous lesions with AFB and no multinucleated cells independent of the aetiological agent involved. Currently, culture remains the only reliable method of identifying the *Mycobacterium sp.*



Oral presentations ESVP/ECVP

PATHOGENESIS OF *CLOSTRIDIUM PERFRINGENS* INFECTIONS: THE POULTRY CASE

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Introduction: Necrotic enteritis is an economically important disease of broilers worldwide. Here, the relation between necrosis inducing capacity of *C. Perfringens* strains on the one hand, and strain origin and bacteriocin production on the other hand, was studied.

Materials and methods: *C. perfringens* strains were isolated from diseased and healthy broilers as well as from cattle. The necrosis inducing capacity was tested in an *in vivo* model and bacteriocin production was studied *in vitro*.

Results: *C. perfringens* strains isolated from clinical cases generally could induce necrosis in the chicken intestine *in vivo*, whereas strains from healthy broilers or from cattle could not. Strains inducing necrosis carried the *netB* virulence gene. Moreover, they were more capable of inhibiting growth of other *C. perfringens* strains in a "spot-the-lawn" test *in vitro*. Using a disc diffusion test, these inhibitory factors were shown to be secreted. One of the inhibitory factors was purified and partly characterized. Amino acid sequence analysis and comparison with genome sequence data showed that this was a C-terminal 11.5kDa fragment of a 22.9kDa protein unrelated to any proteins known to date.

Conclusion: Only specific *C. perfringens* strains expressing specific virulence factors can cause necrotic enteritis in broilers.



Oral presentations ESVP/ECVP

PATHOGENESIS OF *CLOSTRIDIUM PERFRINGENS* TYPE C ENTERITIS: THE PORCINE CASE

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Introduction: Necrotizing enteritis caused by *C. perfringens* type C is a fatal disease of animals and humans. Beta-toxin (CPB) is the essential virulence factor, however its cellular and molecular mode of action is still unknown.

Materials and methods: The effects of purified CPB, *C. perfringens* type C culture supernatants and strains on porcine small intestinal mucosa were studied using different in vitro and in vivo approaches. Investigations ranged from immunohistochemical and ultrastructural to cell biological and biochemical investigations.

Results: *C. perfringens* type C strains rapidly induced necrohemorrhagic lesions in ligated neonatal porcine small intestinal loops. CPB was highly toxic to primary porcine endothelial cells, inducing rapid programmed necrosis in these cells. Moreover, it was able to bind to endothelial cells in small intestinal mucosal explants. However, porcine small intestinal epithelial cells were not affected by the toxin. Nevertheless, culture supernatants were able to induce morphological damage to intestinal epithelial cells.

Conclusion: Endothelial cells are the primary target of CPB. The epithelial damage, required for penetration of the toxin into the tissue is induced by additional virulence factors. Identification of such factors will be important to understand the pathogenesis of *C. perfringens* enteric diseases.



Oral presentations ESVP/ECVP

WADDLIA, PARACHLAMYDIA AND CHLAMYDIACEAE IN BOVINE ABORTION

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Introduction: The etiology remains unknown in many cases of bovine abortion in Switzerland and worldwide. Bacteria of the *Chlamydiales* order are known abortive agents, therefore cases of bovine abortion from three representative regions of Switzerland were investigated in this study.

Materials and Methods: Placenta samples (n=343) were tested for *Chlamydiaceae* and for the *Chlamydia*-like organisms *Waddlia* and *Parachlamydia* by different PCR-methods, immunohistochemistry (IHC) and serology for *Chlamydia (C.) abortus*.

Results: In 67.3% of the 343 cases a necrotizing and/or purulent placentitis was found histologically. By real-time PCR, 0.9% (3/343) of the cases were positive for *Waddlia*, 13.4% (46/343) positive for *Parachlamydia* and 14.6% (50/343) positive or questionable positive for *Chlamydiaceae*. Of these samples, confirmation by IHC was possible in 2/3 cases for *Waddlia*, 25/46 for *Parachlamydia* and 4/50 for *Chlamydiaceae*. Of the 50 cases positive or questionably positive for *Chlamydiaceae*, species-identification by ArrayTube Microarray or 16S rRNA PCR resulted in 41 cases positive for *C. abortus*, whereas the presence of *C. suis* was confirmed in four and *C. pecorum* in one case.

Discussion: This study brought evidence of the importance of different members of *Chlamydiales* in different regions of Switzerland although *Waddlia* is not occurring in a high prevalence.



Oral presentations ESVP/ECVP

HISTOLOGICAL FINDINGS IN BOVINE SKIN AFTER NATURAL INFECTION WITH *BESNOITIA BESNOITI*

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Introduction: *Besnoitia besnoiti*, a cyst-forming coccidian, is the cause of bovine besnoitiosis. Little is known about life cycle and transmission of the parasite. During an infection trial clinical and pathological changes after natural infection with *B. besnoiti* were monitored.

Material & Methods: Five Simmental heifers and one Simmental bull were held on pasture with three Limousin cows chronically infected with besnoitiosis. All animals were examined daily and blood and skin samples were taken at regular intervals. During the trial two additional Limousin cows, suspected to be suffering from acute besnoitiosis were added to the group of trial animals.

Histological sections were stained with H&E and Giemsa. Immunohistochemistry for parasite detection was performed using a polyclonal rabbit antiserum.

Results: Infection with *B. besnoiti* was confirmed by serology and PCR in the two limousin cows added during the trial and three of the Simmental heifers. Parasites were detected in routine stains and *via* immunohistochemistry. Cyst formation from young to mature stages and host immune response to the parasite were monitored.

Conclusion: *B. besnoiti* can be transmitted naturally in our climatic region. Infection can be confirmed by PCR, serology or histology.



Oral presentations ESVP/ECVP

HISTOPATHOLOGICAL FINDINGS IN ANIMALS AFFECTED BY BOVINE BESNOITIOSIS, WITH SPECIAL FOCUS ON MALE GENITAL ORGANS

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Introduction: Bovine Besnoitiosis, caused by *Besnoitia besnoiti*, is an emerging disease in the EU. Although some work has mentioned the presence of orchitis and sterility in bulls suffering from Besnoitiosis, there are no studies describing the testicular lesions.

Materials and Methods: Seventeen animals from the same herd (10 cows and 7 bulls) with skin lesions (scleroderma, hyperkeratosis and multifocal alopecia) were slaughtered for sanitary reasons. Several tissues, including skin, mucose membranes and sexual organs were studied histopathologically, using haematoxylin/eosin and Masson's trichrome stains, and immunohistochemistry (IHC) for factor VIII and Vimentin.

Results: Multifocal, chronic, granulomatous and eosinophilic inflammation, mainly associated with intracellular protozoan cysts, was observed in several tissues. Skin, nasal and oral mucosa, tongue, eye, eyelid, conjunctiva and male genital organs were the most frequently affected ones. In the male genital organs, the lesions were observed within epididymis and/or pampiniform plexus of 3 bulls, but not in the testicular parenchyma. One of the bulls had multifocal and extensive areas of necrosis and mineralization within testicular parenchyma, together with lack of spermatozoa in one epididymis and few in the other one. Often cysts were associated to vascular structures. Immunohistochemistry against the cells containing the cyst was negative for Factor VIII and positive for Vimentin. Masson's trichrome stain marked the cells containing the cysts in red.

Discussion and Conclusions: Present results suggest that cysts of *B. besnoiti* may have a special tropism for vascular walls of pampiniform plexus and epididymis. Used stains and IHC indicated that cysts are allocated within muscular cells of tunica media from the vascular wall, causing compression and vascular stenosis. This situation may cause subsequent ischemia, testicular necrosis, azospermia and infertility.



Oral presentations ESVP/ECVP

Session B Experimental pathology

THE PERILS OF MOUSE PATHOLOGY

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With the advent of genetic engineering, numerous lines of genetically engineered mice (GEM) have been created and used in medical research. Many of these mice develop novel non-neoplastic lesions and tumours in various tissues. These novel lesions, first described in such mice are often observed for the first time, present difficulties in exact morphologic diagnosis and may be lesions without known or proven biological activity. For example, proliferative lesions of the GI tract can be cystic and "invasive" but not possess morphologic and biologic characteristics of neoplasia as it is normally known. Carcinomas may be diagnosed based on morphology alone with little evidence of invasion much less metastases. Apparent lymphomas may be shown to lack characteristics of clonality and reported "leukemias" may be unusual splenic reactive lesions. Normal tissues of mice have been diagnosed as skin teratomas and carcinomas. Often, molecular and biological assays (transplantation) are not performed that may give clues as to their true diagnoses. The diagnosis and terminology for such lesions are often determined by investigators that are not pathologists or lack experience in mouse pathology. Yet, many journals appear to publish manuscripts which include pathology findings, have no pathologist as a co-author, and were not reviewed by pathologists. Examples of several cases will be given.



Oral presentations ESVP/ECVP

ERCC1 DEFICIENT MICE SHOW SEGMENTAL PROGERIA

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Introduction: The *Ercc1* protein is involved in three genome maintenance systems: nucleotide excision repair, interstrand cross-link repair and double-strand break repair.

Materials & Methods: *ERCC1*^{-1/m} mice, having a single copy of a gene coding for a truncated *Ercc1* protein, were generated in a hybrid *c57bl/6**FVB background and necropsies were performed at scheduled time points and end-of-life. Histologic changes were scored semi-quantitatively and score distributions were compared.

Results: *Ercc1* hypomorphic mice are smaller and short-lived (median: 20 wks) compared to wildtype. Histologically, a number of changes that are also observed in aged wildtype mice occur in *ERCC1* deficient mice at an accelerated rate, but with variable distribution among liver, kidney, central nervous system, skeleton and hematopoietic tissues.

Conclusion: We conclude that the *ERCC1* deficient mice show features of segmental progeria.



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UROKINASE-TYPE PLASMINOGEN ACTIVATOR DEFICIENCY PROMOTES COLITIS-ASSOCIATED CARCINOGENESIS IN MICE

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Introduction: Urokinase-type plasminogen activator (uPA) participates in cancer-related biological processes, such as wound healing and inflammation. The present study aimed to investigate the effect of uPA deficiency on the outcome of dextran sodium sulfate (DSS)-induced colitis in mice.

Materials and Methods: *uPA*-deficient (*uPA*^{-/-}) and wild-type (*wt*) Balb/c mice were treated with DSS or remained untreated. Mice were necropsied either 1 week or 7 months after DSS treatment. Colon samples were analyzed by histopathology, immunohistochemistry, ELISA and real-time PCR.

Results: One week post DSS treatment there were typical DSS-colitis lesions in both *wt* and *uPA*^{-/-} mice. The affected colon of *uPA*^{-/-} mice, however, had significantly lower levels of active Tgf- β 1 compared to that of *wt* mice. Importantly, at 7 months, with no colitis evident, half of the *uPA*^{-/-} mice had colon cancer whereas *wt* mice did not.

Discussion and Conclusions: uPA catalyzes the conversion of plasminogen to plasmin, which in turn activates extracellular latent Tgf- β 1. Tumor suppressor roles of Tgf- β 1 are well-established both in humans and mice. The low levels of active Tgf- β 1 due to uPA deficiency may explain inflammatory-induced carcinogenesis in *uPA*^{-/-} mice.



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PEMPHIGUS VULGARIS – DISRUPTED NICHE ADHESION IN STEM CELL ACTIVATION AND REPAIR

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Introduction: The stem cell (SC) niche is the microenvironment that regulates SC homeostasis. To address the role of intercellular niche adhesion, we used a mouse model for the autoimmune blistering disease pemphigus vulgaris. In this model and in the majority of human patients, the desmosomal cadherin desmoglein 3 (Dsg3) is targeted by antibodies, and intercellular adhesion is altered. The hair follicle bulge is the best characterized stem cell niche in skin and is targeted by Dsg3 autoantibodies.

Material and Methods: Eight-week-old C57Bl/6J mice received Dsg3 function disrupting antibodies (AK23) to weaken intercellular adhesion in the telogen bulge. Consequences for SC homeostasis and functionality were monitored by clonal growth assays *in vitro*, label retention studies using K5-tTA;tetO-H2BGFP mice, RNA and protein analyses of SC markers and *in vivo* skin reconstitution assays.

Results: AK23 induced SC activation and proliferation involving PI3K/Akt and β -catenin. The growth potential, an important stem cell characteristic was transiently decreased and the expression of SC markers involved in SC quiescence and maintenance was reduced. Additionally, signaling pathways involved in hair growth were inhibited. In spite of that, SC did not lose their ability to reconstitute skin in the long-term.

Conclusion: Dsg3-mediated SC adhesion is important for niche homeostasis. Therefore, pemphigus vulgaris serves as a disease model to also study human SC dynamics and repair.



Oral presentations ESVP/ECVP

BIODISTRIBUTION OF RADIOACTIVELY LABELED NANOPARTICLES IN THE MOUSE

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Introduction: Fluorochromatic labeling of organic nanoparticles is often used to investigate their biodistribution in animal models. However, this technique is descriptive in nature and does not allow for signal quantification. Furthermore, fluorochromatic labeling changes chemical and physical properties and is therefore thought to change the distribution characteristics of nanoscaled particles. We therefore hypothesized that radioactive labeling facilitates a quantifiable determination of the biodistribution of nanoparticles.

Material and Methods: 47 NMRI-mice received ³⁵S-labeled, 7 ± 1,5 nm sized dendritic Polyglycerol Sulfate (dPGS) or unlabeled dPGS intravenously or subcutaneously. Radioactivity from tissues at different times up to 21 days was analyzed by surface-counts, autoradiography, liquidscintillation, imager-survey and histological photoemulsion.

Results: Radioactivity measurements allowed for a tissue- and time-dependent quantification of the labeled dPGS. Radioactively-labeled dPGS were still quantifiable in liver and spleen after 21 days following intravenous injection. Subcutaneous application resulted in a similar but delayed distribution kinetic.

Discussion: The biodistribution of dPGS was quantitatively determined by all methods used for radioactivity-testing. This approach should provide an innovative, sensitive and adaptable method to detect nanoparticles without changing their biorelevant properties.



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EFFICIENT CENTRAL NERVOUS SYSTEM TRANSDUCTION BY INTRACISTERNAL AAV₁₀ GENE TRANSFER IN RATS

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Introduction: Adeno-Associated Virus (AAV) vectors are promising tools for clinical gene therapy. The current approach to treat neurodegenerative diseases is direct intracerebral delivery of AAV vectors, which is invasive and requires multiple injections. In order to investigate broader central nervous system (CNS) gene transfer methods we initiated a strategy based on intracisternal AAV injection (into the cerebrospinal fluid).

Material and methods: Two groups of rats, neonates and adults, received a single intracisternal administration of AAV₁₀ encoding enhanced Green Fluorescent Protein (EGFP). Four weeks later brains, spinal cords, and peripheral organs were collected to investigate 1/ the cellular and tissular pattern of EGFP expression by laser confocal microscopy, 2/ the eventual histologic lesions by photonic microscopy, and 3/ the biodistribution profile of the AAV₁₀ by qPCR.

Results: AAV₁₀ has a strong tropism for CNS in neonates and adults. Neurons are mainly targeted: EGFP is expressed in pyramidal and subcortical neurons, Purkinje cells, motor neurons from cervical to lumbosacral spinal cord, and sensory neurons in dorsal root ganglia.

Conclusion: We show that intracisternal gene therapy is a minimally invasive, efficient, widespread, safe, and promising approach for the treatment of neurodegenerative diseases.



Oral presentations ESVP/ECVP

Session C – Experimental pathology

NEUROMODULATION AFTER NERVE GRAFTING INTO THE SPINAL CORD IN RATS

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Introduction: Pharmacological neuro-protective and neuro-modulating effects become crucial for restorative surgery in spinal cord (SC) injuries.

Methods: Sural nerve grafting into the SC with co-adaption to the nerve of the abdominal muscle was performed in 30 adult rats. Group I was used as control; the groups II and III received Cerebrolysin[®] or NaCl solution. After three months the rats were monitored for muscle re-innervation and the grafted nerve (GN) was injected with fast blue. The histology and fluorescence microscopy were made on SC and GN. The immunochemistry for transporters into the motor endplates (MEP) was used.

Results: Re-innervation and partial switch from cholinergic to glutamatergic transmission was confirmed. The Cerebrolysin[®] group showed enhanced number of oligodendroglia, and reduced number of astrocytes and macrophages, better preservation of neurons, reduced fibrosis and better axonal regeneration. Fast blue positive neurons were demonstrated in ventral horns of SC.

Discussion and Conclusion: Re-activation of glutamatergic transporters in the MEP after nerve grafting in SC was described by BRUNELLI et al in 2005. We demonstrated that spinal neurons can re-innervate the GN and that Cerebrolysin[®] has neuroprotective effects.



Oral presentations ESVP/ECVP

TOXICO-PATHOLOGICAL EFFECTS ON CHICKS HATCHED FROM EMBRYOS ADMINISTERED WITH OCHRATOXIGENIC FUNGAL EXTRACTS

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Introduction: Ochratoxin A is a cause of chick embryo mortality and pathological lesions in visceral organs of chicks hatched from chicken embryos.

Material and Methods: A total of 7 ochratoxigenic isolates were randomly selected for determination of embryo toxicity potential. Two doses of extracts containing 100 and 1000 ng/egg OTA (20 µl) were separately injected through chorioallantoic membrane into a 12 embryos (96 hours old) of White Leghorn layer breeder hens.

Results&Discussion: Lowest mortality and highest hatchability was observed in chick embryos kept as controls. Embryonic mortality varied from 16.67 to 83.33 percent by 100 & 1000 ng/egg OTA. Grossly liver and kidneys were swollen and hemorrhages were present as compared with control. Microscopically vacuoles were present in cytoplasm of hepatocytes varying from one to multiple in number in each cell. These vacuoles upon staining with Sudan black B dye appeared blue black confirming fatty change. In kidneys necrotic and pyknotic nuclei in tubular epithelial cells of kidneys were consistently present throughout the parenchyma of kidneys in all chicks. In histological scoring of liver and kidney tissues in chicks only individual cell necrosis was significantly higher in OTA-1000 as compared with OTA-100. In kidneys congestion was significantly higher in OTA-1000 than that in OTA-100 group. Tubular necrosis and glomerulopathy were also significantly higher in OTA-1000 as compared with OTA-100 group. There was an increased thickness of inter-follicular connective tissue. Apoptotic bodies increased in the medulla of follicles and follicles appeared smaller in size compared with control chicks.

Conclusion: Histopathological lesions scoring of liver, kidneys and bursa of Fabricius suggested as dose related increase in severity of the pathological alteration.



Oral presentations ESVP/ECVP

IMMUNE PHENOTYPING OF BOVINE PLACENTAS FOLLOWING EXPERIMENTAL INOCULATION WITH *NEOSPORA CANINUM* AT LATE GESTATION

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Introduction: *Neospora caninum* (Nc) is a major cause of bovine abortion but its pathogenesis is not completely understood. Nc infection stimulates cell-mediated immune mechanisms, which may cause placental damage leading to abortion. The aim of this work was to study the distribution of *Neospora* antigen and characterise placental immune response following infection at day 210 of gestation.

Materials and Methods: Cows were culled at 14, 28, 42 and 56 days post inoculation (dpi). Placentomes were examined by immunohistochemistry using antibodies against *Neospora*, macrophages (CD68), T-cells (CD3⁺, CD4⁺, CD8⁺, $\gamma\delta$ TCR), natural killer (NK) cells (CD335) and B cells (CD79).

Results: *Neospora* was detected at 28, 42 and 56 dpi. Macrophages were labelled mainly at 14 dpi. Inflammation was generally mild and mainly characterised by CD3⁺, CD4⁺ and $\gamma\delta$ TCR labelled cells; whereas CD8⁺ cells were less numerous. Few NK cells were only observed in infected animals.

Discussion: Compared with previous studies at earlier stages of gestation, *Neospora* was less widely disseminated in the placenta but the immune cellular recruitment patterns were similar. However, cellular infiltrates were less severe than previously seen. This may explain the milder clinical outcome observed when animals are infected late in gestation.



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COMPARATIVE EVALUATION OF WOUND HEALING EFFICACY OF LEAF EXTRACT OF *HIPPOPHAE RHAMNOIDES L.* AND *HIPPOPHAE SALICIFOLIA* IN SPRAGUE DAWLEY RATS

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Introduction: This study compares the healing efficacy of lyophilized leaf extract of sea buckthorn species, *Hippophae rhamnoides* (HR) and *Hippophae salicifolia* (HS) native to India, in full-thickness cutaneous excision wound in rats.

Materials and Methods: The study used open wound healing model as described by Gal, et al. (2008). Most effective concentration of the extract was found to be 1% (w/w). Sprague dawley (SD) male rats were divided in four groups and each topically treated with propylene glycol, Providine iodine (PI), leaf extract of HR and HS, applied once daily for 14 days. The wound contraction was measured every two days. Six rats from each group were sacrificed on day 2, day 6 and day 14 for semi-quantitative histological & histochemical assessment.

Results and Discussion: HR and HS treated rats showed faster wound contraction up to day 6 and day 8 respectively. Both HR & HS treatments showed anti-inflammatory effect, early and faster angiogenesis, fibroblast proliferation and collagen synthesis until day 6. The treatment with HS revealed much faster wound contraction, epithelial and fibroblast proliferation in comparison to HR. However, both the HR & HS treatments appeared to have delayed fibroblast maturation as observed at day 14.

Conclusion: HS treatment was the best for wound healing when compared to HR and PI.



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Session D – Experimental and tumor pathology

INFLUENCE OF SURFACE COOLING ON CEREBRAL CORTEX LESIONS FOLLOWING EXPERIMENTAL CARDIAC ARREST IN A PIG MODEL

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Introduction: Neurological sequelae are common after cardiac arrest and resuscitation. Mild therapeutic hypothermia is known to be neuroprotective, but the ideal technique to induce and maintain hypothermia has yet to be established.

Materials and Methods: Sixteen pigs underwent artificial cardiac arrest for 10 min followed by 8 min of conventional life support. After successful defibrillation the animals were randomized into two groups (hypothermia, normothermia). The hypothermia group was cooled to 33.0°C for 14 h by surface cooling. At day 9 of the experiment the animals were killed and the brains perfused with formalin. Paraffin-embedded coronary sections were stained with H&E. Frontal, parietal, temporal, occipital and insular cortex was examined regarding type and extent of lesions using a semi-quantitative scoring system.

Results: Between the hypothermia and normothermia groups statistically highly significant differences ($p < 0.001$) were found in frontal, parietal, temporal and occipital cortex and statistically significant differences ($p < 0.05$) in the insular cortex. All animals showed oedema and eosinophilic neuronal necrosis, but to a lesser extent in the hypothermia group. Malacia was found in six out of eight normothermic animals but not in any hypothermic animal.

Conclusions: Surface cooling led to significantly less brain damage after cardiac arrest in this pig model.



Oral presentations ESVP/ECVP

CHOLERA-TOXIN SUPPRESSES CARCINOGENESIS IN A MOUSE MODEL OF INFLAMMATION-DRIVEN SPORADIC COLON CANCER

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Introduction: Dextran sodium sulphate (DSS)-induced colitis is required for the promotion of azoxymethane (AOM)-initiated neoplasia in a mouse model of colorectal cancer (CRC). In the present study, this mouse model of CRC was used to investigate whether the modulation of intestinal immune response by cholera-toxin (CT) has an effect on AOM/DSS carcinogenesis.

Materials and Methods: Balb/c mice were treated with various permutations of AOM, DSS and CT or remained untreated. Mice were necropsied at 3.5 months after the DSS or DSS/CT treatments. Colon samples were analyzed by histopathology, immunohistochemistry and real-time PCR.

Results: At 3.5 months post AOM/DSS treatment colonic polyps were formed but there was no evidence of residual colitis. At this late time-point, inflammatory cells and the expression of cytokines such as IL-6, TNF- α and IL-10 in the colon mucosa were at a baseline level in both CT-pretreated and non-pretreated mice. Nonetheless, CT pretreatment reduced the formation of AOM/DSS colonic polyps by 6-fold.

Discussion/Conclusion: Orally administered CT protected mice from CRC by modulating tumor-promoting inflammatory events. Current studies in our laboratory aim to reveal critical aspects of this modulation.



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TUMOUR CELL PHENOTYPES DIVERGE IN EXPERIMENTALLY INDUCED OVINE PULMONARY ADENOCARCINOMA WHEN COMPARED WITH NATURAL DISEASE.

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Introduction: Ovine pulmonary adenocarcinoma (OPA) is a naturally occurring lung cancer in sheep caused by jaagsiekte sheep retrovirus (JSRV). Dramatic differences have been found in the incubation times in experimental OPA when compared with natural OPA. An immunophenotypical study comparing tumour lesions from natural OPA with experimentally induced OPA lesions was carried out.

Materials and Method: Routine immunohistochemical procedures using antisera against surfactant proteins (A, B, C, D) (SPs), Clara cell protein (CCP), Dendritic cell lysosome-associated protein (DC-LAMP), Ki67 and E-Cadherin were carried out.

Results and Discussion: Variations of the proportion of SPs were only significant ($p < 0.017$) when natural tumours were compared with experimental OPA. SPC and CCP positive cells were found in alveolar and bronchiolar tumours. DC-LAMP numbers of positive cells were significant higher in the natural OPA groups when compared with experimental OPA ($p < 0.026$) and also increased steadily from early to advanced OPA lesions. E-Cadherin positive cells were found in elevated numbers in all tumours being only with reduced expression in the group of early atypical OPA. Ki67 indexes were high in experimental OPA, early OPA and metastases but these differences were not significant. The significance of these results in the pathogenesis of the OPA disease is discussed.

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PROGNOSTIC FACTORS IN CANINE PERIVASCULAR WALL TUMORS

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Introduction: Canine soft tissue sarcomas (STS) constitute 15% of subcutaneous tumors. In canine oncology STS are grouped as a single clinicopathological entity regardless of the specific histotype. The aim of this study was to compare the role of several prognostic variables in canine perivascular wall tumors (c-PWT) as a distinct group of STS.

Materials and Methods: We evaluated tumor site and size, margins, depth of lesions, type of growth, necrosis, mitoses, MIB-1 based proliferation index (PI), grade and follow up in 56 c-PWT. Association among variables and their prognostic role were investigated statistically.

Results: Tumor size was significantly associated with relapse. Cases with clean margins did not recur. Depth was associated with relapse but had a low prediction capability. The association among tumor site, margins, depth, and type of growth identified pathological profiles associated with relapse. Grade, mitosis, PI and necrosis did not correlate with relapses.

Discussion: The lack of association between grade and relapses in c-PWT differs from STS as a group. C-PWT relapses are associated with size and specific pathological profiles suggesting that their distinction from STS is necessary for c-PWT prognosis. Evaluation of other STS types independently is recommended for the definition of prognostic parameters.



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Session E – Tumor pathology

IMMUNOHISTOCHEMICAL AND GENETIC INVESTIGATIONS ON CANINE MAST CELL TUMOURS

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Introduction: Different methods are used for characterization and prognosis in canine mast cell tumours (MCT).

Material and Methods: Solitary MCT from 265 dogs and multiple MCT from 30 dogs were investigated histologically, immunohistochemically (*c-Kit*, n=124; Ki-67, n=80) and genetically (exons 8, 9, 11, 12, n=65).

Results: Solitary MCT were larger than multiple MCT (P=0.001).

Solitary MCT were histologically grade I (56%), II (37%) and III (7%). In cases of multiple MCT all neoplasms on a dog were grade I (40%), II (27%), III (n=5, 17%). In 5/30 cases MCT were grade I & II on the same animal.

Multiple MCT with grade I expressed *c-Kit* pattern 1 in 88.2% of the cases (solitary MCT 54.3%, P=0.016), but multiple MCT grade III expressed *c-Kit* pattern 3 in 81.8% of cases (solitary MCT 27%, P=0.005). Numbers of Ki-67 antigen positive cells were increased (>23 positive cells/1 cm²) in about 50% of the cases in both groups.

Genetic analyses revealed a duplication in exon 11 of the *c-Kit* gene in only one dog with a solitary MCT grade III with *c-Kit* pattern 2 and increased proliferation activity in an 8 years old Retriever.

Conclusion: There are several differences between solitary and multiple mast cell tumours. Mutations of the *c-Kit*-gene were not of diagnostic relevance in our material. Thus prognosis and therapeutic approaches using tyrosinkinase-inhibitors have to be discussed critically.

Oral presentations ESVP/ECVP

KIT RECEPTOR DYSREGULATIONS IN FELINE MAST CELL TUMOURS AND SYSTEMIC MASTOCYTOSIS

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Introduction: Feline mast cell tumours (FeMCTs), overall accounting for 1-9% of feline neoplasms, are characterized by a highly variable biologic behaviour. Frequent post-surgical recurrence, *de novo* development of multiple tumours and concurrent visceral and cutaneous involvement justify uncertainty in differentiating benign from malignant forms, with tendency to systemic spread.

Materials and Methods: A series of FeMCTs with variable clinical presentation were examined by histology (cell morphology, differentiation, growth pattern, mitotic activity), CD117 immunohistochemistry and c-kit mutation analysis (exons 8, 9, 11). Obtained data were correlated with clinical records (clinical signs, TNM stage, haematological abnormalities, 2-year follow-up) to assess their prognostic significance.

Results: 20 cats with 10 solitary cutaneous, 5 multiple cutaneous, and 5 systemic MCTs were included; 9 were still alive at the end of the follow-up period. Overall, 29 tumour samples were examined. There were 21 well-differentiated, 3 pleomorphic and 5 atypical FeMCTs; mean mitotic activity was 9/10 HPFs. Low-to-high CD117 expression was observed in 18 cases. Further C-kit mutations, beside those previously described in FeMCT, were found.

Conclusions: This study investigates the effects of KIT dysregulations on FeMCT biologic behaviour, also potentially allowing the identification of those patients that may benefit from molecular targeted therapies.



Oral presentations ESVP/ECVP

THE INTERLEUKIN-2-RECEPTOR IS EXPRESSED IN CANINE MAST CELL TUMOURS

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Introduction: Previous studies have shown that mutations of the *c-kit*-receptor occur in less than 15 % of canine mast cell tumours (MCT). We have therefore speculated that other molecular mechanisms of mast cell tumour development and malignancy must be involved. To elucidate the potential impact of the pro-proliferative interleukin-2-receptor (IL-2R) signalling, expression levels of IL-2R subunits (CD25, CD122, CD132) as well as interleukin-2 (IL-2) were analyzed in canine neoplastic and non-neoplastic mast cells.

Materials and Methods: mRNA expression levels of all three IL-2R subunits and IL-2 were compared between patnaik grade 1 and grade 3 MCT by quantitative real-time RT-PCR. In addition, protein expression levels of CD25, CD122 and IL-2 were analyzed immunohistochemically and by immunofluorescence in neoplastic and non-neoplastic canine mast cells.

Results: Grade 3 MCT had increased IL-2R mRNA expression whereas protein expression was higher in grade 1 MCT. Interestingly, IL-2 mRNA expression was decreased in grade 3 MCT. Remarkably, non-neoplastic cutaneous mast cells do not express IL-2R.

Discussion and Conclusion: In contrast to non-neoplastic mast cells canine MCT show an atypical immunophenotype by expressing both the IL-2R and its ligand IL-2. IL-2R signalling may therefore be pro-proliferative not only for lymphocytes but also for canine MCT. Furthermore, CD25 may serve as a potential tumour marker that discriminates between well differentiated neoplastic versus non-neoplastic mast cells.



Oral presentations ESVP/ECVP

MOLECULAR MECHANISMS OF TYROSINE-KINASE INHIBITION IN CANINE MAST CELL TUMORS

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Introduction: Tyrosine-kinase inhibitors (TKIs) have recently been introduced for the treatment of canine mast cell tumors. The clinical effect of these compounds has been described precisely. However, the downstream mechanisms of the inhibited tyrosine kinases, mainly the KIT receptor, are unknown in dogs.

Material and Methods: Cell proliferation, metabolic activity, mRNA- and protein expression were analyzed by WST assay, microarray and 2D-DIGE and mass spectrometry in a canine mast tumor cell line (C2) after 12, 24 and 72 hours of treatment with the TKI masitinib.

Results: Treatment with masitinib significantly reduced the metabolic activity and cell division. Transcriptome analysis identified a changed expression of up to 3500 genes including genes associated with cell proliferation, metabolism, and apoptosis. Genes associated with 15 pro-proliferative molecular pathways were up-regulated after 72 hours. Proteome analysis identified 24 proteins with different expression levels after TKI treatment.

Discussion and Conclusion: Masitinib treatment of neoplastic canine mast cells leads to significant changes of the global gene expression. These effects include increased expression of several genes which may constitute alternative pro-proliferative signaling pathways and may be potential targets for a combination therapy with masitinib.



Oral presentations ESVP/ECVP

ROLE OF β -CATENIN IN CANINE OSTEOSARCOMA

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Introduction: The role of Wnt/ β -catenin signaling pathway in the pathogenesis of osteosarcoma (OS) is not yet completely known, as well as the prognostic significance of β -catenin expression. Recently, the Wnt/ β -catenin signaling pathway, known to be essential for proper osteoblast's maturation, has been suggested to be inactivated during OS induction and progression.

Materials and Methods: Immunohistochemical expression of β -catenin was investigated in canine OS samples, using a semi-quantitative method to analyse results. Its expression was correlated to histological grading, survivin and p53 expression, and follow-up data. β -catenin was also evaluated by immunofluorescence in canine OS cell cultures.

Results: Nuclear β -catenin immunolabelling was detected in osteoblasts surrounding normal bone trabeculae. In all cases cytoplasmic and/or membranous immunostaining were observed, while the highest number of nuclear positive cells was found in fibroblastic OS and among spindle cells of mixed OS. Nuclear expression was rarely observed among OS cells lines. Nuclear survivin and p53 positive cells were found in all cases.

Conclusion: Nuclear β -catenin immunolabelling, a hallmark of Wnt pathway activation, in normal osteoblasts and the absent/low expression in most of the OS, suggested that this pathway is not activated in canine OS. Furthermore, statistically significant correlation has been found between nuclear β -catenin immunolabelling and a longer survival time.



Oral presentations ESVP/ECVP

THE HEAT SHOCK PROTEIN 90 INHIBITOR 17-AAG INDUCES ANTIPROLIFERATIVE AND APOPTOTIC EFFECTS IN CANINE OSTEOSARCOMA CELL LINES

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Introduction: Canine and human osteosarcomas (OSA) are highly resistant to chemotherapy and novel therapeutic approaches involve specific HSP90 inhibitors, such as 17-allylamino-17-demethoxygeldanamycin (17-AAG), that interacts with client proteins regulating signalling transduction pathways, which has been successfully used in clinical trials against different tumours.

Materials and methods: Canine OSA cell lines and normal osteoblasts were treated with different concentrations of 17-AAG and cell proliferation/survival, and invasion, apoptosis (Annexin V, caspase 3 activation), mitochondrial depolarization/mass and known HSP90 client proteins (p53, survivin), β -catenin/Wnt pathway, HSP27, HSP72/73 and Hsp90 expression were evaluated by immunofluorescence, WB, RT-PCR, ultrastructure, flow cytometry.

Results: 17-AAG treatment promoted loss of cell viability, cell growth inhibition (with an increase in G1 and a decrease in S-phase of the cell cycle) strong induction of apoptosis, as well autophagy to a lesser extent (ultrastructure, LC-3 immunoeexpression) of OSA cell lines in a time- and dose-dependent manner. Furthermore, 17-AAG down-regulated p53, survivin and β -catenin expressions, whereas decreased Hsp90 and increased Hsp70 expression were also observed.

Conclusions: Our in vitro data confirm that Hsp90 inhibition may represent an useful target in the therapy of osteosarcoma.



Oral presentations ESVP/ECVP

IMMUNOHISTOCHEMICAL EXPRESSION OF COX-2, mPGES-1 AND EP2 RECEPTOR IN CANINE HEALTHY AND REACTIVE BONE TISSUES AND IN OSTEOSARCOMAS

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Introduction: Accumulating evidence suggests that cyclooxygenase-2 (COX-2) is involved in growth, progression and metastasis of human osteosarcomas (OS) and that its expression correlates with a poorer prognosis. The aim of this report was to study the expression of COX-2 in healthy, reactive, and neoplastic canine bone tissues and to investigate the events downstream to COX-2 that lead to PGE₂ production by the evaluation of mPGES-1 and EP₂ receptor expression.

Materials and Methods: COX-2, mPGES-1, and EP₂ receptor expression were assessed by immunohistochemistry in 12 samples of normal bone tissues, 14 reactive bones, and 27 appendicular OS. The streptavidin-peroxidase method was used. The results were quantified according to previously described scores.

Results: In healthy tissues no immunoreactivity to COX-2, mPGES-1 and EP₂ receptor was observed. Fifty percent of reactive bone samples scored positive for COX-2, and 57% for mPGES-1 and EP₂ receptor, although with a weak staining intensity. Ninety-three percent of OS expressed COX-2, mPGES-1 was expressed in 85% and EP₂ receptor in 89% of tumours.

Oral presentations ESVP/ECVP

Session F – Infectious diseases and Neuropathology

INVESTIGATIONS OF ENCEPHALITOOZONOSIS IN RABBITS

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Introduction: In the present study different detection methods (histology, immunohistochemistry and *real time*-PCR) for post mortem diagnosis of *Encephalitozoon cuniculi*-infections in rabbits were investigated.

Materials and Methods: Tissue samples from intestine, brain, heart, liver, lung and kidney of 81 rabbits were examined histologically and by immunohistochemistry. Tissue samples of the above mentioned organs of 55 animals were examined by *real time*-PCR to detect *E. cuniculi*-DNA.

Results: Histologically, lesions due to an infection with *E. cuniculi* could be observed in brain, kidney, heart, lung, liver and eye of the examined rabbits. The diagnosis encephalitozoonosis was made, when typical histopathological lesions could be detected (granulomatous encephalitis and interstitial nephritis) and/or *E. cuniculi* were detectable by at least one of the detection methods. According to this definition, an *E. cuniculi*-infection could be proven in 47% (38/81) of the examined rabbits.

Conclusion: To diagnose encephalitozoonosis, the histopathological examination, as well as the investigation using *real time*-PCR, are suitable. The detection of *E. cuniculi* is insufficient by histological examination alone. Immunohistochemistry is a more sensitive method and suitable for routine diagnostic. *Real time*-PCR is suitable to detect DNA of *E. cuniculi* in tissue probes and proved to be the most sensitive method of all methods investigated.



Oral presentations ESVP/ECVP

INTERLEUKIN-10 EXPRESSION IS ASSOCIATED WITH DELAYED VIRAL ELIMINATION FROM THE BRAIN OF SJL MICE IN THEILER'S MURINE ENCEPHALOMYELITIS

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Introduction: Protective immunity in Theiler's murine encephalomyelitis virus (TMEV)-infected SJL mice is reduced by immunomodulatory leukocytes, which leads to viral persistence and demyelination. The aim of the present study was to compare the phenotype of brain-infiltrating leukocytes and cytokine expression profile in susceptible SJL and resistant C57BL/6 mice associated with TMEV-induced acute polioencephalitis.

Materials and Methods: Brains of TMEV-infected SJL and C57BL/6 mice were investigated by histology and immunohistochemistry to detect T cells (CD3), regulatory T cells (Foxp3), B cells (CD45R/B220) and microglia/macrophages (CD107b) at 7 and 14 days post infection. Further, transcripts of TMEV, Foxp3, TNF, IFN- γ , TGF- β 1, IL-1, IL-2, IL-10 and IL-12 were measured by quantitative RT-PCR.

Results: SJL mice showed an increased number of Foxp3⁺ regulatory T cells and CD45R/B220⁺ B cells associated with elevated Foxp3 and IL-10 mRNA levels during the early infection phase. In contrast, resistant C57BL/6 mice exhibit higher TNF- α mRNA and reduced TMEV RNA levels in the brain in comparison to SJL mice.

Conclusion: Results of the present study substantiate the hypothesis that an increased regulatory T cell and B cell function during the initiating TMEV infection phase leads to an imbalanced cytokine milieu which contributes to ineffective antiviral immunity in animals with a susceptible genetic background.



Oral presentations ESVP/ECVP

PERIVENTRICULAR BRAIN LESION DEVELOPMENT AND AXONAL PATHOLOGY IN A VIRAL MURINE MODEL FOR MULTIPLE SCLEROSIS

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Introduction: The Theiler's murine encephalomyelitis virus (TMEV) infection of mice is a widely used animal model for demyelinating disorders, such as multiple sclerosis (MS) in humans. The aim of the present study was to identify axonal pathology in the central nervous system (CNS) of experimentally TMEV-infected susceptible SJL/J mice and resistant C57BL/6 mice.

Materials and Methods: The phenotype of TMEV-infected cells was identified by confocal laser scanning microscopy. Inflammatory responses and demyelination within the CNS were determined by histology using semiquantitative scoring systems. Furthermore, axonal damage was quantified by amyloid precursor protein and non-phosphorylated neurofilament immunohistochemistry (IHC). Axonal density was determined by morphometric analyses of phosphorylated neurofilament IHC and Bielschowsky's silver stain.

Results: An early infection of ependymal and periventricular cells followed by inflammation and demyelination as well as axonal pathology around the fourth ventricle in susceptible SJL/J mice was shown. While periventricular demyelination and axonal damage was transient, white matter lesions of the spinal cord progressed.

Conclusion: Summarized, the demonstration of ependymal infection and subjacent spread into the brain parenchyma as well as regional virus clearance despite ongoing demyelination and transient axonal damage in other CNS compartments allows new insights into TME pathogenesis.



Oral presentations ESVP/ECVP

ORIGIN OF CSF ANTIBODIES INDUCED BY INTRATHECAL IMMUNIZATION AND APPLY TO RABIES CONTROL IN EXPERIMENTAL ANIMALS

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Introduction: Intrathecal (IT) immunization involves injecting antigens directly into the intraventricular, subarachnoid spaces or brain, to induce antigen-specific antibodies (Ab) in the cerebrospinal fluid (CSF). The objective of the present study was to investigate the origins of CSF Ab after IT immunization.

Materials and Methods: Rabbits were immunized IT with inactivated rabies virus. Serum Ab, cytokine expression, histopathology and immunohistochemistry of brains were analyzed and compared with control. Mice were challenged rabies virus intracerebrally, to estimate the prophylactic effect of IT immunization within central nervous system (CNS).

Results: CSF Ab was rapidly induced after second IT immunization and TNF-alpha expression was also increased. Mononuclear cells including Ab-producing cells infiltrated multifocally around the blood vessels of the brain and leptomeninges. Furthermore, subcutaneous (SC) immunization prior to IT immunization induced rapid and magnified Ab responses in the CSF compared with IT immunization alone. These results were confirmed by the fact that mice immunized SC prior to IT were resistant to intracerebral virus challenge.

Conclusion: The origin of CSF Ab is speculated to represent both influx from serum and local production within CNS. Further, combined SC and IT immunization might be a more effective vaccination protocol for prophylaxis and treatment of rabies.



Oral presentations ESVP/ECVP

ORGANOTYPIC BRAIN SLICE CULTURES AS A TOOL FOR THE INVESTIGATION OF LISTERIOSIS IN RUMINANTS

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Introduction: Central nervous system (CNS) infections in ruminant livestock, such as listeriosis, are of major concern for veterinary and public health. To date no pertinent host specific *in vitro* models for ruminant CNS infections are available. Here we evaluated the suitability of organotypic brain slices of ruminant origin as an *in vitro* model to study mechanisms of *Listeria monocytogenes* (LM) infection.

Material and methods: Brains were obtained from young ruminants at the slaughterhouse. Hippocampal and cerebellar brain slices were cut with a vibratome and cultured up to 32 days. Viability was assessed with live/dead cell stains weekly. The composition of cell populations was determined by immunofluorescence on whole slices and in sections. Slice cultures were infected with LM, and infection was monitored by bacterial titration and immunofluorescence.

Results: Viable neurons, astrocytes and microglia were observed up to day 32. LM replicated in the brain slices, and bacteria were observed in astrocytes, microglia and associated with neurons.

Conclusion: Brain slice cultures from young slaughtered animals remain viable for several weeks in culture. Moreover, they are permissible to LM infection and replication. Therefore, this *in vitro* system has great potential for an ethically sustainable and inexpensive model to study host-pathogen interactions in listeriosis and possibly other neuroinfectious diseases in ruminants.



Oral presentations ESVP/ECVP

AN OVINE NEURODEGENERATIVE SYNDROME ASSOCIATED TO REPETITIVE VACCINE ADMINISTRATIONS

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Introduction: It is known that some vaccine adjuvants are potent neurotoxic agents, causing neurodegeneration at least in mice. The objective of this work is to describe an ovine neurodegenerative syndrome seen in commercial flocks that could be linked to the use of vaccine adjuvants.

Materials and Methods: Natural cases were obtained since 2007 and studied by pathological means. Additionally, 6 experimental sheep were used, three of them being repeatedly vaccinated with standard doses of adjuvant-containing commercial vaccines, over a period of 8 months.

Results: Naturally-affected sheep showed weight loss leading to extreme cachexia. An array of progressive behavioural and neurological symptoms including restlessness, compulsive wool biting, neurogenic muscular atrophy, stupor, ataxia and death were observed. At post-mortem, a marked serous atrophy of fat was detected but lesions of known cachetic diseases were not found. Microscopically, a diffuse neuronal degeneration was observed, mainly in the spinal cord. Experimentally-vaccinated animals showed similar but less severe clinical symptoms and pathological findings. Detection of aluminium and mercury in the CNS is currently being studied using Morin stain, chemical analysis and energy dispersive X-ray spectrometry.

Discussion & Conclusion: It is concluded that repeated use of adjuvant-containing commercial vaccines could lead to a, not yet well recognized, neurodegenerative process in sheep.



Oral presentations ESVP/ECVP

CHARACTERIZATION OF BETA AMYLOID DEPOSITION IN CATTLE BRAIN

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Introduction: Brain aging is mainly associated with senile and/or diffuse Beta-amyloid (β A) plaques in the brain tissue of both humans and animals. In particular, no studies in cows have yet investigated the presence of amyloid. The aim of this study was to assess the presence and the distribution of deposits of β A and tau protein in this species.

Materials and methods: Formalin-fixed samples obtained from 4 brain regions, belonging to 4 groups of 15 animals each were studied. Each group included healthy and diseased young cows as well as healthy and diseased old cows. Brain tissues were studied by immunohistochemistry (IHC) and by Western Blot analysis.

Results: By IHC most frequently β A deposits were observed intracellularly but also sporadic amyloid aggregates were found. Immunoblot analysis showed that β A was represented by both fragments β A 1-40 and 1-42 in selected brain areas.

Discussion and conclusion: This study demonstrates the presence of β A peptides in brain cattle at different ages and characterizes the phenotype of β A distribution and deposition in the nervous tissue. Therefore, the study of brain cattle represents a valid animal model for understanding the pathogenic mechanisms of neurodegeneration.



Oral presentations ESVP/ECVP

Session G – Tumor pathology

CO-EXPRESSION OF PDGFR ALPHA AND BETA IN CANINE OSTEOSARCOMAS CELL LINES AND TISSUES: NEW TARGETS FOR INNOVATIVE THERAPEUTIC STRATEGIES

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Introduction: PDGFR α and PDGFR β are tyrosine kinases receptors overexpressed in 70-80% of human osteosarcoma (OSA) that represent a suitable target for clinical use of specific kinases inhibitors. Canine OSA is considered a model in comparative oncology. In this study we investigated PDGFR α and PDGFR β expression in canine OSA tissues and primaries canine OSA cell lines.

Materials and Methods: PDGFR α and PDGFR β transcripts were evaluated in 7 OSA cells line by q-PCR. PDGFR α and PDGFR β expression was evaluated by western blot on 7 cells lines lysates and by immunohistochemistry on 28 cases of canine OSA.

Results: Molecular studies revealed that PDGFR α and PDGFR β transcripts are over-expressed respectively in 4/7 OSA cells line and in 2/7 OSA cell lines if compared to normal osteoblastic cell lines. Immunohistochemistry revealed that canine PDGFR α and PDGFR β are expressed in 71.4% and 82.1% respectively

Conclusion: These data showed that expression and distribution of the PDGFR α and PDGFR β in canine osteosarcomas are similar to human suggesting the potential therapeutic target of this receptors and remarking the important role of canine model in testing innovative approaches for human osteosarcomas therapies.



Oral presentations ESVP/ECVP

HEPATOSPLENIC AND HEPATOCYTOTROPIC T CELL LYMPHOMA – TWO DISTINCT TYPES OF T CELL LYMPHOMA IN DOGS

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Introduction: Hepatosplenic T cell lymphoma (HS-TCL) has recently been adopted as a new entity in the canine WHO classification. However, only two cases have been reported in this species so far precluding identification of general characteristics of HS-TCL in dogs.

Material and Methods: The clinical, clinicopathologic and pathological findings of nine dogs with T cell lymphoma centered on the liver and spleen without involvement of peripheral lymph nodes were assessed.

Results: The findings in seven dogs were consistent with a diagnosis of HS-TCL and closely recapitulated the human disease. Two dogs differed in the pattern of hepatic involvement and immunophenotype of neoplastic lymphocytes as well as in clinicopathologic data. Based on the marked tropism of neoplastic lymphocytes for hepatocytes, the term „hepatocytotropic T cell lymphoma” (HC-TCL) is proposed.

Conclusions: This study highlights the diagnostic hallmarks of HS-TCL and supports its classification as a WHO entity in dogs. In addition, a new type of lymphoma was recognized and termed HC-TCL. HC-TCL had distinct architectural, immunophenotypic and clinicopathologic features, indicating that it is a separate biological entity rather than a histopathologic variant of HS-TCL.



Oral presentations ESVP/ECVP

A NOVEL CLONALITY ASSAY FOR THE ASSESSMENT OF CANINE T CELL PROLIFERATIONS

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Introduction: Molecular clonality assays targeting the T cell receptor γ (TRG) locus are of growing importance as an adjunctive tool to differentiate neoplastic from reactive T cell populations in companion animals. A recent description of the full canine TRG locus revealed that existing assays were based on incomplete sequence data. As a consequence, an assay will yield a false negative result if it does not cover the rearranged genes.

Materials and Methods: A new multiplex polymerase chain reaction based clonality assay was developed that covers all rearranged canine TRG genes. The performance of the new assay was compared with an assay described by Vernau and colleagues.

Results: The new assay detected an average of 3 – 4 rearrangements per tumor. The detection rate of randomly selected clonal samples was 13/27 (48%) with the existing assay and 25/27 (93%) with the new assay. The difference in detection rate was even more pronounced in a series of hepatic T cell lymphomas of presumed $\gamma\delta$ origin, in which the new assay detected 9/9 (100%) cases as opposed to 1/9 (11%) with the existing assay.

Conclusions: The new multiplex-based clonality assay has a superior sensitivity over traditional assays and is ideally suited for canine T cell clonality diagnostics.



Oral presentations ESVP/ECVP

Session H – Infectious diseases/Neuropathology

PATHOMORPHOLOGICAL FINDINGS IN RATS (*RATTUS NORVEGICUS*) AFTER EXPERIMENTAL COWPOX VIRUS INFECTION

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Introduction: Cowpoxvirus (CPV) is endemic in Europe and wild rodents are considered as reservoir hosts. An increase of virus transmission via pet rats was reported recently as cause for human disease. Natural infection of rats with poxviruses manifests in three clinical patterns: a peracute pulmonary, a milder dermatitis and a mixed form.

Materials and Methods: To investigate the impact of the infection route on clinical course, development of lesions and tropism experimental intradermal versus intranasal CPV infections were performed in rats. A combination of both routes was included as well an assignment of sentinel animals. Immunohistochemistry was performed for antigen detection.

Results: The study demonstrates a correlation of clinical manifestation and pathomorphology with the infection route: intradermal and contact exposure yielded in a mild, dermal form, characterized by development of vesiculopustular dermatitis. In contrast, intranasally infected rats died peracutely showing dyspnoea only. Occasionally, a mixed form was observed. Immunohistochemical antigen detection was restricted to the upper respiratory tract and/or affected skin areas only; no systemic distribution of CPV was noted.



Oral presentations ESVP/ECVP

EXPRESSION OF CCSP AND SPLUNC₁, PUTATIVE ANTI-INFLAMMATORY PROTEINS, FOLLOWING MURINE RESPIRATORY VIRAL INFECTION *IN VIVO*.

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Introduction: Short Palate Lung and Nasal Epithelium clone 1 (SPLUNC₁) and Clara Cell Secretory Protein (CCSP) are expressed by respiratory epithelial cells; SPLUNC₁ predominantly in the upper respiratory tract and CCSP is expressed by Clara cells, which are more numerous within bronchioles. Both proteins are thought to have an anti-inflammatory role.

Materials and Methods: We examined the expression of both proteins in respiratory viral infections of different pathogenicity, i.e. in MHV-68 infected woodmice (*Apodemus sylvaticus*) and BALB/c mice infected with Respiratory Syncytial Virus (RSV), Sendai Virus and Influenza A virus.

Results: After MHV-68 infection, a decrease in the bronchiolar expression of both CCSP and SPLUNC₁ was seen at 7 dpi, but by 14 dpi both proteins were upregulated. RSV did not induce remarkable differences in expression of CCSP. Conversely mice infected with Sendai and Influenza A Viruses showed a decrease at 7 dpi, notably in bronchioles with peribronchiolar inflammation. There were minimal alterations in SPLUNC₁ expression.

Conclusions: Our results provide evidence that both proteins, but in particular CCSP, play a role in the respiratory response to injury and are downregulated during an inflammatory response.



Oral presentations ESVP/ECVP

INTRALESIONAL DETECTION OF A BIRNAVIRUS-LIKE AGENT IN FIELD AND EXPERIMENTALLY REPRODUCED CASES OF TRANSMISSIBLE VIRAL PROVENTRICULITIS (TVP)

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Introduction: Transmissible viral proventriculitis (TVP) is an infectious disease with characteristic microscopic lesions within proventriculus. Recently, a new birnavirus-like agent, named *Chicken proventricular necrosis virus*, has been proposed as the causative agent of TVP.

Materials and Methods: Several chickens from 4 different broiler farms experiencing slight increases in mortality were submitted for diagnosis. After necropsy, the proventriculus was studied by light and electron microscopy, and by specific *Infectious bursal disease virus* (IBDV) detection techniques, such as immunohistochemistry (IHC) and polymerase chain reaction (PCR). Finally, a homogenate of six proventriculi from one of the studied flocks was filtered and inoculated in 29 specific pathogen free 1-day-old chickens via oculonasal route. Birds were euthanized and necropsied at 5, 7, 9 and 11 dpi, and histopathological and molecular studies were performed.

Results: Characteristic histopathological TVP lesions, including multifocal necrosis of oxynticopeptic cells along with moderate intraglandular multifocal lymphocytic infiltration, were observed in chickens from the 4 field cases and in 3 out of 5 experimentally inoculated chickens necropsied at 9 dpi. IBDV IHC showed positive intranuclear and intracytoplasmic staining in necrotic oxynticopeptic cells. Electron microscopy showed several viral particles, which size and morphology were compatible with virus from *Reoviridae* or *Birnaviridae* families, within the cytoplasm of necrotic epithelial cells. IBDV PCR gave negative results.

Discussion and conclusions: This is the first report describing the specific intralesional detection of a birnavirus-like agent in natural and experimental cases of TVP. Molecular studies are needed to further characterize and corroborate that a new type of birnavirus is the etiological agent of this condition.



Oral presentations ESVP/ECVP

Session K – Tumor pathology

HISTOLOGICAL GRADES OF MALIGNANCY AND PROGNOSIS IN CANINE MAMMARY TUMOURS

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Introduction: Histological diversity of canine mammary tumours (CMT) makes their diagnosis difficult. Moreover, the relationship of the different subtypes with prognosis is still uncertain. The histological malignancy grade, has been proposed to enable the pathologist to provide accurate tumor information, although its relation to prognosis has never been proved before.

Materials and Methods: A prognostic study of 60 female dogs with malignant CMT was performed. This cohort of animals were clinically evaluated and surgically treated during 2008. Dogs were followed up until the time of writing. Histological diagnosis and tumor malignancy grade were performed using a new classification system for CMT (Goldschmidt, Peña et al., 2011). In patients with more than one malignant CMT, only one was selected for prognostic evaluation (grade I, n=28; grade II, n=16; grade III, n=16). Epidemiological, clinical, histological and follow-up variables were considered. Statistical analyses were performed with a significant level $p < 0.05$.

Results: Grading system was related to the follow-up parameters analyzed. Dogs with grade III tumors had significantly lower overall survival time compared with dogs with grades II and I. Dogs with grade I tumors survived longer than 2 years and none of them died due to the mammary cancer. Recurrences and/or metastases during follow-up were significantly more frequent in dogs with grade III tumors compared to grades II and I.

Discussion and Conclusion: Histological grading system is an accurate method to predict the outcome of dogs with mammary cancer.



Oral presentations ESVP/ECVP

EXPRESSION PATTERNS OF P-GLYCOPROTEIN IN CANINE MAMMARY GLAND TUMORS RELATED WITH MYOEPITHELIAL CELLS

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Introduction: Permeability (P)-glycoprotein is a membrane-bound multi-drug resistance associated protein that is influential in the induced chemotherapy resistance in numerous cancers. The protein is well-known and has been widely-studied in human cancer research, but is less studied in veterinary medicine. The purpose of the study was to identify P-glycoprotein expression in canine mammary gland tumors and to correlate the protein with several histopathological parameters.

Methods: For the detection of P-glycoprotein, immunohistochemistry with monoclonal antibody C219 and reverse transcription-polymerase chain reaction (RT-PCR) was performed.

Results: P-glycoprotein expression was inversely correlated with histopathological parameters, and localized in two different cell types: epithelial cells and myoepithelial cells. Epithelial expression was more intense, but the expression in either cell type was not significantly relevant as a prognostic factor. RT-PCR results were inconsistent with immunohistochemistry results. Negative immunoreactive tumors demonstrated positive signals in RT-PCR, similar to findings with human breast cancers.

Conclusion: P-glycoprotein displayed negative correlation with histopathological factors so that its expression was not considered as poor prognosis. It was a novel findings that the different localizations of P-glycoprotein which clinical implications presumably related with myoepithelial cells. Further investigations required in the chemotherapy received groups.



Oral presentations ESVP/ECVP

CLONAL ANALYSIS OF COMPLEX CANINE MAMMARY TUMORS

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Introduction: Complex mammary tumors are composed of more than one type of neoplastic cells. It is not known whether this tumor type originates from a single progenitor cell with the ability to differentiate into several different phenotypes, or whether the tumor originates from multiple progenitor cells. By analysis of X-chromosome inactivation, clonality of complex tumors can be revealed.

Material and Methods: Frozen sections of canine mammary glands from 15 individuals were screened for zygosity and primary tumors from 5 heterozygous cases were analyzed. Fragment analysis was performed on laser capture micro dissected tumor cells, following selective cleavage of the active unmethylated X-chromosome.

Results: A benign mixed tumor and a complex adenoma appeared monoclonal, with tumor cells of epithelial, cartilage and spindle cells/connective tissue phenotypes. Two cases of complex carcinomas contained both monoclonal and polyclonal epithelium. The fifth tumor, an atypical adenoma had a polyclonal pattern in two tested parts.

Conclusion: The X-chromosome inactivation analysis revealed that complex canine mammary tumors might be of polyclonal origin. We found that two benign complex tumors were monoclonal whereas two complex carcinomas were composed of both monoclonal and polyclonal tumor cell populations.



Oral presentations ESVP/ECVP

IMMUNOEXPRESSION OF PROTEINS RELATED TO EPITHELIAL-MESENCHYMAL TRANSITION IN CANINE MAMMARY TUMORS

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Introduction: Epithelial-mesenchymal transition (EMT) is defined as switching of polarized epithelial cells to a migratory fibroblastoid phenotype. EMT is known to be involved in the progression and metastasis of various cancers in humans, but this specific process is still little explored in the veterinary literature. The aim of this research was to evaluate the expression of EMT-related proteins in canine mammary carcinomas (CMCs).

Materials & Methods: The expression of EMT-related proteins (Snai-1, S100A4, cytokeratin, E-cadherin, N-cadherin, and matrix metalloproteinase-2) was evaluated by immunohistochemistry in CMC of 94 female dogs. Histopathological characteristics (vascular invasion, stromal invasion, mode of growth and histological grade) were compared with the expression of EMT-related proteins in CMCs.

Results: Loss of epithelial proteins and/or acquisition of the expression of mesenchymal proteins were observed, particularly in tumors with evidence of stromal invasion; however, significance regarding anatomopathological characteristics was only observed between S100A4 immunoreexpression and vascular invasion. Snai-1 was expressed in mammary luminal cells of histologically malignant tumors and in myoepithelial cells of benign and malignant complex tumors and was significantly related to E-cadherin loss.

Conclusions: Loss of epithelial proteins and/or the acquisition of mesenchymal proteins are associated with EMT and may have an important role in the evaluation of CMC patients.



Oral presentations ESVP/ECVP

ADRENAL CORTICAL CARCINOMAS IN BEEF CATTLE AT SLAUGHTER

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Introduction: Despite being a common neoplasm, bovine adrenal gland neoplasia is not well characterized presumably because they do not present with clinical disease or cause mortality. These neoplasms are therefore best studied in slaughterhouse surveys of mature animals.

Methods: Ten adrenal cortical carcinomas from beef cattle were collected at slaughter. Pancytokeratin, S-100, melan-A, a-inhibin, chromogranin-A, neuron specific enolase and synaptophysin immunocytochemical staining was used to characterize the neoplasms.

Results: Carcinomas were in <0.03% of mature, cull beef cows. Large adrenal adenomas were in ~0.2% of cows. All carcinomas invaded the vena cava and metastasized to lung, liver and/or lymph nodes. Neoplasms were characteristically soft, mottled red/yellow and occasionally gritty. Primary neoplasms often were >1.5cm in diameter and similar to many large adrenal adenomas. Metastases were best noted in the lungs, but small, pulmonary lesions were occasionally not noted. Neoplastic cells had typical morphology with little anaplasia and two cases had calcific granules in both primary and metastatic neoplasms. Carcinomas, both in the primary lesion and metastases, stained for melan A and a-inhibin. The normal bovine adrenal cortex stained strongly for S-100, but only carcinomas with calcific granules stained positively for S-100.

Conclusion: Slaughterhouse-derived neoplasms are a convenient source of material for study of the molecular pathogenesis of adrenal neoplasia.



Oral presentations ESVP/ECVP

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF BOVINE ADRENAL GLAND TUMORS

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Introduction: Tumors of the bovine adrenal glands are found relatively frequently during routine meat inspections. However, the knowledge on the aetiology, pathogenesis and epidemiology of bovine adrenal gland tumors is sparse, and no studies have defined their immunohistochemical characteristics.

Materials and Methods: Thirty-four bovine adrenal tumors, submitted from Danish abattoirs to the Department of Veterinary Disease Biology from 1999-2010, were examined. Normal bovine adrenal gland tissue and selected bovine adrenal tumors were immunostained for vimentin, pan-cytokeratin, synaptophysin, chromogranin A, inhibin alpha, calretinin and melan A. In addition, ultrastructural examination was performed on two of the tumors.

Results: Following immunohistochemical examination, the tumors were classified as adrenal adenomas, adrenal carcinomas, pheochromocytomas, and ganglioneuromas.

Adrenal adenomas and carcinomas labeled positive for melan A (12/20), vimentin (12/20) and cytokeratin (9/20). The pheochromocytomas labeled positive for chromogranin A (2/2) and synaptophysin (2/2), and the ganglioneuroma labeled positive for vimentin and S100. Ultrastructural examination of two adrenocortical tumors revealed that the tumor cells were hormone producing.

Conclusion: Adrenal adenomas and carcinomas are the most common tumors of the bovine adrenal gland, and an antibody panel consisting of melan A, synaptophysin and chromogranin A is applicable for classifying bovine adrenal gland tumors.



Oral presentations ESVP/ECVP

EVIDENCE OF BOVINE PAPILLOMAVIRUS TYPE 2 IN THE PLACENTA OF COWS WITH URINARY BLADDER TUMOURS

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Introduction: Bovine Papillomavirus type 2 (BPV-2) infection resulting in tumours of the urinary bladder is very common in some breeds of cattle living at pasture and grazing on bracken fern-infested lands. Papillomavirus are believed to be highly epitheliotropic but new host cell types are emerging. Here we describe a productive infection of BPV-2 in the placenta of cows who also have urinary bladder tumours.

Materials and Methods: Placentomes were sampled at public slaughterhouses from four pregnant cows suffering from tumours of the urinary bladder in which E5 oncoprotein was in vivo detected in blood by immunoprecipitation.

Results: The expression of E5 was detected in the placenta by immunoprecipitation. Morphologically E5 was found to be colocalized with PDGF β -r. The latter appears to be phosphorylated (activated). L1 protein was detected by western blot.

Discussion and Conclusion: Our study shows that the complete life cycle of BPV-2 appears to occur in the placenta from cows suffering from urothelial tumours. Like humans, trophoblasts appear to be the preferential targets for BPV infections too. Our findings support the view that BPVs are not strictly keratinocyte-specific and strengthen our recent results showing L1 in peripheral blood cells.



Oral presentations ESVP/ECVP

FRACTAL DIMENSION OF THE HEPATOID ADENOMAS AND CARCINOMAS IN DOGS

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Introduction: The fractal dimension (FD) provides an excellent explanation of the roughness and self-similarity of natural objects and has been exploited for various biomedical recognition applications, including some which are used in research pathology. The aim of our study was to evaluate histopathological features in tissue specimens from canine hepatoid gland adenoma and carcinoma using a specific computer-based fractal image analysis approach.

Materials and Methods: In order to better analyze architectural differences we determined an optimal staining (H&E, Van Gieson and IHC /vimentin/), magnification, and image analysis technique.

Results: There was a significant difference ($p < 0.05$) with regards to tumor type in the vimentin stained tumors. Carcinomas presented significant higher mean values of FD compared to adenomas and to normal circumanal gland.

Conclusion: These results indicate that malignancy of canine hepatoid gland tumor can be characterized by describing the complex pathologic architecture of tumors using fractal image analysis approach and, what is even more important, because of its cheapness and accuracy this method could be used in the analysis of other tumours as well.



Oral presentations ESVP/ECVP

Session L – Infectious diseases/Natural occurring diseases

BONE MARROW PATHOLOGY IN HAEMORRHAGIC SYNDROMES IN YOUNG CALVES

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Introduction: An emerging haemorrhagic syndrome of calves less than 1 month old has been recorded in Europe since 2007/2008 (bovine neonatal pancytopenia, BNP). Bone marrow examinations on 350 calves <1month with multisystemic haemorrhage, presented to AHVLA and SACVS for investigation, formed part of a multidisciplinary approach.

Materials and methods: The protocol included histopathology of standardized bone marrow sites (femoral cavity, sternbrae and ribs). Sternal and femoral sites were compared in 35 calves.

Results: Trilineage hypoplasia (TLH), involving extensive depletion of erythroid and myeloid precursors and megakaryocytes (aplastic anaemia), was observed in 333 (95.1%) animals, and was best assessed in sternum. Bilineage hypoplasia (late stage erythroid series cells and megakaryocytes) occurred in 4 animals. One calf had intermediate lesions. Evidence of acute bovine virus diarrhoea virus infection was detected twice. Diffuse regenerative responses or no unequivocal change was found in 10 animals.

Discussion and conclusion: The predominant bone marrow lesion in this series was TLH, the characteristic lesion of BNP. The sternum is the site of choice for detection of these lesions. The presence of TLH indicates injury to haematopoietic stem cells. Two of the calves with bilineage hypoplasia originated from herds in which at least one other calf in the herd had confirmed TLH, therefore megakaryocyte/erythroid hypoplasia may represent a variant of BNP.



Oral presentations ESVP/ECVP

FATAL RESPIRATORY TRACT NECROSIS IN PRE-WEANED LAMBS

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Introduction: Rapidly fatal respiratory signs and sudden deaths in 3-8 week old lambs, occurring shortly after completion of management tasks including administration of mineral (copper, selenium and cobalt) drenches, were investigated.

Materials and methods: Three lambs from flock 1 and 6 lambs from flock 2 were necropsied. Investigations included histological examination of trachea and lung and analysis of copper and selenium concentrations in lung, compared with age matched controls.

Results: In 6/9 lambs, profuse straw-coloured pleural fluid and pulmonary consolidation were found. Histological examination revealed multifocal coagulative necrosis of airway epithelium forming detached rafts of ciliated cells, patchy peribronchiolar alveolar haemorrhagic necrosis and marked protein-rich pulmonary oedema in 7 lambs. The pulmonary copper concentrations were elevated in the 7 lambs with airway necrosis (274-1543 μ mol/kg DM, n=7) compared with controls (28-98 μ mol/kg DM, n=5).

Discussion and conclusion: The airway epithelial lesions were consistent with a surface-acting injury following inhalation of a necrosis-inducing chemical. High local concentrations of copper are known to cause necrosis in a wide range of tissues including liver, neuraxis and skeletal muscle. The findings suggest that the pulmonary lesions in these lambs result from inadvertent inhalation of the drenches, and hence exposure of airway epithelium to high concentrations of copper, and possibly of selenium, resulting in airway necrosis.



Oral presentations ESVP/ECVP

JAAGSIEKTE SHEEP RETROVIRUS POSITIVE CELLS ARE FOUND IN TISSUES OF VERY YOUNG LAMBS NATURALLY FEED WITH COLOSTRUM FROM INFECTED EWES

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Introduction: Ovine pulmonary adenocarcinoma (OPA) is a contagious lung cancer of sheep caused by jaagsiekte sheep retrovirus (JSRV). JSRV can also be found in the lymphoid organs, and colostrum/milk can contribute to the natural infection of lambs. In this study we detected JSRV positive cells in lamb tissues naturally feed with colostrum from JSRV infected ewes.

Materials and Methods: 22 ewes positive to JSRV PCR-blood test, with no clinical signs of OPA, and 30 lambs from these animals were used in this study. Colostrum and milk serial samples were taken throughout lactation and tested by specific JSRV-PCR. Lambs were naturally fed with colostrum and milk from their mothers and serially euthanized at 12h, 24h, 48h, 72h, 5 days and 10 days after birth. Tissue samples from several organs were processed for immunohistochemistry (IHC) and evaluated with polyclonal and monoclonal antibodies against JSRV proteins by routine methods.

Results and Discussion: IHC results showed a very low number of labelled mononuclear cells in mesenteric lymph nodes of lambs aged 24h and older. In ruminants, mononuclear cells from colostrum can pass intestinal barrier and reached the circulation of newborn and it is therefore speculated that this is a mechanisms for JSRV transmission.



Oral presentations ESVP/ECVP

IMMUNOCOMPETENT CELLS IN THE LYMPH NODES OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS-INFECTED PIGS

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Introduction: Despite the efforts in accumulating knowledge of Porcine Reproductive and Respiratory Syndrome (PRRS), the role of immunocompetent cells still remains unclear. The goal of this study was to evaluate the changes in the subpopulations of antigen presenting cells and T lymphocytes in the lymph nodes of PRRS virus (PRRSV) infected pigs.

Materials and Methods: Twenty-eight piglets were distributed in batches of four and killed at different time points. Four control pigs were used and killed at the end of the study. Lymph node samples were collected and fixed in 10 % buffered formalin and Bouin solution. Antibodies against PRRSV, SWC3, S-100, HLA-DR and CD3 antigens were used in the immunohistochemical study.

Results: Viral antigen showed an increase at 3 and 7 days post-inoculation (dpi) in retropharyngeal and mediastinal lymph nodes, respectively. Antigen presenting cells followed an undulating kinetic, without marked changes. A decrease in the expression of HLA-DR and CD3 was observed at 3 and 7 dpi in both lymph nodes.

Conclusion: A failure in the establishment of an effective immune response in PRRS may be related to a downregulation of MHC-II and a lack of activation of immunocompetent cells.



Oral presentations ESVP/ECVP

EMERGENCE OF A CANINE DISTEMPER VIRUS STRAIN WITH MODIFIED MOLECULAR SIGNATURE AND ENHANCED NEURONAL TROPISM ASSOCIATED TO HIGH MORTALITY IN WILD CARNIVORES

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Introduction: An ongoing canine distemper virus (CDV) outbreak characterized by high morbidity/mortality reached Switzerland in April 2009.

Materials & Methods: Wild carnivores including red foxes (*Vulpes vulpes*), Eurasian badgers (*Meles meles*) stone and pine martens (*Martes foina*, *Martes martes*) and one Eurasian-lynx (*Lynx lynx*), were examined between April 2009 and August 2010.

Results: 74 were CDV-positive. Most common gross findings included emaciation and lung consolidation. Bronchointerstitial pneumonia and an uncommon Snyder-Hill-like-associated polioencephalitis were frequent histological findings. Intracytoplasmic inclusions were seen in the lung of all affected species, while syncytial cells were common in foxes, less frequent in badgers and absent in martens. Neuronal inclusions were most common in foxes but were also seen in martens and badgers. Phylogenetic analysis based on the hemagglutinin-protein revealed a common progenitor shared by the Swiss-CDV-strains and a 2004-Hungarian-CDV-strain. Functional analysis of the hemagglutinin-protein, of a Swiss isolate, revealed higher surface expression and more efficient binding to SLAM-receptor than that of the reference strain A75/17.

Conclusions: These changes are considered part of a molecular signature of the Swiss-CDV-strains, which might have contributed to the high morbidity and mortality of this outbreak.



Oral presentations ESVP/ECVP

INVESTIGATION OF A UNIQUE SHORT OPEN READING FRAME WITHIN THE 3' UNTRANSLATED REGION OF THE CANINE DISTEMPER VIRUS MATRIX MESSENGER RNA

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Introduction: Increasing evidence suggests that the long “untranslated” region (UTR) between the matrix (M) and the fusion (F) proteins of morbilliviruses has a functional role. Unique to canine distemper virus (CDV), a short putative open reading frame (ORF) has been identified within the wild-type CDV-M 3' UTR (termed M2). Here, we investigated whether M2 was expressed from the genome of the virulent and demyelinating A75/17-CDV strain.

Materials & Methods: An expression plasmid encoding the M2 ORF tagged both at its N-terminal (HA) and C-terminal domains (RFP), was first constructed. Then, a recombinant virus with its putative M2 ORF replaced by HA-M2-RFP was successfully recovered from cDNA (termed recA75/17(green) -HA-M2-RFP). As positive control a recombinant virus was created, where the expression of M2 was ensured by placing it into another location with its own mRNA. M2 expression in cells transfected or infected with these mutants was studied by immunoprecipitation, immunofluorescence, immunoblot and flow cytometry analyses.

Results: Although fluorescence was readily detected in HA-M2-RFP-transfected cells, absence of red fluorescence emission (except in the positive control virus) in several recA75/17(green)-HA-M2-RFP-infected cell types suggested lack of M2 biosynthesis, which was confirmed by the other techniques. Consistent with these data, no functional role of the short polypeptide was revealed by infecting various cell types with HA-M2-RFP over-expressing or M2-knockout recombinant viruses.

Conclusions: Thus our data provided evidence that the CDV-M 3' UTR does not express any polypeptides.



Oral presentations ESVP/ECVP

HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF VITREORETINOPATHY IN SHIH TZU DOGS WHOSE EYES WERE REMOVED FOR MEDICAL REASONS

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Introduction: The objectives of this paper are to describe the histopathological features of vitreoretinopathy in the eyes of Shih Tzu dogs removed for medical reasons and to suggest possible mechanisms of this ocular disease.

Materials and Methods: A total of 50 cases of Shih Tzu ocular vitreoretinopathies were selected from the COPLOW collection. Histopathological and immunohistochemical techniques were performed in order to detect the abnormalities.

Results: The most characteristic histological abnormalities seen in 50/50 cases were the presence of retinal detachment and extensive retinal tears. In 31/50 cases an extracellular, eosinophilic matrix material admixed with few spindle cells and minimal phagocytes and erythrocytes in the vitreous attached to the posterior lens capsule, was detected. A pre-iridal fibrovascular membrane (PIFM) was identified in 34/50 globes. Goniodysgenesis, was detected in 4/50 globes. Secondary glaucoma, was noted histopathologically in 26/50 globes. In 13/50 globes, hypermature and subcapsular cataract were detected. In 5/50 globes a cyclitic membrane was detected while in 30/50 globes chronic superficial keratitis with evidence of prior ulceration was also detected.

Discussion: This study provides the first demonstration of histopathological features of vitreoretinopathy in Shih-Tzu dogs. Vitreous degeneration is seen probably secondary to abnormal vitreous development. This in turn leads to retinal detachment and tear with secondary neovascular glaucoma and intraocular hemorrhage.



Oral presentations ESVP/ECVP

EVALUATION OF PREPUTIAL CYTOLOGY IN DIAGNOSING ESTROGEN PRODUCING TESTICULAR TUMOURS IN DOGS

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Introduction: Increased numbers of superficial cells in preputial smears have been proposed as a marker for estrogen producing testicular tumours in dogs.

The purpose of this study was to evaluate the diagnostic sensitivity and specificity of preputial cytology in estrogen producing testicular tumours in dogs.

Materials and methods: Forty-five dogs with palpable testicular masses and 30 healthy control dogs were included. Dogs were evaluated for signs of alopecia and/or feminization. Analysis of preputial cytology, hematology and serum estradiol was performed. Dogs with testicular masses were neutered and the testes were submitted for histopathologic examination.

The dogs were divided into three groups; 1) control dogs (n=30), 2) testicular mass and serum estradiol <40 pmol/l (n=35), 3) testicular mass and serum estradiol >40 pmol/l (n=10).

Results: >20% superficial cells in preputial smear was significantly associated with serum estradiol >40 pmol/l ($P=3 \times 10^{-5}$) with a sensitivity of 80% and a specificity of 98%. 7/10 dogs in group 3 and 1/35 in group 2 had clinical signs of alopecia, which resolved after neutering. Number of superficial cells was significantly increased ($P=4 \times 10^{-5}$) in preputial smears from dogs with alopecia.

Discussion and conclusion: It appears that preputial cytology has a high sensitivity and specificity for the diagnosis of estrogen producing testicular tumors in dogs.



Poster Abstracts

P1: DEVELOPMENT OF VETERINARY FORENSIC PATHOLOGY FROM CRIME SCENE TO COURT

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Goal: A secure, transparent, high quality process where obtained forensic results are correctly understood in court.

Introduction: Veterinary forensic science is an emerging field in veterinary medicine. A greater legal interest with more laws and resources available to prosecute crimes toward animals increases the demands on the veterinary profession regarding post-mortem examinations, laboratory analyses and legal documentation. We want to consolidate forensic veterinary pathology in Sweden by development of crime scene routines, improvement of pathology/diagnosis, introduction of evidence evaluation using the logical (Bayesian) approach and introduction of a new structure for veterinary expert statements intended for the legal system.

Methods: Forensic trace recovery, analysis and evidence evaluation will be studied using two models in different settings: (i) intentional poisoning of small animals (cats and dogs) with rodenticides as model poison, and (ii) intentional infection of groups of animals (cattle) employing type scenarios. A survey- and interview-based study (iii) directed to legal officials will also be done. In (iii) we will both investigate how veterinary reports/statements are understood and used today and how the reports can be improved in future.



Poster Abstracts

P2: EVALUATION OF ²¹³BI TOXICITY IN MICE AS PRECLINICAL APPROACH OF RADIO-IMMUNOTHERAPY

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Introduction: ²¹³Bi is a radionuclide proved to be efficient against isolated cells due to their high lineic energy transfer of alpha particles. It has been proposed for several indications, both in haematological diseases and solid tumours. ²¹³Bi coupled with specific antibodies is considered as a promising anticancer drug. In this preclinical study, ²¹³Bi coupled with bovine serum albumin (BSA) was injected to mice to determine its toxicity.

Materials and methods: Systemic injections of increasing doses of ²¹³Bi-BSA were administered to mice and a histological evaluation of mouse organs was then undertaken.

Results: Liver and spleen shows increased extramedullary haematopoiesis testifying a deficient central haematopoiesis (medullary toxicity). The liver shows isolated cellular necrosis, centrolobular fibrosis and periportal inflammatory cell infiltrations. The kidney shows tubular injury characterized by basophilic tubules, karyomegaly, cytomegaly, tubular dilation. Interstitial lesions are also noticed: inflammatory cell infiltrations, fibrosis. Finally glomerular structures are affected too, revealing glomerulosclerosis and increased proteinuria.

Conclusion: ²¹³Bi coupled with BSA shows mainly and hepatotoxicity (necrotizing hepatitis) and nephrotoxicity (glomerular, interstitial and tubular injuries).



Poster Abstracts

P4: EVALUATION OF HEPATOTOXICITY USING NUCLEAR MORPHOMETRY IN RATS TREATED WITH POLYPHENOLIC EXTRACTS

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Introduction: The evaluation of hepatotoxic or hepatoprotective agents is based on structural and functional parameters. The objective of the present study was to evaluate the hepatoprotective activity of polyphenolic extracts of vegetal material based on morphology of hepatocytes and hepatocyte morphometry.

Material and methods: Adult Wistar rats were inoculated with Walker 256 carcino-sarcoma. Subsequent chemotherapy was provided for all inoculated rats. Polyphenolic extracts of *Viscum album*, *Aristolochia clematitis* and *Lycopodium clavatum* were orally administered. Routine histopathology was completed with assessment of area and perimeter of hepatocyte nuclei (Olympus Cell^B program).

Results: Acute hepatotoxicity was revealed by focal randomly distributed hepatocyte necrosis, hepatocellular megalocytosis, characteristic features for apoptosis and hepatocyte mitosis. The biggest number of clearly outlined nuclei was recorded in the control group (an average of 55 nuclei/digital image x400). The number of nuclei ranged between 30 and 40 in inoculated rats. Nuclear area and perimeter recorded the biggest values in cases with hepatocellular megalocytosis (nuclear area: $91.90 \pm 25.64 \mu\text{m}^2$ and nuclear perimeter: $41.44 \pm 5.83 \mu\text{m}$). Polyphenolic extracts of plants provided a partial hepatoprotective effect, proved by decrease and disappearance of hepatocyte mitoses; all the aforementioned hepatocyte alterations were noticed in all experimental rats.

Conclusions: The assessment of hepatoprotective effect of polyphenolic extracts of plant in rats with tumoral disease and treated with cytostatic drugs using computered morphometry reduce the risk of subjective evaluation. We consider that this method is complementary with histopathological investigation.



Poster Abstracts

P5: EXPRESSION OF ENZYMES INVOLVED IN XENOBIOTIC METABOLISM IN EQUINE RESPIRATORY TISSUES

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Introduction: Respiratory tissues are exposed to inhaled harmful substances which may be converted into toxic metabolites causing oxidative stress and inflammation. Enzymes contributing to the bioactivation or detoxification processes are cytochrome P₄₅₀ (CYP) enzymes, glutathione-S-transferases (GST) and superoxide dismutases (SOD).

Material and Methods: In abattoir material of horses, gene expression and cellular localization of CYP1A1, GSTM1 and EC-SOD were investigated in the trachea, primary bronchi and lungs. The lungs were histopathologically examined. Some horses had a history of recurrent airway obstruction (RAO).

Results: All the examined enzymes were expressed and predominantly localized in airway epithelia. The highest gene expression of CYP1A1 and EC-SOD occurred in the lungs, whereas GSTM1 was mostly expressed in the extrapulmonary airways. Horses with an RAO history in general had chronic bronchitis and bronchiolitis. Inter-individual variations in the levels of expression of the enzymes were considerable, but a trend towards a higher expression of CYP1A1 and a lower of GSTM1 in horses with inflamed small airways was apparent.

Conclusion: The trend towards changed enzyme expressions in horses with inflamed small airways suggests that respiratory tract enzymes involved in the formation and detoxification of toxic metabolites might have a role in chronic equine bronchitis/bronchiolitis.



Poster Abstracts

P6: DELIBERATE PARACETAMOL POISONING IN TWO PUPPIES

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Introduction: Cases of factitious illness by proxy in pets occur rarely in the literature. To our knowledge, this is the first reported case of deliberate paracetamol intoxication in pet dogs.

Case Histories: Two puppies were diagnosed with paracetamol intoxication with fatal outcome in one of them. These cases are considered deliberately intoxicated and therefore referred to as fabricated or induced illness (FII).

Results: Paracetamol intoxication is limited to organs containing enzymes for bioactivation. Within certain dose levels the liver will show typical but unspecific centrilobular hepatocellular necrosis. In this case the diagnosis was based on morphological changes and confirmed by gas chromatography-mass spectrometry of liver.

Conclusions: The most severe stage of FII is induced illness, as described here, compared to cases of falsified history and fabrication of laboratory results. All scenarios can be harmful to the animal, either directly or indirectly. This case contains the two most salient warning signs of FII which involve improvement of health during separation and death of those who remain under the perpetrators care. Perpetrators are usually females with attention-seeking behaviour. 'Veterinarian shopping' is also described. The small animal practitioner is usually the first to meet this kind of owners. However, for the pathologist FII can be confusing cases where the owner is dishonest and your requesting colleague might have been misled.



Poster Abstracts

P7: HISTOPATHOLOGICAL LESIONS IN CHICKEN BROILERS AND LAYERS IN POLAND – A RETROSPECTIVE STUDY OF 189 CASES

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Introduction: The significance of histopathology in the diagnosis of avian diseases is undisputed. The present study aimed to estimate the prevalence of histopathological lesions in chicken broilers and layers in relation to age of birds and the lesion site.

Materials and Methods: The analysis related to 189 cases (126 broilers, 63 layers) submitted during 1999-2010. HE stain and others methods were used.

Results: Histopathologic changes occurred most frequently in the liver and lymphoid organs. In 23% cases (mean age 22 days) intranuclear inclusions were found in hepatocytes, and in 58% cases (m.a. 23 days) proventriculitis was also observed. Evidence of parasitic or fungal infection and amyloid deposition were rare. Lesions associated with Marek disease, lymphoid leukosis and fowl pox were recognized only in material derived from layers and respectively in 3,2%, 6% and 1,1% of all cases (mean age 176d, 205d, 131d).

Conclusion: To our knowledge this is the first such analysis made in Poland



Poster Abstracts

P8: IMPAIRED PLACENTAL VASCULARIZATION AND EMBRYO GROWTH AFTER IN VITRO MANIPULATION IN SHEEP: A MORPHOMETRICAL STUDY

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Introduction: Aberrant placentation occurs early during embryonic development after assisted reproductive techniques. To further understand this failure, vascular morphometry of ovine placentas and embryo growth after *in vitro* activation (IVA) and *in vitro* fertilization (IVF) at 20 and 22 days of gestation was performed.

Results: Crown-ramp measure (mm) after *in vitro* manipulation is reduced at 20 (CTR-controls: 3.84; IVF: 3.48; IVA: 3.46) and 22 days (CTR: 4.42; IVF: 3.84; IVA: 3.74), as well as placental vessel number/field (20-22 days: IVF: 1.25-1.93; IVA: 1.24-1.71; CTR: 3.11-3.48). At 20 d, stage 1 vessels (early vasculogenesis) were prevalent in IVA samples (IVA: 26.67% of total vessels; IVF: 10.55%; CTR: 3.54%), stage 2 (early tube formation) in IVF (IVF: 77.82%; CTR: 70.31%; IVA: 66.33%) and stage 3 (late vasculogenesis) in CTR (CTR: 26.15%; IVF: 11.63%; IVA: 10%). At 22 d, more vessels in stages 1 (18.18%) and 2 (72.72%) occur after IVA than IVF (1: 5.24%; 2: 64.51%) and CTR (1: 2.62%; 2: 53.59%), while CTR had more stage 3 vessels (43.79%) than IVF (30.25%) and IVA (9.1%).

Discussion: *In vitro* manipulation leads to delayed maturation and reduced density of placental vessels, that affect post-implantation embryo growth.



Poster Abstracts

P9: DIAGNOSING BARITOSIS WITH GRANULOMATOUS PNEUMOCONIOSIS IN AN ECLECTUS PARROT (*ECLECTUS RORATUS*), DUE TO BARIUM SULFATE ASPIRATION

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Introduction: Baritosis, barium aspiration resulting in pneumosilicosis, is very rarely reported in animals. The presented psittacine biopsy case includes a diagnostic pathology challenge, as there is no histochemical stain specific for barium.

Material and Methods: Radiographs showed areas of increased radio-opacity in the lung of a seven year old eclectic parrot (*Eclectus roratus*). Endoscopic and surgical follow-up examination showed several whitish nodules in the lung and adhesions of the air sac wall on one side. Submitted formalin-fixed lung tissue biopsies were examined with multiple histochemical staining methods.

Results: The lung masses were multiple pneumoconiosis granulomas composed of densely packed macrophages with the cytoplasm distended by golden brown granules, expanding and replacing normal lung tissue, within a sparse fibrous stroma. There was a lack of other inflammatory components. Special stains; PAS, Grocott, Prussian blue and Ziehl-Neelsen, were negative. The cytoplasmic granules showed no birefringence. Additional clinical information was provided and included previous examination where barium sulfate (BaSO₄) was given *per os*. Radiographs of the biopsy paraffin block showed obvious radio-opacity of the granulomas, confirming the suspicion of baritosis, with aspiration of a significant amount of barium sulfate as the underlying cause.

Conclusion: Suspected baritosis can be confirmed by radiographing the paraffin blocks containing barium-laden organs.



Poster Abstracts

P10: EXPRESSION OF ANTI-APOPTOTIC MOLECULES IN THE BRAIN OF DOGS WITH GRANULOMATOUS MENINGOENCEPHALITIS

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Introduction: Granulomatous meningoencephalitis (GME) is a common inflammatory disease of the canine central nervous system with an unknown, probably immune-mediated etiology. The aim of the present study was to test the hypothesis that an increased expression of anti-apoptotic molecules contributes to lesion progression in GME.

Materials and Methods: Brains of eight dogs affected by GME were investigated by histology and immunohistochemistry (IHC) using markers for T cell (CD3), regulatory T cells (Foxp3), B cells (Pax5) and histiocytic cells (lysozyme). Furthermore, the expression of the anti-apoptotic mediators survivin, Bcl-2 and cIAP-2 was quantified within GME lesions by IHC.

Results: Early GME lesions were dominated by perivascular and meningeal CD3⁺ T cell infiltrates, with the majority of lymphocytes expressing Bcl-2. In comparison, advanced lesions, characterized by granuloma formation were associated with survivin expression predominantly in epithelioid macrophages.

Conclusion: Results of the present study demonstrates the occurrence of cell type specific expression of apoptosis inhibiting molecules which have the ability to protect infiltrating inflammatory cells from elimination by apoptotic cell death. Accordingly, these molecules might represent contributing factors for prolonged inflammation and lesion progression in GME .



Poster Abstracts

P11: ASSOCIATION OF AROMATASE AND 3 β HSD WITH CUPRIZONE-INDUCED DEMYELINATION AND REMYELINATION IN C57BL/6 MICE

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Introduction: The cuprizone model for toxic demyelination is commonly used to investigate mechanism of remyelination in central nervous system. Aromatase and 3 β HSD are steroidogenic enzymes that are thought to play a role in myelination. The aim of the study was to investigate relationship between aromatase and 3 β HSD after experimentally induced demyelination and remyelination in mice.

Material and Methods: C57BL/6 mice were fed a diet of 0.2% cuprizone for 6 weeks. Remyelination was assessed returning mice to normal diet for 4 weeks after 6 weeks cuprizone treatment and mice fed normal diet were used as control. The severity of demyelination was determined in corpus callosum with histological sections stained with luxol fast blue. Intensity of aromatase and 3 β HSD were detected by Western blot analyses.

Results: Histologically, severe demyelination was observed in demyelination group, but results from remyelination group resembled those of control mice. Aromatase was expressed in 38, 44, 55 kDa mw, and the highest level of aromatase was expressed in the demyelination group compared with remyelination and control groups. 3 β HSD was expressed in 42 kDa mw as low concentration in demyelination group whereas it was not expressed in remyelination and control groups.

Discussion and Conclusion: The result suggest that increased aromatase and 3 β HSD levels may be compensatory mechanism for new myelin formation in demyelination.



Poster Abstracts

P12: PROTECTIVE MECHANISMS OF A GRAPE SEED EXTRACT (BURGUND MARE VARIETY) ON CHRONIC ULTRAVIOLET B IRRADIATION-INDUCED SKIN DAMAGE IN SKH-1 HAIRLESS MICE

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Introduction: The aim of this study was to evaluate the protective mechanisms of a topically applied grape seed extract (BM variety), with 2 concentrations (2.5 and 4 mg polyphenols/cm²), in a subacute *in vivo* model of skin UVB exposure.

Materials and Methods: Sixty female mice SKH-1 were randomly divided in six groups of 10 animals. The extract was applied 30 minutes before UVB irradiation (240 mJ/cm²). Exposures were made on 10 consecutive days. At 24 hrs after the last UVB exposure dorsal skin biopsies from each mouse were used for histopathological evaluation of apoptotic keratinocytes (sunburn cells) and skin inflammation. Immunohistochemistry to detect cyclobutane pyrimidine dimers (CPDs) formation and epidermal hyperplasia (anti Proliferating cell nuclear antigen – PCNA) was performed on paraffin-embedded skin samples.

Results and Discussion: CPDs, sunburn cells, inflammation and epidermal hyperplasia were markedly increased in all irradiated groups, but reduced in both BM treated groups compared to unprotected ones.

Conclusion: Topically applied BM extract favours the formation of apoptotic cells which are being replaced by hyperproliferative cells, creating a milieu in which the apoptotic process is favoured, by this protecting the skin against malignancy.



Poster Abstracts

P13: GRANULOMATOUS LESIONS EXPERIMENTALLY INDUCED BY *PROTOTHECA* IN MICE

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Introduction: Granuloma formation is a chronic inflammatory reaction where macrophages and other inflammatory cells are involved. The aim of the study is to evaluate the ability of *Prototheca* a unicellular algae, to induce granulomatous lesions in mice.

Material and Methods: Two groups of 2 months old BALB/c mice were established: group I inoculated with a *P. wickerhamii* reference strain and group II inoculated with a *P. zopfii* isolate. The experiment was completed at 4 weeks postinfection. To confirm the presence of algae in the affected nodular internal lesions, microscopic, microbiological and histopathological (PAS stained) examinations were performed. To appreciate the lesion severity a scoring system of lesions was used, obtaining a systemic lesional score for each animal. The data were statistically processed using “t” test.

Results: Lesions caused by *Prototheca* algae consisted of granuloma and pyogranuloma on skin, liver, pancreas, gut and diaphragm. By all the examinations performed we identified the etiologic agent of protothecosis. Using “t” test in order to compare the medium values of lesional systemic score, lesion category between two groups was statistically significant ($p < 0.05$).

Conclusion: In group inoculated with *P. zopfii* the lesions were more severe than those observed in *P. wickerhamii* infected group.

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Poster Abstracts

P14: EFFECT OF HYPERTENSION ON EXPERIMENTAL PERIPEHRAL NEUROPATHY

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Introduction: Relationships between hypertension and diabetic peripheral neuropathy (DPN) have been recently reported in clinical research. It remains unclear whether hypertension is a risk factor for DPN. To investigate the effects of hypertension on DPN, morphological features of peripheral nerves in alloxan-induced diabetic WBN/Kob rats were evaluated.

Materials and Methods: Male WBN/Kob rats were divided into 3 groups: (1) alloxan-induced diabetic rats (AL group); (2) alloxan-induced diabetic rats with deoxycorticosterone acetate-salt (DOCA-salt) treatment (ADN group); and (3) non-diabetic rats with DOCA-salt treatment (DN group). Animals in AL group were sacrificed at 23 weeks after dosing, but those of ADN and DN groups were sacrificed at 9 – 18 weeks after dosing as they developed a poor body condition.

Results: Systolic blood pressure in ADN and DN group treated with DOCA-salt was significantly elevated compared to that in AL group, but there was no significant difference between two hypertensive groups. Morphologic analysis showed reduction of myelinated fiber size due to reduced axonal caliber of the sciatic and tibial nerves in rats of AL and ADN groups, but these changes were less severe in the sural nerve. Endoneurial blood vessels in ADN and DN groups exhibited endothelial hypertrophy and narrowing of vascular lumen compared to AL group without hypertension.

Conclusion: These results suggest that combined diabetes and hypertension could not intensify DPN in WBN/Kob rats.



Poster Abstracts

P15: NASAL SWABS AS A SOURCE OF SAMPLES USEFUL IN SCREENING FOR JSRV INFECTION IN SHEEP

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Introduction: Jaagsiekte sheep retrovirus (JSRV) is an exogenous type D-related retrovirus recognized as the aetiological agent of contagious lung cancer of sheep (adenocarcinoma). The virus cannot be propagated routinely *in vitro* so virus isolation for diagnostic purpose cannot be used. Serological tests are also not available because of an absence of specific antibodies in the blood. Therefore diagnosis of the virus infection relies on the clinical history and histopathological examination of affected lungs. Application of ante-mortem diagnostic PCR is limited by low sensitivity in individual cases (the virus load in blood is extremely low and labile). The study aimed at testing the possibility of using nasal excretions from JSRV infected sheep as a source of samples for PCR based ante-mortem diagnosis of JSRV infection.

Materials and Methods: The source of material were small amounts of nasal excretions collected using swabs from nostrils of five sheep experimentally infected with JSRV. The small amounts of excretions were used for both DNA and RNA preparation and subsequently for PCR and RT-PCR amplification.

Results: First positive results of PCR and RT-PCR analysis of samples collected from nostrils of experimentally infected sheep were observed two months after infection and usually were coincident with the increased amount of nasal excretion. Testing the samples collected subsequently at the different time points showed consistently similar results.

Conclusion: The positive results of both PCR and RT-PCR amplification of JSRV genetic material present in nasal fluid of infected sheep make possible ante-mortem diagnosis of infection and is probably applicable to field conditions. The approach can be useful in screening OPA suspected flocks based on apparent clinical signs. Estimation of sensitivity and efficacy of the proposed approach in individual cases of suspected (clinically affected) animals requires the study of larger numbers of sheep.



Poster Abstracts

P16: PRENEOPLASTIC AND NEOPLASTIC MAMMARY AND NON-MAMMARY LESIONS ASSOCIATED WITH INFLAMMATION IN CHEMICALLY INDUCED CARCINOGENESIS IN WISTAR RATS

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Introduction: Recent data have expanded the concept that inflammation is an indispensable participant in the neoplastic process, fostering proliferation, survival and migration. The aim of the study is to follow up the incidence of chronic inflammation in chemically induced mammary carcinogenesis.

Material and methods: There were three groups of 37 days old Wistar female rats: group I inoculated with N-methyl-N-nitroso-urea (MNU), group II with MNU+astaxanthin (ASTA) in diet, and group III with ASTA. ASTA was administered orally (50µg astaxanthin/rat/day, 7 months). The experiment was finished at 14 months from MNU intake. Samples harvested for histopathology exam were processed using paraffin technique.

Results: Mammary tumor induction determined by MNU was reduced (33,3% – 37,5%). There were diagnosed several other tumor types in several organs (cholangiocarcinoma, nephroblastoma, lung carcinoma). Both groups inoculated with MNU encountered precancerous hyperplastic mammary lesions (simple adenosis, typical lobular epitheliosis), but no inflammation in mammary parenchyma. A chronic inflammation has been associated with hyperplasia and/or cancer in lungs, liver and kidneys.

Conclusion: highA strong association between chronic inflammation and MNU-induced carcinogenesis exists. Chronic inflammatory state may lead to environments that foster genomic lesions and tumor initiation.

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Poster Abstracts

P17: PATHOLOGIC STUDY AND DETECTION OF VIRUS IN GUINEA PIG ORGANS AFTER INOCULATION FOOT-AND-MOUTH DISEASE VIRUS TYPE O

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Introduction: The aim of this study was to determine the duration of persistence of FMD virus in different organs of guinea pig after intra dermal route of administration in plantar surface of leg as well as determining the histopathological lesions induced by the virus.

Materials and Methods: At first, we adapted the virus to the guinea pig, by injecting the FMD virus into 10 guinea pigs. After observations of generalization of the FMD virus and preparation of adapted virus to guinea pig, 30 other guinea pig were divided randomly into 5 groups, each group consisted of 6 animals. 5 animal in each group were injected with the adapted virus and one guinea pig in all groups was injected with normal saline. On 2, 4, 14, 30 and 60 days post inoculation (PI), all the animals from one of the groups were bled to determine antibodies to FMD virus by serum neutralization method. Then the animals were euthanized and samples of different organs (heart, lung, liver, spleen, pancreas, retropharyngeal lymph node, plantar epithelium, inguinal lymph node and tongue epithelium) were collected. One part of the each samples underwent a histopathologic examination. Virus isolation, Elisa and PCR tests were performed on the other part of the tissue samples.

Results: Based on our study the most outstanding lesions due to FMDV were observed in plantar epithelium, lung, tongue epithelium and spleen, respectively. Intracellular spongiosis, vacuolization and pyknosis of cells in spinous layer, intercellular edema in the spinous layer of Plantar and tongue epitheliums, increased size of interalveolar septa in the lung and decreased of white pulp in comparison with control animals were the most obvious lesions in histopathologic examination of the sampled tissue. The result of Sandwich Elisa tests showed that there was only low amount of virus in the spleen the on day 2 PI. Subsequently presence of virus was detected in the heart, lung, pancreas, non-inoculated plantar epithelium on day 4 PI. The quantity of the virus in non-inoculated plantar epithelium was higher than other organs. On day 14 PI, presence of the virus was detected in most organs. However, there was no sign of presence of virus in any organs on 30 and 60 days PI. Totally the results of Elisa tests and presence of virus, was well-matched with the lesions in plantar epithelium, lung, tongue epithelium and a little with the lesions in the spleen. Description of PCR assay result on 2 days PI showed weak bands from PCR product of spleen. Whereas, on day 4 PI same bands were observed in Heart, Lung, Pancreas, and plantar epithelium. On day 14 PI those band were observed in heart, lung, liver, pancreas, plantar epithelium. However, there was no band in any organs on days 30 and 60 PI. On 2 days PI rising of antibodies titer, reason for generalization virus in guinea pig s body, began to increase which was Continued until 4 days PI and reached to peak on 14 days PI. However, it was decreased on days 30 and 60 PI.

Discussion and Conclusion: Generally according to the results of present study, it is believed that the major sites of FMD virus persistence in guinea pig tissues are plantar epithelium, lung, and tongue epithelium respectively. The most increased level of virus persistence was on days 14 and 30 PI, respectively.

Poster Abstracts

P18: IMMUNE RESPONSES OF PROGENY OF HENS FED AFLATOXIN CONTAMINATED RATIONS

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Introduction: The present experimental study was conducted to determine the immunological responses of progeny of hens kept on aflatoxin (AFB₁) contaminated rations with or without vitamin E (VE).

Material and Methods: Layer breeder hens were divided into 12 groups and fed rations containing different possible combinations of AFB₁ (0.00, 0.01, 0.05, 2.5, 5.0 and 10.0 µg/kg) along with VE (0.0 and 100 mg/kg). Hens were inseminated artificially with semen of males kept on basal ration. Experimental feeds were offered for three weeks. Fertile eggs were incubated to obtain the progeny chicks from AF intoxicated hens.

Results & Discussion: Body weights of chicks of AFB₁ fed hens were significantly lower than controls. Antibody titers against SRBC were significantly lower in the chicks from the hens fed higher doses of AFB₁. Lymphoproliferative response to PHA-P of the chicks was significantly lower in progeny of the hens fed 0.50 µg/kg or higher levels of AFB₁. Peritoneal macrophages of the chicks fed AFB₁ showed significantly low phagocytic potential and nitrite production when challenged with LPS. Significant ameliorative effects of VE were found upon AF induced damage to RBC engulfment and NO production by peritoneal macrophages and anti-oxidant potential as determined by azo-bis compound.



Poster Abstracts

P19: AN EXPERIMENTAL STUDY OF HISTOPATHOLOGICAL AND FUNCTIONAL CHANGES OF THYROID GLAND DUE TO COADMINISTRATION OF SOY EXTRACT(SE) AND VITAMIN D₃ (CHOLECALCIFEROL) IN MICE

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Introduction: Soy is suspected to induce hypothyroidism. Vitamin D₃ (VD₃) decreases serum TSH.

Materials & Methods: 42 adult female mice were kept for 35 days in 7 groups. Oral administration of soy hydro-alcoholic extract and VD₃ was by gavage. Group 1 - 5ml/kg/day normal saline; Group 2 - 5g/kg/day SE; Group 3 - 100µg/kg/day VD₃; Group 4 - 5g/kg/day SE+100µg/kg/day VD₃; Group 5 - 10g/kg/day SE; Group 6 - 200µg/kg/day VD₃; Group 7 - 10g/kg/day SE+200µg/kg/day VD₃. The mice were bled to determine serum calcium, T₃, T₄, TSH and their thyroids were dissected for histopathological examinations. Data were analyzed by one way ANOVA followed by post-hoc Tukey test (p < 0.001).

Results: In mice receiving SE (2,5), decrease of T₃, T₄, increase of TSH, decrease of follicular epithelial cell height and increase of differences between follicle diameters were observed. Mice receiving VD₃ (3,6), showed increase of calcium, decrease of TSH and no important changes in follicular morphology. Mice receiving SE and VD₃ (4,7), showed decrease of T₃, increase of TSH, decrease of follicular epithelial cell height and increase of differences between follicle diameters.

Conclusions: This study confirms that soy can cause hypothyroidism. Extraction couldn't prevent the goiterogen factors in soy. Coadministration of SE and VD₃ could balance serum calcium and T₄, but the histopathological changes in these groups were present yet.



Poster Abstracts

P20: TELOMERASE ACTIVITY IN CATTLE INFECTED WITH BOVINE LEUKEMIA VIRUS (BLV)

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Introduction: Telomerase is a telomere synthesizing reverse transcriptase. This enzyme compensates the loss of telomere associated with cell division. Telomerase adds new telomeric sequences to the end of chromosomal DNA in order to overcome the end-replicating problem. In humans and other vertebrates, the telomeric sequence contains TTAGGG repeats. Telomerase activity is present in embryonal and germ cells, but is undetectable in most somatic cells, but many tumor cells have a high level of telomerase activity. Telomerase reactivation in tumor cells has been observed in some mammals. Therefore telomerase activity has been proposed as a tumor marker in these animals. The bovine leukemia virus (BLV) is an oncogenic B-lymphotropic retrovirus that causes enzootic bovine leukemia, the most common tumor in cattle.

Materials: Investigations were performed on the group of 80 cattle infected with BLV and specific antibodies and proviral DNA were detected in their sera by ELISA and PCR respectively. Telomerase activity was measured in sera, plasma, and cell lysates: lymphocytes, spleen, lymph nodes, bone marrow and supernatants of these cells, cultured *in vitro*. The same investigations were performed with materials taken from 21 control healthy cows.

Methods: Telomerase activity was determined with the use of commercial ELISA kit (Cusabio), according to the producer recommendation.

Results: The concentrations of telomerase in the sera of BLV-infected cows were determined from 0,119 ng/ml to 0,354 ng/ml. In the plasma, the telomerase concentrations were at levels of 0,105 to 0,279 ng/ml. In the supernatants from the *in vitro* cultured lymphoid cells these concentrations were estimated from 0,177 ng/ml to 0,482 ng/ml. In samples of control animals the telomerase activity was undetectable .

Conclusions: Similar to many tumors in humans, telomerase activity was detected in cows infected with bovine leukemia virus and this activity can be a useful marker for tumor development or therapeutic target.



Poster Abstracts

P21: EFFICACY OF ENDIGENOUS VACCINE AGAINST JOHNE'S DISEASE

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Introduction: The present study was conducted to see the protective efficacy of an endogenous vaccine in protecting for MAP infection in goats.

Materials & Methods: For this study goats was divided into two groups. Group I comprising 10 goats (Sham-immunized) and Group II comprising 15 goats (Indigenous vaccine) were challenged twice with 3×10^9 MAP Bison type strain S5 on 50 DPV and with 5×10^9 MAP Bison type strain S5 on 270 DPV.

Results: The goats of group II gained higher body weight as compared to sham-immunized goats while there was no significant difference in body weight gain between the vaccinated groups. The studies of cell mediated immunity revealed the impact of vaccinated and experimental infection by MAP S5 strain on the proliferation of PBMCs. The CMI response (SI value) increased at 30 DPV and showed down regulation from 90 DPV and onwards in vaccinated (group II) and control goats (group I). The studies on humoral immune response revealed that at 180 DPV seroconversion rates increased significantly in vaccinated goats and were maintained till 450 DPV. MLN collected at 200 DPV from sacrificed goat revealed presence of oedematous fluid and focal infiltrations of mononuclear cells with scattered presence of epitheloid cells and few giant cells. At 450 DPV infiltrations of large numbers of MNC and epitheloid cells forming sheet like arrangements with multinucleated giant cells were observed. The study of body score at 200 and 450 DPV on the parameters of body conformation, carcass components fat measurements revealed better marks in vaccinated animals (groups II) than control (group I).

Conclusions: Endogenous Vaccine was effective for controlling Johne's disease.



Poster Abstracts

P22: EXPERIMENTAL STUDIES OF PATHOGENECITY OF CHICKEN INFECTIOUS ANAEMIA VIRUS (3 ISOLATES) IN IRAN

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Introduction: Chicken Anaemia Virus (CAV) is a small non-enveloped icosahedra virus with a negative sense, single stranded circular DNA genome. It has been classified as the only member of the genus Gyrovirus of the family Gyroviridae.

Materials and Methods: The aim of this experiment was to evaluate pathogenicity of 3 Chicken Anemia Virus isolates, CV1, CV2 and CV3. 30 one day old SPF chicken were grouped and received an intramuscular inoculation of one isolate per group. Two other groups (control groups) were inoculate with a live vaccine virus and normal saline respectively. The packed cell volumes were determined on blood samples from each bird. Antibodies were measured using the Competitive Eliza Test . The liver, bursa of Fabricius, spleen, thymus and skeletal muscle organs were fixed in neutral buffered 10% formalin, processed and embedded in paraffin. The blocks were sectioned (5µm) and stained with Hematoxylin and Eosin. The lesions of bursa and thymus were evaluated for lymphocyte depletion and scored as 1: normal, 2: mild, 3: moderate and 4: severe infections.

Results: The birds in first three groups showed ruffled feathers, depression and body weight reduction. After 18 days they were weighed, bled and euthanized. Three birds were found dead during the experiment (one in each test group). Hematocrit values of the three tested groups were below normal. Grossly the thymus and bursa tissue were severely atrophied. Bone marrow was yellow and pale. Severe atrophy and depletion in thymus, bursa of Fabricius and bone marrow tissue was observed and this was statistically significant in comparing with control groups ($p < 0.05$).

Discussion and conclusion: While CAV infection is understood to be most pathogenic in young growing birds, until now the infection has only been traced in slaughter age chickens in Iran. The present work showed pathogenicity of CAV in day-old chicken and displayed the detrimental impacts of CAV on immune system of chickens with apparent concentration in thymus.



Poster Abstracts

P23: IMMUNOHISTOCHEMICAL CHARACTERISATION OF IMMUNE CELL SUBSETS ON LYMPH NODES FROM WATER BUFFALOES

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Introduction: Water buffaloes (*Bubalus bubalis*) play a crucial role in Asian agriculture and their importance is increasing in western nations. They are susceptible to similar aetiological agents of disease as cattle but the outcome may be different. This may be due to differences in the responses of the immune systems in the two different species. The aim of this work was to characterise immune system cell subsets on fixed lymph nodes from buffaloes.

Materials and Methods: Immunohistochemistry was performed on zinc salts fixed paraffin-wax embedded lymph nodes from healthy water buffalo. Monoclonal antibodies (mAbs) were selected from those used in other species or reported previously for water buffalo tissues using other techniques.

Results: Specific labelling was observed using mAbs previously unreported in buffalo tissues [EBM11 (macrophages), CC58 (CD8 T-cells), IL-A29 ($\gamma\delta$ TCR), NKp46 (NK-cells) and HM57 (B cells)] or using clones previously described for use in flow cytometry [MMIA (CD3 T cells), IL-A11 and CC30 (CD4 T-cells)].

Discussion: The results from this study provide a new panel of mAbs to investigate the buffalo inflammatory response to diseases in fixed tissues. Other mAbs previously used in ruminants could also be examined to provide further tools for use in water buffalo tissues.



Poster Abstracts

P24: SYSTEMIC CANDIDIASIS IN A HOVAWART DOG

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Introduction: Over a two week period, a 3-year-old female Hovawart developed progressive neurological symptoms, polyuria/polydipsia and was euthanized and submitted for necropsy.

Materials and Methods: Gross and microscopic examination and mycology culture were performed.

Results: Numerous white, solid masses were seen in the renal cortex, pelvis and medulla. The left axillary lymph node and deep caudal cervical node were moderately enlarged, and meninges were slightly yellow. Histologically the renal tissues were disrupted by multiple granulomas with rich fungal colonization. Granulomas had a central area of caseous necrosis with a capsule of fibrous granulation tissue richly infiltrated with leukocytes (macrophages, epithelioid cells, multinucleate giant cells, lymphocytes and plasma cells). Fungal organisms were elongate and had budding pseudohyphae endowed with thick-walled, oval to round spores. *Candida* species was isolated from the left kidney, and further identification is ongoing. The brain, spinal cord, meninges and enlarged lymph nodes displayed scattered granulomas with numerous fungal organisms.

Discussion: This dog had systemic candidiasis but its pathogenesis is unclear. Fungal organisms were observed in lymphatic vessels and veins, but not in arterioles, suggesting lymphatic or venous dissemination, possibly aided by phagocytosis and transport of viable intracellular organisms by circulating macrophages. Systemic mycosis develops most often in debilitated or immunosuppressed patients. Polyuria/polydipsia suggests that the dog may have had diabetes mellitus but this could not be verified.



Poster Abstracts

P25: E-CADHERIN EXPRESSING SCHWANN CELLS OFFER A PORT OF ENTRY FOR *LISTERIA MONOCYTOGENES* NEUROINVASION IN RUMINANT RHOMBENCEPHALITIS

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Introduction: In listeric rhombencephalitis of ruminants, LM likely enters the brain via cranial nerves, but involved host cell receptors are not known. We investigated the putative role of E-cadherin, the host cell receptor for the major LM surface ligand internalin A, in brainstem invasion and intracerebral spread of LM.

Material and methods: E-cadherin expression and localization of LM in the nervous system of ruminants with and without natural listeric brainstem encephalitis was determined by immunohistochemistry and double-immunofluorescence.

Results: E-cadherin is expressed in choroid plexus, meningotheilium and restricted neuropil areas of the medulla, but not in the endothelium. In cranial nerves and ganglia, E-cadherin is expressed in Satellite cells and myelinating Schwann cells. Expression does not overlap with the presence of microabscesses in the medulla. LM is observed in phagocytes, axons, Schwann cells, Satellite cells and ganglionic neurons.

Conclusion: The E-cadherin expressing oral epithelium and glial cell compartment of cranial nerves provide a port of entry for free bacteria offering a site of intracellular primary replication, from where the bacterium may invade axons by cell to cell spread. It is likely that intracerebral spread is independent of E-cadherin and relies primarily on axonal migration.



Poster Abstracts

P26: COX2 EXPRESSION INCREASES IN SKIN BIOPSIES FROM DOGS AFFECTED BY NODULAR CUTANEOUS LEISHMANIOSIS

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Introduction: Cyclooxygenase 2 (COX-2) and endothelial growth factor (VEGF) expression in dogs affected by nodular skin leishmaniosis was evaluated, because they are less resistant to the infection due to a lack of priming of T lymphocytes to leishmania by langerhans cells and MHC-II+ cheratinocytes.

Materials & Methods: 8mm skin biopsies were sampled from 8 dogs affected by nodular cutaneous leishmaniosis, 3 dogs affected by “sterile” chronic active dermatitis and 5 healthy dogs. Serial tissue sections were incubated with a set of antibodies in order to assess the COX2 (Goat pAb anti-COX2, ab23672, ABCAM) and VEGF (Mouse anti-Canine VEGF mAb, clone 247109, R and D Systems) expression.

Results: COX2 and VEGF expression was significantly higher in *Leishmania* positive (L+) samples than in L- ones, and almost absent in the healthy skin. L+ samples showed strong COX2 and VEGF expression in the endothelial cells of dermal and hypodermal capillaries as well as nervous terminations while L- samples displayed COX2 positiveness only in macrophages, small groups of dermal fibroblasts and rare neutrophils. COX2 and VEGF expression was occasionally seen in few fibroblasts and endothelial cells in control samples.

Conclusions: Strong local COX2 and VEGF expression in dermatitis induced by *Leishmania* sp. could be correlated to a weaker macrophage reactivity as well as to their insufficient ability to carry out the parasite “binding” and “killing” activity probably due to higher local synthesis of PGE2.



Poster Abstracts

P27: GENERALIZED PARVOVIRUS-INFECTION OF THE CNS IN A PUPPY

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Introduction: Canine parvovirus-infection (CPV) typically leads to katarrhalic enteritis, lymphocytic depletion and panmyelophthisis. The detection of parvovirus in the central nervous system (CNS) of dogs has been discussed controversially but recent data indicate that brain cells could be more often affected than anticipated.

Materials and Methods: A 9-weeks-old male labrador puppy died of parvovirus-infection and pneumonia. Organs were fixed in formalin, embedded in paraffin and used for HE-staining, immunohistochemistry (IHC) and *in situ* hybridization (ISH). IHC was performed using a monoclonal and a polyclonal anti-parvovirus-antibody, ISH was carried out applying digoxigenin (DIG)-labelled probes (DNA-probe 315 bp, RNA-probe 222 bp) detecting CPV-virusprotein (VP) 1 and VP2-DNA and mRNA stretches.

Results: Gross lesions consisted of a katarrhalic enteritis with depleted Peyers patches, saggy spleen and deep red bone marrow. Histologically, hemorrhagic pneumonia, shortened, sticky villi with moderate lymphohistiocytic infiltration of the intestinal tract and moderate follicular depletion of lymphatic tissues was present. Parvovirus antigen was detected in the small intestine, spleen, mesenterial lymphnode, liver, kidney and lung. Interestingly, there was a widespread infection of neurons, cerebellar granule cells, glial cells and endothelial cells in the CNS. By ISH fewer CNS-cells were labelled, predominantly in the hippocampus and cerebellum.

Conclusion: The CNS can be infected in case of generalized parvovirus infection of the dog. Whether canine CNS manifestation depends on the virus subtype, host factors such as age, vaccination status or immune status has to be further investigated employing respective animal cohorts.



Poster Abstracts

P28: INTRACEREBRAL SPREAD OF *LISTERIA MONOCYTOGENES* ALONG AXONS IN RHOMBENCEPHALITIS OF RUMINANTS

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Introduction: Listeriosis is an important food-borne infection in humans and ruminants caused by *Listeria monocytogenes* (LM). In ruminants, rhombencephalitis is the most common clinical form of listeriosis. The knowledge about its neuropathogenesis, and particularly how LM invades and spreads within the brain, is only fragmentary. The aim of this study was to establish an exact mapping of microabscesses and to investigate the cellular localization of LM in the brain in order to determine whether LM spreads intra-axonally from the brainstem into rostral areas.

Material and methods: Topographical mapping of microabscesses in 16 sheep, 12 goats, and 7 bovines with listeriosis was performed on H&E sections. Brain tissues were analyzed with triple-immunofluorescence (IF) using antibodies against LM and neurofilaments, and TOTO-3.

Results: In all three species a selective topography of microabscesses was observed with affection of specific white matter tracts. Grey matter lesions were predominantly located in nucleus interstitialis, nucleus ruber, and nuclei of the IX, III, V CN (in descending order). Using triple-IF, LM could be shown closely associated with and parallelly oriented to neurofilaments.

Conclusions: The peculiar topography of microabscesses with predominant affection of certain white matter tracts is highly indicative for intracerebral spread along axons. Intra-axonal migration of LM is supported by our IF-results.



Poster Abstracts

P29: A CASE REPORT OF TUBERCULOSIS IN PEAFOWL (*PAVO CRISTATUS*)

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Introduction: Tuberculosis in peafowl is a rare event supported by the reports of a few experimentally and naturally induced infections. This insufficiency of data is consistent with the lack of knowledge about pathological features as well as peafowl susceptibility to this disease.

Material and methods: One 3-year-old peahen was submitted for necropsy. Imprints of organs with granulomas were used for performing bacteriological investigation (Ziehl-Neelsen stain). Organs were sampled for routine histopathology and special histological stains (Masson trichromic, PAS and Ziehl-Neelsen).

Results: Extensive miliary and large caseous granulomas were identified in trachea, lung, thoracic wall (*mm. intercostalis*), liver, spleen, limit between esophagus and proventriculum, intestine and subcutaneously (inferior cervical region). Granulomatous lesions were associated with a chronic posttraumatic ventriculitis (total rupture of wall opened into a large fibrous diverticulum). Particularly, histological features presented typical, solid and well-organised granulomas with various degrees of inflammation and peripheral fibrous wall associated with non-encapsulated granulomatous reaction between confined lesions. Some of multinucleated giant cells mimicked the features specific for Langhans cell of mammals' bacterial granuloma, placed far from necrotic foci.

Discussions and conclusions: Pulmonary lesions are rare in birds, because of the lack of binding affinity of *Mycobacterium avium* for respiratory epithelium. Multiple pathway of contamination may be considered for this case: digestive (sustained by the lesions of the intestine, liver and spleen) and respiratory (lung). Subcutaneous granuloma was probably generated *via* perforation of ventriculum, posttraumatic diverticulum being located adjacent the mycobacterial lesion.



Poster Abstracts

P30: APOPTOSIS IN LYMPHOID TISSUES OF PRRSV INFECTED PIGS DETECTED BY TUNEL AND CLEAVED CASPASE-3 IMMUNOHISTOCHEMISTRY

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is characterized by the immunosuppression of infected animals, however, the mechanism of induction of apoptosis in PRRS has not been elucidated. This study focuses on the evaluation of the apoptosis phenomena by microscopic examination, cleaved caspase 3 (CCasp3) immunohistochemistry and TUNEL method in lymphoid organs of PRRSV infected pigs

Materials and Methods: Twenty-eight pigs were inoculated with PRRSV and killed sequentially until 24 days post-inoculation (dpi). As controls, four other pigs were inoculated with 1 ml of sterile medium and killed at 24dpi. CCasp 3 and TUNEL immunolabelling was carried out with commercial kits (Signal Stain-Cleaved Caspase 3 Asp175, Cell Signaling; and, *In situ* cell death detection, POD, Roche, respectively)

Results: Apoptotic bodies and/or pyknotic nuclei, were observed from 3 dpi onwards, coinciding with the beginning of PRRSV expression, which reduced later. In contrast, CCasp3 expression and TUNEL positive results were seen only in few animals at 24 dpi in lymphoid organs.

Conclusion: The early detection of apoptotic phenomena and PRRSV expression together with the delayed and scattered positivity shown to CCasp3, suggests that apoptosis may be triggered by caspase 3 independent pathway in PRRS.



Poster Abstracts

P31: *BALANTIDIUM COLI* INFECTION IN A BELGIAN WARMBLOOD FOAL

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Introduction: *Balantidium coli* is a ciliated protozoan that is considered as a common commensal parasite of the large intestine of man, rodents, swine and nonhuman primates, but becomes rarely a pathogenic opportunist by invasion of tissues that have been damaged by other diseases.

Case history: A five month old Belgian Warmblood foal was presented with tachypnoea, stridor and fever. After therapy with antibiotics and anti-inflammatory drugs, the foal died after 8 days. Necropsy was performed and showed a hemorrhagic necrotizing typhlocolitis, catarrhal enteritis and interstitial pneumonia.

Material and methods: At necropsy, samples of the large intestine, small intestine and lung were taken for histopathology.

Results: Hemorrhagic necrotizing typhlocolitis with an accumulation of trophozoites of a *Balantidium coli*-like protozoan in the mucosa were found histologically. The protozoal trophozoites were 30-150 µm long with one large macronucleus and cilia on the external surface. There was an intense diffuse infiltration of lymphocytes, plasma cells and eosinophils in the mucosa and submucosa. In the small intestine there were sporadic macro- and microgamonts of *Eimeria* spp. present.

Conclusion: *Balantidium coli* can be associated with hemorrhagic and necrotizing typhlocolitis in horses. In guinea pigs there is evidence that *Balantidium coli* is a pathogenic opportunist secondary to *Eimeria caviae* infection. This has not yet been described in horses.



Poster Abstracts

P32: DOWNREGULATION OF ANTI-INFLAMMATORY CYTOKINES IN BRAIN DURING CANINE VISCERAL LEISHMANIASIS

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Introduction: Canine visceral leishmaniasis (VL) is caused by the intracellular parasite *Leishmania chagasi* (= *infantum*). Similar to other protozoan diseases, the peripheral infection culminates with disturbances in the nervous system, histopathologically represented by leptomeningitis and choroiditis.

Materials and Methods: Brain samples of 10 infected and of 5 uninfected dogs were collected and IL-10 and TGF- β gene expression were measured by real time RT-PCR. The results were given using the $2^{-\Delta Ct}$ method and differences between groups were assessed by Mann Whitney test.

Results: The infected dogs revealed lower levels ($P=0.0109$) of IL-10 (4.16×10^{-5}) when compared with the control group (5.32×10^{-4}). TGF- β was also lower ($P=0.0047$) in the infected group (2.58×10^{-3}), in comparison with the control group (1.36×10^{-2}).

Discussion and Conclusion: IL-10 and TGF- β possess an immunoregulatory role mainly involving the suppression of the Th1 response. In brain, they prevent leukocyte entry, glial activation and promote neuronal and glial survival. These results are compatible with our previous findings of high numbers of T cells and increased glial reactivity in the CNS of dogs with VL. Taken together, these data reflect the loss of the neuroprotective effects of these cytokines as well as a compartmentalized profile of cytokine in the brain during VL.



Poster Abstracts

P33: GENERALIZED TOXOPLASMOSIS IN FIVE CATS

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Introduction: Felids are the only definitive hosts for *Toxoplasma gondii*. Feline infections are usually subclinical, but severe and even fatal infections have also been described.

Materials and Methods: All feline cases submitted for necropsy and diagnosed with toxoplasmosis between 2008 and 2010 (Faculty of Veterinary Medicine, University of Helsinki) were retrospectively investigated. The original necropsy reports and histological tissue sections were reevaluated. In addition, immunohistochemistry (IHC) with *Toxoplasma gondii* epitope-specific antibody was performed from all tissues available.

Results: During the three-year period, five (3.2 %) of 157 cats were diagnosed with generalized toxoplasmosis. Main pathological lesions were multifocal to diffuse interstitial pneumonia, multifocal necrotizing hepatitis, and multifocal nonsuppurative meningoencephalitis with glial granulomas. In addition, necrotizing lymphadenitis and splenic red pulp hyperplasia were common findings. Occasional mild inflammatory foci were seen in heart, pancreas, skeletal muscle and adrenal glands. IHC demonstrated mild to massive parasite burdens not only in tissues with pathological lesions, but also in unaffected tissues.



Poster Abstracts

P34: INTESTINAL MYIASIS IN PIGS

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Introduction: Myiasis is the infestation of live human and vertebrate animals with dipterous larvae, which, at least for a certain period, feed on the host's dead or living tissue, liquid body substances, or ingested food. Broadly, myiasis can be divided into three types: cutaneous myiasis, body cavity myiasis, and accidental myiasis. Intestinal myiasis, an accidental phenomenon occurring when fly eggs or larvae are ingested with food and excreted with faeces, is usually transient and asymptomatic; however in some cases infestation can be associated with symptoms.

Material and Methods: During the final autopsy of an experimental study in piglets, fly larvae were found in the intestinal tract of the negative control animals housed in a separate box. Several pigs (15 weeks old) showed presence of larvae in the ileum and caecum and some animals had intestinal inflammation. Except for the presence of larvae, no prominent changes in the faeces consistency could be noticed. Samples of the whole intestinal tract were taken for histopathological examination.

Results: will be discussed in the poster

Discussion: This is the first time that intestinal myiasis is described in pigs. The major cause in the rare cases described in humans is attributed to the *Musca domestica* or common house fly.



Poster Abstracts

P35: OTITIS MEDIA ASSOCIATED WITH CHOLESTEOMA AND LEPTOMENINGITIS IN A CAT DUE TO A STREPTOCOCCUS INFECTION

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Introduction: Otitis media is usually due to an extension of infection from the external ear canal or to penetration of the tympanic membrane by a foreign object. It is seen in all species but, is most common in dogs, cats, and rabbits. Hematogenous spread of infection to these areas is possible but rare. Otitis media may lead to otitis interna and inflammation of the inner ear structures. Chronic otitis media (COM) can be divided in two subtypes: COM with and without cholesteatoma (including precholesteatomatous states). The first is an aggressive form of otitis which can lead to labyrinthine or cerebromeningeal complications. It has been described in human and dogs but never before in cats.

Material and Methods: Our case concerned an adult, black female domestic long-haired cat which was found astray with signs of cachexia, moaning when manipulated, apathy, depression and weakness, circling and leaning to the right and ataxia. Due to bad health status of the animal it was decided to euthanize the cat. Necropsy was performed and lesions noted. Samples of the right bulla tympanica wall, as well as from the cerebrum, cerebellum and brainstem, liver and spleen were taken for bacteriological (including 16SrDNA sequencing) and histopathological examination.

Result: Will be presented on the poster.



Poster Abstracts

P36: IMMUNOHISTOCHEMICAL LOCALIZATION OF HAPTOGLOBIN IN PORCINE SALIVARY GLAND AND DIAPHRAGM TISSUES

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Introduction: Changes in the concentration of haptoglobin (Hp) have been reported in saliva and meat juice samples; however the extrahepatic localisation of this protein is uncertain. In this study, immunohistochemistry was employed to localise haptoglobin.

Materials and Methods: Five healthy and five diseased conventional pigs from a finishing unit which was seropositive to PRRSv, PCV2, *Mycoplasma hyopneumoniae* and *A. pleuropneumoniae* were used in the present study. Samples from liver, salivary gland and diaphragm muscle were collected and fixed in 10% buffered formalin. Immunohistochemical study was performed following an ABC method by using an in-house monoclonal antibody against porcine Hp.

Results: In diseased animals, the expression of Hp was confirmed by means the immunolabelling in the liver. In the salivary gland, Hp was detected in the cytoplasm of scattered glandular epithelial cells, as well as within the cytoplasm of duct epithelial cells. A multifocal immunostaining of myofibers of skeletal muscle was observed.

In contrast healthy pigs only displayed a mild to poor immunolabelling of Hp in salivary gland and diaphragmatic muscle.

Conclusion: The extrahepatic localization of Hp observed would suggest a contribution of these tissues to the increment of Hp levels found in saliva and meat juice samples in inflammatory conditions.



Poster Abstracts

P37: ISOLATION AND CHARACTERISATION OF NOVEL MAP STRAINS FROM UGANDAN CATTLE

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Introduction: *Mycobacterium avium subspecies paratuberculosis* (MAP) infection has been confirmed only recently among Ugandan cattle. Hence, no information exists on the diversity of MAP strains from the country. The aim of our study was to isolate and characterise MAP from faeces of ELISA positive cattle and tissues with histologically suspected lesions of Johne's disease.

Materials and Methods: 21 MAP isolates, confirmed by their mycobactin dependence on Herrold's egg yolk medium and IS900 PCR, were characterised using molecular markers comprising Short Sequence Repeats (SSR, loci 1, 2 and 8), mycobacterial interspersed repeat units (MIRU, loci 2 and 3), Variable number tandem repeat units (VNTR, locus 32) and IS1311 PCR-REA analysis.

Results and Discussion: The 21 isolates were differentiated into 10 different strains using a combination of all the markers. These strains were distributed throughout the country. The results show the existence of the cattle and bison type strains in the country. Two isolates showed a yet unreported IS1311 pattern designated as X type. The study has also shown the existence of new SSR genotypes of MAP previously unreported in cattle elsewhere.



Poster Abstracts

P38: FIRST REPORT OF ALEUTIAN DISEASE IN A LEAST WEASEL (*MUSTELA NIVALIS*)

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Introduction: Aleutian disease (AD) is a slow infection caused by a parvovirus (ADV) and almost exclusively concerns the mink, although antibodies have been demonstrated in other species.

Materials and Methods: Gross pathology, histopathology and PCR were used to establish the diagnosis of AD in an eight-month-old male least weasel.

Results: The animal was found dead at the Thessaloniki Zoo. It had decreased appetite for 3-4 days. It had been kept as a pet and was donated to the zoo two months prior to its death. In both places it was housed alone. On necropsy, it was cachectic and showed diffuse alopecia. Multiple small whitish foci were scattered throughout the lungs. The liver and spleen were severely enlarged. Histologically, severe multifocal or diffuse plasma cell infiltrations of various organs were observed. Using PCR, ADV DNA was detected from various organs.

Discussion and Conclusion: The source of infection in the present case is unknown; given the fact that no mink or other Mustelidae were kept at the zoo. It is possible that it was another animal carrying the virus, that came in contact with the weasel, most likely while it was kept as a pet. AD is reported for the first time in this species.



Poster Abstracts

P39: DIAGNOSIS OF CAPRINE TUBERCULOSIS USING ESAT-6/CFP-10 PEPTIDES IN PERSISTENTLY INFECTED HERDS

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Introduction: A caprine tuberculosis eradication-programme based on the comparative tuberculin skin test is being implemented in the South-East of Spain. Although initial progress was promising, the programme subsequently stalled. Two observations made were that the presence of paratuberculosis in the flocks and the desensitisation caused by repeat tuberculin skin test could lead to a decrease in the skin test sensitivity.

Material and Methods: To evaluate the efficacy of the alternative blood-based IFN- γ assay in conjunction with peptides derived from the specific antigens ESAT-6/CFP-10 for the diagnosis of caprine tuberculosis, two goat herds with persistent tuberculosis co-infected with paratuberculosis were selected for study. The results obtained using these antigens were compared with skin test and the IFN- γ assay using avian and bovine tuberculin. Several rounds of testing with the three techniques were carried out in each herd and test-positive goats were killed after each test round to establish the presence of tuberculosis infection by macroscopical and microscopical analysis of lesions and *M. caprae* isolation.

Results and Conclusion: The IFN- γ assay using ESAT-6/CFP-10 performed better (72% of sensitivity and 90% of specificity) than the other skin test and IFN- γ assay using PPD (sensitivities of 40% and 68%, specificities of 45%, and 84%, respectively). The false negative animals detected in the study had small single lesions, generally fibrocalcified, that could correspond with non-active/latent infections.



Poster Abstracts

P40: MYCOBACTERIOSIS IN PSITTACINES: A POTENTIAL FOR ZOOONOTIC DISEASE?

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Introduction: *Mycobacterium spp.* is still a problem, especially in ornamental fowl and pet birds and, for underlying the relevance of this infection in the latter, the authors report a retrospective study on mycobacteriosis in 123 psittacines.

Materials and Methods: These cases were analyzed by means of gross examination, histopathology (haematoxylin and eosin, Ziehl-Nielsen stain) and 23/123 cases through a Polymerase Chain Reaction-Restriction Enzyme Digestion (PCR-RED) based upon Hsp65 gene for the identification of *Mycobacterium (M.)* species.

Results: The most commonly affected species were Amazon parrots and grey-cheeked parakeets. Lesions were observed in liver, spleen, intestine, lungs, air sacs, conjunctiva, eyelid, skin, infraorbital sinus, heart, pancreas, kidney, testes, ovary and, only histologically, in adrenal, bone, skeletal muscle, thymus, brain, pancreas, synovium, parathyroids, thyroid, perineurium. Most commonly, infiltration of numerous epithelioid cells or foamy macrophages, with or without multinucleated cells, containing acid-fast bacilli, occurred. An interesting lesion was the granulomatous aortitis in a cockatiel concurrent with atherosclerosis. 20/23 cases submitted for molecular diagnosis were positive for *M. genavense*, 2/23 for *M. avium* and 1/23 had mixed infection.

Conclusions: *Mycobacterium genavense* represents the primary agent of mycobacteriosis in psittacines and the potential for zoonosis must be considered, especially in immunocompromised persons, children and pet birds' breeders.



Poster Abstracts

P41: EVALUATION OF PATHOGENICITY OF TWO RANAVIRUSES (RCV-JP, HNV) ISOLATED FROM THE BULLFROG AND A SALAMANDER FOR JAPANESE NATIVE AMPHIBIANS

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Introduction: Five mass die-offs in wild amphibians due to ranavirus occurred in 2009 and 2010 successively in 4 Prefectures. Additionally, an outbreak occurred in a protected colony of *Hynobius nebulosus*, and 2 rana virus strains (RCV-JP, HNV) were isolated and established. The aim of this study is to evaluate the pathogenicity of these 2 ranaviruses for Japanese native amphibians.

Materials and Methods: We prepared 13 native species (8 salamanders and 5 frogs, n = 486 individuals) for challenge experiments, and inoculated them by bath and /or intraperitoneally with virus. The animals were then examined with histopathological and molecular biological techniques.

Results: The mortality rate of RCV-JP was 100% in salamanders and 33 – 100 % in frogs. The mortality rate of HNV was 0-100 % with high mortality in all salamander species except *H. nigrescens*. Additionally, mortality was greatest at elevated temperatures, especially in larvae. In gross findings, systemic edema, multi-centric ulceration and hemorrhage of skin and necrosis of toe tips and/or tail were observed in larvae. There were body cavity dropsy, hepatic enlargement and atrophy of spleen in adults. Necrotic changes in the parenchyma of organs was the dominant histological lesion. Sometimes, intra-cytoplasmic basophilic inclusion bodies were present in hepatocytes and epithelial cells of the kidneys.

Conclusions: These two viruses have the potentiality to affect the Japanese ecological system, because the viruses showed high pathogenicity for native amphibian species.



Poster Abstracts

P42: *CRYPTOSPORIDIUM BAILEYI*-INFECTION IN RED-BREASTED MERGANSER DUCKLINGS

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Introduction: *Cryptosporidium (C.) baileyi* is a coccidian parasite, which infects different avian species including ducks. The primary site of the parasite is the intestinal tract including the Bursa of Fabricius (BF).

Materials and Methods: Necropsy and histopathology (H&E, modified Ziehl-Neelsen stain) was performed on five, two to three weeks old, hand-reared Red-breasted Mergansers (*Mergus serrator*) that had died during one week in a zoological garden. The cryptosporidial species was identified by PCR and partial sequencing of the 18S rRNA gene. In addition, in-situ hybridization (ISH) specific for cryptosporidia was carried out.

Results: At necropsy, all five ducklings were emaciated and anemic. Histologically, high numbers of cryptosporidia were attached to the apical surface of the bursal epithelial cells and present inside the lumen of the BF. The epithelium was moderately hyperplastic, irregular structured and showed a mild heterophilic infiltration. With the modified Ziehl-Neelsen stain only few luminal cryptosporidia were positive. In contrast, with ISH numerous cryptosporidial stages could be detected easily.

Discussion: This is the first report of bursal cryptosporidiosis in the Red-breasted Merganser. The infection with *C. baileyi* induced an irregular hyperplasia of the bursal epithelium and mild heterophilic bursitis in these ducklings, which is in agreement with the lesions found in other duck species after natural and experimental infection.



Poster Abstracts

P43: PATHOLOGY OF AVIPOXVIRUS INFECTION IN PIGEONS

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Introduction: The present study describes pathological and electron microscopic findings of pox lesions in two naturally infected pigeons.

Results: The most prominent external examination was small focal to coalescing wart-like nodular lesions on the featherless areas of skin. At necropsy, there were no significant internal lesions. Histopathologically, the epithelial cells of skin were proliferative with ballooning degeneration, many of which had eosinophilic intracytoplasmic inclusion bodies known as Bollinger bodies. Ultrastructurally, inclusions with virions around periphery and virus-filled inclusions as well as free virions were observed in cytoplasm of the affected cells. Avipoxvirus was confirmed by identifying 250-350 nm virions with a dumbbell-shaped and typical thickness of chorio-allantoic membranes (CAMs) infected with virus.

Conclusions: This study showed that pathology played a key role not only in identification but giving information of the virus simultaneously.



Poster Abstracts

P44: PATHOLOGIC FINDINGS IN RED-LEGGED PARTRIDGES (*ALECTORIS RUF*A) AND COMMON PHEASANTS (*PHASIANUS COLCHICUS*) NATURALLY INFECTED WITH BAGAZA VIRUS (BAGV) IN SPAIN

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Introduction: *Bagaza virus* (BAGV) is a mosquito-borne Flavivirus, belonging to the Ntaya group that has been isolated from mosquitoes in Africa and India. Antibodies against BAGV have been detected in humans in India, but its pathogenicity for humans is still unknown. It had not been detected in Europe, until August 2010 when an outbreak caused by BAGV started among game birds in the south of Spain (Cádiz).

Materials and Methods: Detailed necropsies of six red-legged partridges (*Alectoris rufa*) and three common pheasants (*Phasianus colchicus*) were carried out. A complete set of tissues was taken and fixed in 10% neutral buffered formalin for processing for histology.

Results: Macroscopic lesions included injection of cephalic and coronary vessels, pallor of the pancreas, pectoral muscle and the myocardium, and altered consistence and color of the spleen. Microscopic lesions were found mainly in the central nervous system consisting of congestion, gliosis, necrosis and perivascular cuffing. Most of the individuals had congestion and/or inflammation in the gross intestine, the proventriculus, the pancreas, the liver, the kidney and the heart. Necrotic foci, thickened capsule and granulocytic infiltrates were found in the spleen.

Conclusions: BAGV was highly pathogenic for Spanish game birds causing macroscopic and microscopic lesions, showing an important tropism for the nervous system.



Poster Abstracts

P45: *HALICEPHALOBUS GINGIVALIS* INFECTION IN A HORSE

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Introduction: *Halicephalobus gingivalis* is a saprophytic free-living nematode that is infrequently identified in horses and humans. *Halicephalobus gingivalis* appears to have a tropism for the central nervous system and kidney in horses.

Case history: A five year old Connemara mare was presented with facial swelling and weight loss. After five days the horse had trouble breathing. Due to the poor condition of the horse, the owners elected to have the horse euthanized. Necropsy showed a diffuse inflammatory swelling of the head, mainly involving the mandibula and maxilla.

Material and Methods: At necropsy, samples of the gingiva were taken for histopathology.

Results: Microscopic lesions consisted of granulomatous inflammation and fibrosis of the gingiva. Numerous *Halicephalobus gingivalis* larvae were present within the lesion. The larvae were 50-100 µm long with presence of a characteristic oesophagus with a corpus, isthmus and bulb. The inflammatory infiltrate consisted of macrophages, lymphocytes, plasma cells, neutrophils and multinucleated giant cells.

Conclusion: *Halicephalobus gingivalis* should be considered as a cause of facial swelling and mandibular bone deformation in horses. The mechanism by which *Halicephalobus gingivalis* establishes an infection remains speculative.



Poster Abstracts

P46: CORRELATION BETWEEN TISSUE INVADED BY *CANDIDA ALBICANS* AND CYTOKINES SECRETION IN EXPERIMENTAL ANIMALS

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Introduction: Systemic candidiasis is an important infection in patient with innate immunodeficiency. The aim of this study was to evaluate the expression of TLR-2, INF- γ , TNF- α , IL-2 and IL-12 and also histopathological finding of Candida invasion to different organs

Materials & Methods: Thirty male BALB/c mice were studied in three groups: Group 1(G1) which received intramuscular cortisol (1 mg/kg) for three days and then, they were challenged with Candida albicans (1×10^5 cells) intraperitoneally. Group 2(G2) received only Candida at the same dose of G1. Normal saline were administrated to group 3 (G3) via intraperitoneal injection. After ten days TLR-2 and cytokines were evaluated in sera samples by standard methods and also histopathological studies were done after euthanasia.

Results: In this study a significant decreasing was observed in expression of TLR-2, INF- γ , TNF- α , IL-2 and IL-12 in G1 compared to G2 and G3. The invasion of Candida albicans to kidney resulted in tissue necrosis, haemorrhage and infarction. In lung tissue the presence of the yeasts in vessels and air sacs were dominated. In G2 animals moderate injuries were observed just in kidney.

Conclusion: Our finding indicated that cytokines can stimulate innate immunity against systemic Candidiasis while corticosteroids inhibit Th1 cytokines expression and functions and animals are in danger to systemic opportunistic fungal infections.



Poster Abstracts

P47: UNUSUAL MANIFESTATION OF CRYPTOCOCCOSIS IN A SWAMP WALLABY (*WALLABIA BICOLOR*)

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Introduction: Cryptococcosis is caused by an infection with *Cryptococcus neoformans*, a fungus which is usually harbored in soil and the manure of some birds. A ten years old, female swamp wallaby presented with clinical signs of lethargy, weight loss and prior to death a head tilt, nystagmus and paresis of the hindlimbs was noted.

Materials and Methods: The animal was submitted for necropsy. Fixed tissue samples had been processed by routine methods and stained with hematoxylin and eosin. Additionally, periodic acid-Schiff (PAS) reaction and Grocott special stain were performed on several tissue sections.

Results: Macroscopically, only a moderate internal hydrocephalus was seen. In the histological examination, a variable, mainly moderate and pyogranulomatous inflammation in the cerebrum, cerebellum, spinal cord and unilaterally in the petrosal bone was detected. In these organs and also in the thyroid gland, lung, and conjunctiva, up to 30 µm in diameter, spherical, PAS- and Grocott-positive, fungal organisms with a large halo and occasional narrow-based budding were present.

Conclusion: An unusual manifestation of an infection with fungal organisms, morphologically identified as *Cryptococcus neoformans*, in a swamp wallaby from a zoological garden in Northern Germany is presented. The suspected portal of entry for the causative agent into the brain is via the Eustachian tube.



Poster Abstracts

P48: H5N1 SUBTYPE HIGHLY PATHOGENIC AVIAN INFLUENZA IN EURASIAN EAGLE OWL (*BUBO BUBO*) IN SOUTH KOREA

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Introduction: Highly pathogenic avian influenza (HPAI) viruses of subtypes H5 and H7 characteristically induce fatal systemic infection in poultry. South Korea experienced outbreaks of HPAI in 2003-2004, 2006-2007, 2008 and 2010-2011. The Eurasian eagle owl (*Bubo bubo*) is a species of horned owl resident in much of Europe and Asia including South Korea. This case is the first report in HPAI in Eurasian eagle owl.

Materials and methods: From January to February 2011, four dead eagle owls were found in 4 provinces (Kyunggi, Chungnam, Chunnam and Kyungnam). The dead eagle owls were submitted to us for diagnostic investigation and examined using pathological and microbiological methods.

Results: The eagle owls were mild to moderately dehydrated and in poor body condition. The gross findings were multiple distinct white spots in the pancreas and the spleen, enlargement of the spleen and uric acid deposition in the kidneys. Microscopically, there were multiple foci of necrosis with mild infiltrates of heterophils in multiple organs, including pancreas, spleen, heart, liver and brain. Influenza viral antigen was demonstrated within pancreatic acinar epithelium, mononuclear cells in spleen, myocardiocytes, hepatocytes and neurons. Sometimes, alveolar macrophages, endothelial cells and renal tubular epithelial cells displayed viral antigen. The H5N1 subtype isolated from the eagle owls had a series of basic amino acids at the HA cleavage site (RERRRKR). This series is characteristic of influenza viruses that are highly pathogenic to chicken. There was no bacterial growth in cultures from the liver.

Discussion and Conclusion: We diagnosed an H5N1 subtype HPAI in Eurasian eagle owl. To our knowledge, this is the first report of HPAI in Eurasian eagle owl (*Bubo bubo*) in the world. The Eurasian eagle owl feeds on small mammals. Considering the feeding habits, we suggest that eagle owls were secondarily infected with HPAI through the ingestion of infected dead wild birds or domestic poultry.



Poster Abstracts

P49: ABORTIONS IN RUMINANTS ATTRIBUTED TO SELENIUM DEFICIENCY

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Introduction: Selenium deficiency is associated with a number of conditions in ruminants including retention of the placenta in cattle and mastitis, however its role in the occurrence of abortions alone or in combination with other mineral imbalances or deficiencies or infectious agents is poorly understood.

Materials and Methods: A high rate of abortions was observed in a 400 head dairy sheep flock (190 abortions), a 500 head goat flock (60 abortions) and a 370 head beef cattle herd (50 abortions) in Greece. Clinical examination, microbiological and parasitological examinations of blood sera from aborting animals and, gross and histological examination of aborted foetuses were performed to identify the causative agent.

Results: Examinations for the presence of *Toxoplasma* spp, *Neospora* spp and a range of bacteria commonly associated with abortions in ruminants were negative. The aborted foetuses examined showed pale discolouration of skeletal muscles and the myocardium, lesions indicative of muscular dystrophy. Histology confirmed extensive and severe muscular degeneration. Administration of selenium at a dose of 0.1 mg/kg resulted in the cessation of the abortions and in healthy newborns.

Discussion: The abortions observed in sheep, goats and cattle were attributed to selenium deficiency.



Poster Abstracts

P50: AGING HISTOPATHOLOGICAL LESIONS IN BOVINE MUSCLES

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Introduction: The aetio-pathogenesis of sarcopenia is complex and probably involves several hormonal, metabolic and nutritional factors, as well as physical inactivity. The aim of this work was to study the age-related lesions in the skeletal muscles of cattle.

Materials and methods: Muscle samples (diaphragm and sternomastoid muscle) from 34 aged cows (7-20 years), regularly slaughtered in the Piemonte region, were submitted for histological, histochemical and immunohistochemical staining to evaluate morphology, oxidative activity and inflammatory reactions. Animals were grouped into 3 age groups and possible associations between the histological findings and age were investigated.

Results: Internal nuclei, angular fibers, fiber atrophy, necrosis, focal sarcosporides, non suppurative inflammatory infiltrates and increases of connective tissue were the most important features detected. The spectrum of positivity for CD4, CD8, CD79 and MHC1 was also established.

The only significant difference among age groups was the higher number of internal nuclei found in the diaphragm of older animals (ANOVA, $P < 0.01$).

Discussion and conclusion: Most of the observed findings are similar to those described in aged people. Degenerative and regenerative changes in the muscle or denervation suggest similarities between sarcopenia in humans and cattle. Further investigations are needed to better understand the mechanism of these muscular changes.



Poster Abstracts

P51: CHRONIC PROLIFERATIVE RHINITIS ASSOCIATED WITH *SALMONELLA ENTERICA* SUBSPECIES *DIARIZONAE* IN SHEEP IN SPAIN.

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Introduction: Parasites and fungi are common causes of chronic rhinitis in small ruminants. However, only in a few occasions have *Salmonella* species been associated with chronic rhinitis in sheep. An outbreak of upper respiratory chronic pathology in five sheep of the same Spanish flock associated with *Salmonella enterica* subspecies *diarizonae* is described.

Materials and Methods: The study was conducted in a 750 sheep flock under traditional rearing system. All animals were clinically monitored and 5 animals showing signs of chronic obstructive disease were separated from the flock. They were euthanized and samples from nasal swabs were cultured following routine procedures. Resulting colonies were selected and identified by biochemical tests. Serotyping was carried out by Central Veterinary Laboratory (Algete, Madrid). Tissue samples were also obtained and tested using routine immunohistochemical procedures using a mouse monoclonal antibody anti *Salmonella*.

Results and discussion: In all cases *Salmonella enterica* subspecies *diarizonae* serovar 61:k:1,5,(7) was isolated. Histopathology revealed a chronic proliferative rhinitis with marked hyperplasia of epithelial cells which contained intracytoplasmic organisms labeled positive with the mouse monoclonal anti *Salmonella* reagent. The relevance of this bacteria as an important pathogen causing rhinitis in sheep in Spain is discussed.



Poster Abstracts

P52: OSTEOCHONDROSIS, PATHOLOGICAL ALTERATION OF THE BULLS FIGHTING

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Introduction: Osteochondrosis (OCD) is characterized by failure of endochondral ossification involving the articular-epiphyseal cartilage complex. Articular pathologies are frequent in these bulls as they are bred to be overweight animals. This disease has been linked to risk factors including incorrect feeding, grazing hard surfaces, hereditary factors, conformation defects and trauma.

Materials and Methods: The study was carried out on 200 bulls fighting (3 to 4 years-old) where the joint surfaces of the carpal and metacarpal bones III+IV, were analyzed with x-ray, and scanning electron microscope. Areas of interest for histopathological examination were cut and fixed in 10% formalin. Bone samples were decalcified in 11% hydrochloric acid. Tissues were stained with Haematoxylin-Eosin and Fraser Lendrum.

Results: In 80% of the samples of cartilage of the joints there was significant histopathological changes consistent with OCD. In all the affected cases, the lesion was bilateral, appearing on the surface of the cartilage that corresponds to areas of friction within the joint.

Conclusion: Osteochondrosis (OCD) in this fighting bull is characterized by abnormalities in the endochondral ossification of the cartilaginous complex of articular-epiphyseal. The etiology most likely relates to trauma or biomechanics factors on cartilage that has been weakened by nutritional or hormonal imbalance, vascular disruption and genetic factors.



Poster Abstracts

P53: SYSTEMIC SPREAD OF INFECTION IN TAIL BITTEN PIGS

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Introduction: Tail biting causes inflammation of the tail and is associated with abscesses especially in the lung and spine of finishing pigs. Three spreading routes of infection from the tail have been proposed previously: venous, lymphatic and cerebrospinal. The aim of this study was to test those routes for systemic spread of infection in tail biting.

Materials and methods: 35 growing, tail bitten pigs and 21 age-matched, unbitten controls were necropsied. Internal organs, tail and central nervous system were histologically examined and tissues with inflammatory lesions were cultured.

Results: Venous route: Chronic purulent or necrotizing interstitial or bronchopneumonia with (n=7) or without (n=2) abscesses was associated with severe tail damage. Bacteriology revealed primary and secondary pathogens, such as environmental bacteria. Pulmonary actinobacillosis, mild lymphocytic interstitial pneumonia and mild lymphocytic infiltrations in several organs were evenly present both in bitten and controls.

Lymphatic route: Enlarged and mildly reactive lumbar lymph nodes were present in two pigs; both had a severely bitten tail.

Cerebrospinal route: Mild multifocal meningeal lymphocytic infiltrates were present both in bitten and control pigs.

Conclusions: Systemic spread of infection in growing, tail bitten pigs occurs mainly *via* the venous route, targeting the lungs, and to a lesser extent *via* lymphatic spread. Tail biting seems also to predispose the pig to opportunistic lung infections.



Poster Abstracts

P55: OUTBREAK OF FATAL FASCIOLOSIS IN GUINEA PIGS

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Introduction: Guinea pigs are susceptible to infection with *Fasciola hepatica* but the pathologic potential of this fluke species in this host is unknown. Around 30 adult Guinea pigs suddenly died over a period of one month in a breeding facility in the Bern region (Switzerland).

Material and Methods: Complete necropsy and routine histologic examination of selected formalin fixed tissues was performed. Trematodes from the liver lesions were identified by morphology and PCR/sequencing.

Results: Macro- and microscopic findings were similar in all the animals examined. They were characterized by severe ascites, wide areas of acute hepatic necrosis associated with portal to bridging fibrosis and bile duct hyperplasia, vasculitis and thrombosis and multifocal chronic peritonitis. Adult *F. hepatica* flukes were morphologically identified in the hepatic lesions and the diagnosis was genetically confirmed.

Conclusion: The sudden death in these animals was attributed to severe hepatic compromise and consequent hepatic failure caused by *F. hepatica*. Guinea pigs are no natural hosts for *F. hepatica* and, to the author's knowledge, this is the first report of fatal fasciolosis in this animal species. Similar changes were described in experimental infections of Guinea pigs with *Fasciolides magna* flukes which further support the etiology in these cases of fatal distomosis.



Poster Abstracts

P56: A CASE OF MULTICENTRIC B-CELL LYMPHOMA IN AN ALPACA

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Introduction: Alpaca has progressively gained popularity as a companion animal in Sweden. Reports of neoplasia in New World camelids are uncommon, and most reports are of neoplasia in llamas. This case report describes the pathological features of a multicentric lymphoma found in an alpaca.

Material and Methods: A 10 year-old male alpaca that had been used for breeding purpose presented with a history of progressive weight loss and strongly enlarged palpable lymph nodes. Following euthanasia a complete necropsy was performed at the National Veterinary Institute, Uppsala. Selected tissues were fixed in 10% buffered formalin, sectioned, and processed routinely for histopathology. Immunohistochemical staining was performed on samples from the tumour-laden lymph nodes with CD3 and CD79a.

Results: At gross pathological examinations a marked generalized lymph node enlargement was found with replacement of normal lymph node architecture by homogeneous pale tan, firm tissue. The animal was emaciated. Histopathological examinations of tissue samples from lymph nodes revealed a neoplastic proliferation of lymphocytes, characterized by a dense packeting of round to slightly elongated, pleomorphic mesenchymal cells in a fine fibrous stroma. Mitotic activity was relatively high. Immunohistochemical staining with CD79a showed strong positive staining on a large proportion of neoplastic cells.

Discussion: Based on these features the tumour was diagnosed as a multicentric B-cell lymphoma.



Poster Abstracts

P57: APROSENCEPHALY AND OTOCEPHALY IN A LAMB WITHOUT OTX2 MUTATION

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Introduction: Aprosencephaly is a rare condition in veterinary and human medicine characterized by the complete absence of telencephalon and diencephalon and can be associated with severe facial dysmorphism designated as otocephaly. Dysfunctional mutation leading to *otx2* gene heterozygosity is associated with this described phenotype in mouse models.

Materials and Methods: Morphologic abnormalities of the skull and central nervous system (CNS) are described in a stillborn lamb by computer tomography, magnetic resonance imaging and pathological examination. Cells of developed parts of the brain are characterized immunohistochemically and DNA is isolated for *otx2* gene sequencing.

Results: Craniofacial alterations comprise a severely reduced and dysplastic splanchnocranium with pinnae fusing in the midventral part of the brain (otocephaly). The microencephalic brain lacks the whole forebrain (telencephalon and diencephalon) while cerebellum reveals a normally developed layered and matured cortex. No polymorphic sites are recognized within the *otx2* gene which was fully sequenced for the first time in *Ovis aries*.

Conclusion: CNS malformations may have varied etiologies in veterinary and human medicine. In this case of true aprosencephaly, no mutations of *otx2* gene were found. Thus, metabolic and oxygenic disturbances might be possible teratogenic noxi for this naturally occurring condition.



Poster Abstracts

P58: HERITABLE MYOPATHY IN LABRADOR RETRIEVER

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Introduction: Muscular dystrophy is a term used to refer to the skeletal muscle disease that results in progressive degeneration, limited regeneration and fibrosis of myofibers. With the advent of immunohistochemical analysis and molecular diagnostics, more specific classification of various muscular dystrophies are now possible. Some of them in young Labrador retrievers, may result from deficiency of dystrophin, while another one is type II fiber deficiency in which dystrophin is present.

Materials and Methods: Samples of muscle tissue from a 3 months old necropsied male Labrador retriever were fixed and embedded routinely, cut and stained with standard H&E, and histochemical stains. Immunohistochemistry was performed on selected sections with antibodies against C terminus of dystrophin (Labvision) and against myosin type II (Labvision).

Results: Histopathological changes included variability in myofiber size, degeneration and type II fiber deficiency. However, all myofibers were immunopositive for dystrophin. Type II fiber deficiency was the most prominent histopathological finding. Creatine kinase level was not elevated.

Conclusion: This report describes the case of a Labrador retriever puppy with early-onset of muscle weakness associated with type II fiber deficiency, but not dystrophin deficiency. Normal creatine kinase level, type II fiber deficiency and presence of dystrophin indicate heritable myopathy in Labrador retriever (HMLR), different from canine X-linked muscular dystrophy (CXMD) with dystrophin deficiency.



Poster Abstracts

P59: *SOLANUM BONARIENSE* INTOXICATION IN CATTLE: FIRST REPORT IN ARGENTINA

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Introduction: *Solanum bonariense*, a native shrub found in flooding grasslands in South America, has been associated with outbreaks of cerebellar dysfunction in cattle. This work describes the first documented case of *S. bonariense* intoxication in cattle in Argentina.

Materials and Methods: The affected herd consisted of 76 Aberdeen Angus cows and 55 nursing calves which were grazing on forestry land on an island of the Parana River delta. During autumn-spring 2009 after an 8-month grazing period 20 cows showed clinical signs including head tilt, muscle spasms, ataxia, hypermetria and recumbency. One affected cow was euthanized and necropsied. Tissues were collected for histopathology and transmission electron microscopy.

Results: No gross lesions were observed. Microscopically (H&E and Holmes-Luxol fast blue stains) there was diffuse cerebellar Purkinje cell degeneration and loss, with chromatolysis, peripheral perikaryal cytoplasmic vacuolation and peripheral nuclear displacement. Similar changes were observed focally in neurons of the brainstem nuclei. There was axonal swelling and demyelination in the cerebellar white matter. Ultrastructurally there were numerous 0.5-1µm electron-dense membrane-bound intracytoplasmic vesicles (dilated lysosomes) in Purkinje cells.

Discussion: Epidemiological information, clinical signs, microscopic and ultrastructural changes in the cerebellar Purkinje cells are consistent with an acquired storage disease previously described in cattle poisoned with *S. bonariense*.



Poster Abstracts

P60: CAUSES OF BOVINE ABORTION IN ARGENTINA

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Introduction: Abortion is a major cause of economic loss in livestock production and its diagnosis is usually difficult. The aim of this work was to identify causes of spontaneous abortion in bovine fetuses submitted to diagnostic laboratories at INTA Balcarce from 2007 to 2010.

Materials and Methods: *Post mortem* examination was performed on 135 fetuses from dairy (23.0%), beef (61.5%) and mixed (15.5%) herds. Samples were tested for pathogenic organisms and processed for histopathological examination. When lesions were compatible, immunohistochemistry against *Neospora caninum* (Nc) was performed. Anti-Nc, Bovine viral diarrhea virus (BVDV) and bovine Herpesvirus (BHV-1) antibody titers were determined in foetal fluids.

Results: Etiological diagnosis was established on 37.0% of the fetuses. Infectious agents were identified on 31.8%, including *Campylobacter fetus* (12.6%), Nc (8.9%), and *Brucella abortus* (3.0%). Noninfectious causes were determined in 5.2%. Antibodies against Nc, BVDV and BHV-1 were found in 13.5%, 6.7% and 3.0%, respectively. Of the 63% fetuses with undefined etiology, histopathological examination revealed lesions compatible with infectious agents in 87.8%.

Discussion: Association of different techniques allowed establishment of an etiology in a similar percentage of fetuses compared to previous studies. *Campylobacter* was the most frequently isolated bacteria and despite national eradication programs, *B. abortus* is still a relevant cause of abortion in Argentina.



Poster Abstracts

P61: IMMUNOHISTOCHEMICAL STUDY OF TNF IN CANINE CYSTIC ENDOMETRIAL HYPERPLASIA

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Introduction: Tumour Necrosis Factor (TNF) has been identified in the uterus of several species and show changes in expression in some pathological conditions. The aim of this study was to evaluate TNF immunoexpression in canine endometrium with Cystic Endometrial Hyperplasia (CEH; n=20) and to compare it with postpartal samples (PP; n=5).

Materials and Methods: Canine uteri presenting CEH were submitted to histological classification according to Dow's (I, II, III, IV). Immunohistochemistry with a specific monoclonal primary antibody raised against canine TNF molecule (sc-80386; Santa Cruz Biotechnology),n, was used at a 1:50 dilution. An immunostaining intensity score (1-3) was used in the superficial and glandular epithelia (SE and GE) and in cystic epithelium (CE).

Results: Our results found more heterogeneous TNF immunoreaction in almost all the CEH samples, in comparison with PP, that might be associated with the inflammatory infiltrate in the CEH uterus (II, III and IV). Overall TNF positivity was different between CEH and PP samples. In CEH, stronger intensities were found in the SE than in GE, whilst in the CE, lower scores were observed. Higher intensities of immunoreaction against TNF were particularly found in early stages of CEH, and that might be involved in the pathology of the process.



Poster Abstracts

P62: SEVERE NEPHROPATHY WITH CRYSTALLURIA IN BRITISH ZWARTBLES SHEEP

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Introduction: Subacute to chronic nephropathies with intralesional oxalates in ruminants can be observed in cases of a variety of poisonings, hepatic and intestinal disease as well as in primary hyperoxaluria. This poster describes a retrospective investigation of nephropathies in sheep with particular reference to crystalluria.

Materials and Methods: Seven Zwartbles sheep aged between 1 month and 2 years with renal crystal deposition at histopathology were identified by scanning surveillance. The findings were compared with 6 age matched control sheep of a variety of breeds in which a nephropathy with crystalluria had been mentioned in the histopathology report.

Results: In Zwartbles and control sheep, the predominant clinical presentation was diarrhoea, weight loss and/or illthrift. Histopathology of the kidneys showed severe chronic nephropathy with large numbers of intratubular prismatic crystals in Zwartbles sheep. Control sheep showed minimal to mild intralesional crystal deposition. Six of the seven Zwartbles sheep were female. Review of the case files did not reveal any particular cause to explain the pronounced intra-lesional crystal deposition.

Discussion and Conclusion: Due to the retrospective character of the research, much of the data is incomplete. Nevertheless, these data show that crystalluria in Zwartbles sheep is more severe than in other breeds suggesting a breed related predisposition to develop exaggerated oxalate deposition following an unknown insult.



Poster Abstracts

P63: PORCINE PROLIFERATIVE ENTEROPATHY AND PORCINE CIRCOVIRUS 2 INFECTION IN ESTONIA

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Introduction: *Lawsonia intracellularis* is the causative agent of porcine proliferative enteropathy (PPE). PPE is characterized by different syndromes and histopathological features.

PCV2 has been associated with a number of syndromes and it has been described as post-weaning multi-systemic wasting syndrome (PMWS).

Material and methods: Materials were collected from 11 swine herds. For *L. intracellularis* detection, DNA was purified and amplified by PCR. Histological specimens were stained by haematoxylin-eosin, Warthin-Starry silver and by immunohistochemical method.

The organ samples were tested for PCV2 by RT-PCR and immunohistochemical stainings.

Bacteriological investigations were done using the standard bacteriological procedures.

Results: Seven of the 11 investigated herds with signs of post-weaning wasting and diarrhoea were infected with *L. intracellularis*, five herds with *E. coli* and all herds with PCV2.

Proliferative intestinal inflammation caused by *L. intracellularis* was found in the distal part of the jejunum and ileum, but the inflammatory changes were also in caecum and colon.

In PCV2 infections, the most common pathological changes were in lymph nodes where there was a granulocyte infiltration, depletion of lymphocytes and the presence of giant cells.

Discussion and conclusion: The results of this study indicate that *L. intracellularis* and PCV2 are more often diagnosed in herds where the piglets are distressed after weaning.



Poster Abstracts

P64: NATURAL SCRAPIE IN SHEEP. PATHOGENESIS OF AMYLOIDOSIS

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Introduction: Scrapie is a slowly progressive, fatal neurodegenerative disease of small ruminants. Amyloidosis is a non-constant pathologic feature of ovine scrapie. The main objective of this study was to investigate the occurrence and the distribution of amyloid deposits in the brain of naturally infected sheep and to suggest the possible pathogenic mechanisms.

Materials and Methods: Sheep with clinical signs of scrapie were euthanized and the brain was removed and fixed in 10% formalin. Brain tissue was taken at eight levels and processed by routine methods. Sections (4 μ m) were stained with haematoxylin and eosin, Congo-red and Alcian-blue. Immunohistochemistry was performed using the specific monoclonal anti-PrP antibody 2G11. Additionally, double immunostaining for prion protein scrapie (PrP^{Sc}) and amyloid β precursor protein (A β PP) was performed. Molecular techniques including P C R for genotyping and Western blotting in order to confirm the diagnosis were applied in blood and brain samples, respectively.

Results: Immunohistochemistry in sections of the mesencephalon and rostral brain revealed the accumulation of PrP^{Sc} within the vessel walls and perivascularly. Plaque-like areas positive for PrP^{Sc} in the neuropil, located mainly perivascularly, were also observed.

Discussion and Conclusion: In natural scrapie, amyloid was deposited mainly perivascularly following a diffuse pattern, a finding that indicates the association of perivascular cells with the amyloid fibril formation.



Poster Abstracts

P65: GM2-GANGLIOSIDOSIS (TAY-SACHS DISEASE) IN EUROPEAN JACOB SHEEP

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Introduction: GM2-gangliosidosis is a group of inherited lysosomal storage diseases in which GM2 ganglioside accumulates as a consequence of defective coding for hexosaminidase (Hex). We describe the pathology and confirmatory blood biochemistry in Jacob sheep in Europe.

Materials and Methods: Necropsy examination were performed on two 8 month-old male Jacob lambs which presented with progressive hindleg weakness, ataxia and impaired spatial awareness. Formalin fixed paraffin embedded and cryoprotected frozen sections were stained with haematoxylin and eosin (H&E), Periodic Acid Schiff (PAS), Luxol Fast Blue (LFB) and Sudan Black (SB). Electron microscopy on brain and analyses of serum and plasma Hex and HexA activities were undertaken.

Results: In central and peripheral nervous system diffuse marked neuronal perikaryon swelling with pale granular material and/or micro-vacuolar change was noted. Accumulated cytoplasmic material stained positively using LFB on wax embedded material and was strongly PAS and SB positive on cryoprotected tissue. Membranous cytoplasmic bodies were seen in lysosomes ultrastructurally. HexA activity in serum and plasma was markedly deficient compared to controls but not as low as that seen with Sandhoff's variant.

Discussion and Conclusion: Histological, ultrastructural and biochemical findings confirm GM2 gangliosidosis (Tay-Sachs disease). Assay of serum and plasma HexA activity provides a useful diagnostic test in live animals. This spontaneous form of Tay-Sachs disease is a potential model for the human disease.



Poster Abstracts

P66: EFFECT OF PROPOLIS AND POLLEN SUPPLEMENTATION IN DIET ON CHICKENS LIVER MORPHOLOGY DURING *SALMONELLA ENTERITIDIS* NATURAL INFECTION

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Introduction: Propolis and pollen have positive effects on biological activities in human and animals. The aim of the study was to investigate the effect of their supplementation on chicken liver morphology.

Material and methods: 64 nestlings Ross 308 were divided into 4 groups (n=6): 1 (control group) standard feeding, 2 – 250 mg propolis/kg fodder, 3 – 5 g pollen/kg fodder, 4 – 5 g pollen and 250 mg propolis/kg fodder. Propolis and pollen were used during the 2 first weeks of breeding. *S. enteritidis* natural infection was detected in the end of experiment. Specimens of liver were taken for microscopic examination after 2nd, 5th and 6th week of breeding.

Results: Parenchymatous and vacuolar degeneration (group 2) together with necrosis (group 1, 4) were observed in liver. Proliferation of biliary ductules was mild to moderate in group 1 and 4. Cholangitis was often seen in every group, least in 2. Thickness of the arterial walls was noted together with eosinophilic and/or foam cells. Proliferation and/or oedema of endothelial cells and adventitia oedema were observed occasionally and were the mildest in group 2.

Conclusion: Results showed the protective effect of propolis on liver morphology in chicken. The mildest alterations caused by *S. enteritidis* were observed in chicken fed with propolis supplementation.



Poster Abstracts

P67: EXPRESSION OF INDUCIBLE NITRIC OXIDE, NITROTYROSINE AND MANGANESE SUPEROXIDE DISMUTASE IN DOGS WITH INFLAMMATORY BOWEL DISEASE

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Introduction: Studies on colonic lavage fluid from dogs with IBD revealed increased concentrations of nitrites suggesting that the disease is associated with increased activity of iNOS. In this investigation the expression of iNOS, nitrotyrosine (NT) and manganese superoxide dismutase (Mn-SOD) was characterized in intestinal biopsies of dogs with IBD.

Materials and Methods: Samples from 15 dogs with IBD and 14 control dogs were examined immunohistochemically with antibodies to iNOS, NT and Mn-SOD.

Results: Strong expression of iNOS, NT and Mn-SOD was predominantly localized in epithelial and inflammatory cells. In control dogs, a similar staining pattern of markedly lower intensity was found.

Conclusion: IBD in dogs is associated with increased expression of iNOS, NT and the antioxidant enzyme Mn-SOD. As in human beings with IBD, epithelial cells were the main source of these products. Detection of NT suggests that also peroxynitrite, a toxic derivative of NO, is produced. The possible role of these products in canine IBD is not clear. For human IBD and experimental animal models of colitis both harmful and protective functions of NO are under discussion. Our results in control dogs indicate that, in contrast to findings in human control subjects, weak expression of iNOS, NT and Mn-SOD occurs.



Poster Abstracts

P68: DETECTION OF *TRITRICHOMONAS FOETUS* AND *PENTATRICHOMONAS HOMINIS* IN THE INTESTINE OF CATS IN AUSTRIA

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Introduction: Two intestinal trichomonads, *Pentatrichomonas hominis* and *Tritrichomonas foetus*, are described in cats. Whereas *P. hominis* is regarded as commensal, *T. foetus* has been determined as the causative agent of feline large-bowel diarrhea, especially in juvenile pure-bred cats.

Materials and Methods: In this study 102 cats under two years of age with clinical diarrhea were examined for the presence of trichomonads in intestinal tissue sections using chromogenic *in situ* hybridization (ISH). Three different oligonucleotide probes were used on serial tissue sections. The probes were specific for all members of the order Trichomonadida (OT probe), for the family of Tritrichomonadidae, and for *P. hominis*, respectively.

Results: In total, four of the 102 cats were found to be positive with the OT probe. Of these positive pure-bred cats between two and eight months of age, one was positive for *P. hominis*, and three for *T. foetus*. With either parasite mild to moderate non-suppurative enteritis or colitis was associated. All together, a prevalence of intestinal trichomonosis in the examined cats of 4 % or within pure-bred cats of 13 % was found.

Conclusion: In this study, the suitability of chromogenic ISH to detect intestinal trichomonads in cats was shown. Additionally, the specific detection of *P. hominis* using ISH was established.



Poster Abstracts

P69: INFECTION OF A QUAIL (*COTURNIX COTURNIX*) WITH A PUTATIVE NEW INTESTINAL TRICHOMONAD SPECIES

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Introduction: In the intestine of a common quail (*Coturnix coturnix*) a high-grade infection with parasite-like objects associated with a moderate lymphocytic inflammation was found. Specific molecular methods were used for the identification of the protozoa.

Material and Methods: Chromogenic in-situ hybridization (ISH) was performed on paraffin embedded tissue sections with oligonucleotide probes targeting a part of the 18S ribosomal RNA (rRNA) –gene of the order Trichomonadida (OT probe), *Histomonas meleagridis*, *Tetratrichomonas gallinarum* or *Trichomonas gallinae*, respectively. Further, DNA was extracted from the paraffin embedded tissue and the entire 18S rRNA-gene, ITS-1 region, 5.8S rRNA-gene, ITS-2 region and a part of the 28S rRNA-gene was sequenced using primer walking. The acquired sequence was subjected to the Basic Local Alignment Search Tool (BLAST).

Results: In the ISH the parasite-like objects revealed strong positive signals only with the OT probe. The trichomonads were found on the mucosal surface, inside the crypts and migrating into the lamina propria. BLAST analysis showed the highest similarity to *Tritrichomonas foetus* with 95 % homology. Further phylogenetic analyses placed the present nucleotide sequence within the family of Tritrichomonadidae.

Conclusion: The authors report the detection of a putative new *Tritrichomonas* sp. in the intestine of a common quail associated with a lymphocytic inflammation.



Poster Abstracts

P70: STRUCTURAL AND FUNCTIONAL CHANGES OF THE FELINE ENTERIC NERVOUS SYSTEM IN CATS WITH CHRONIC GASTROINTESTINAL DISEASES

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Introduction: Inflammatory Bowel Disease (IBD) is a complex of chronic gastrointestinal disorders of unknown etiology and its pathogenesis is poorly understood. In this investigation, the possible involvement of the enteric nervous system (ENS) in cats with chronic gastrointestinal symptoms is described.

Materials and Methods: Immunohistochemical examinations of plexus submucosus and myentericus were performed on intestinal biopsies of cats suffering from IBD (n=23) or intestinal lymphoma (n=10) by using antibodies directed against neuron-specific enolase (NSE), non-phosphorylated neurofilaments (NPN), phosphorylated neurofilaments (PN), vasoactive intestinal peptide (VIP) and glial fibrillary acidic protein (GFAP).

Results: In lymphocytic-plasmacytic enterocolitis (LPE) a significant reduction of GFAP and VIP and mostly of NSE was present, whereas in eosinophilic gastroenterocolitis (EGEC) only PN was reduced. In fibrosing enteropathy (FE) reduced expression of NSE, NPN, PN and VIP was noted. Cases with intestinal lymphoma had only reduction of PN with increase of NPN.

Conclusion: In LPE changes reflect alterations of enteric glial cells and neurons, whereas in EGEC disturbance in neuronal cytoskeleton is suggested. Only neuronal disturbance is present in FE, whereas lymphomas are associated with direct damage or interference of ENS. Structural and functional alterations of the ENS may contribute to clinically evident signs of vomiting and/or diarrhoea.



Poster Abstracts

P71: HEPATIC CIRRHOSIS IN TWELVE DOGS AFTER EXPOSURE TO INAPPROPRIATE COMMERCIAL DOG FOOD

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Introduction: Twelve dogs of different breeds and ages, from the same kennel, with a history of severe liver failure were presented to our Faculty's Pathology Department.

The aim of this study was to assess the lesions and determine the possible etiology of this condition.

Materials and Methods: Routine necropsy exam was performed, liver samples were taken, fixed in formalin, embedded in paraffin and examined using usual and special stains. Immunohistochemistry for α -smooth muscle actin (SMA) and vimentin was performed. Food samples were sent for biochemical and toxicological analysis.

Results: Macronodular hepatic cirrhosis with associated lesions such as ascitis, jaundice and subcutaneous edema were found in the necropsy exam. At microscopic examination diffuse fibrosis with the presence of regenerative nodules, lipogranulomas and pigment loaded macrophages were seen. SMA positive cells were present in the fibrous septa, in the periportal region and in some cases in the perisinusoidal region.

Conclusion: Immunohistochemistry for SMA permits the identification of extracellular matrix components secreting myofibroblasts. The number of these cells is correlated with the degree of the liver fibrosis.

Acknowledgments: This work was financed by the Ministry of Education, Research and Youth by PNCDI2 Program (12-131/2008).



Poster Abstracts

P72: PATHOGENESIS OF FAILED CLOSURE OF OPTIC FISSURE IN FLS MICE WITH OCULAR COLOBOMA: ZYMOGRAPHIC ANALYSIS OF COLLAGENASE ACTIVITY

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Introduction: It is well known that disturbed basement membrane disintegration at optic fissure causes ocular coloboma in rodents. Our previous studies clarified that almost all FLS fetuses developed ocular coloboma due to failure of optic fissure closure. This study was designed to explore the relationship between collagenase activity and disturbed basement membrane disintegration at optic fissure in FLS mice.

Materials and Methods: Serial coronal sections of eyes from FLS fetuses and F1 fetuses between FLS and CBA mice were examined by *in situ* FITC-conjugated zymography.

Results: Positive collagenase activity was increased at GD 12.0-12.5 and it was undetectable at GD 13.5 around the fusing optic fissure in normal F1 fetuses, whereas collagenase activity was weakly positive or indistinguishable during GD 12.0-13.5 at unfused optic fissures in FLS fetuses.

Conclusion: Decreased collagenase activity may cause the disturbed basement membrane disintegration at optic fissure responsible for ocular coloboma.



Poster Abstracts

P73: SEASONAL CONGENITAL LESIONS OF THE CENTRAL NERVOUS SYSTEM IN CALVES.

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

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

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

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



Poster Abstracts

P74: COINCIDENCE OF MEIBOMIAN ADENOMA AND SQUAMOUS CELL CARCINOMA IN A SHEEP: HISTOPATHOLOGIC & IMMUNOHISTOCHEMICAL STUDY

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Introduction: Meibomian adenoma is benign tumor of tarsal glands (meibomian glands) which are located on the inner aspects of eyelids.

Materials & Methods: A 7 years old female crossbreed sheep presented with protrusion of the right eye and swelling of upper eyelid . Routine enucleating was performed.

Results: Histopathological evaluation of eyelid and globe revealed the co-existence of two kinds of neoplasm. Firstly part of the mass was composing of multiple lobules of sebocytes which were separated by connective tissues. Eosinophilic material which were resemble to keratin and sebum was seen within lobules. This was identified as meibomian adenoma. The second area revealed infiltrating islands of neoplastic squamous epithelium extending through the basal lamina of the epithelium. Infiltration of neutrophils and plasma cells, and fibroplasia was also seen. The second part was noted as squamous cell carcinoma. Immunolabelling for cytokeratin was positive.

Conclusion: According to the literature this is the first report of meibomian adenoma in sheep co-existing with a squamous cell carcinoma.



Poster Abstracts

P75: ICHTHYOSIS FETALIS IN A CALF

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Introduction: Ichthyosis is quite a rare heterogenous hereditary disorder of cornification of the skin, which is characterized by the accumulation of large amounts of scales on cutaneous surfaces. Two forms of ichthyosis, ichthyosis fetalis (IF) and ichthyosis congenita, have been recognized in cattle. The present report describes a case of IF in a calf.

Materials and Methods: The case was a male newborn Holstein calf from normal parents, but died within 12 hours after birth. The necropsy was performed. The tissue samples were stained with H&E and Masson trichrome.

Results: The skin was generally alopecic and covered by folded thick and scaly plaques that were separated by deep grooves or clefts with a reddish base. The calf had small ears, ectropion, eclabium. Histologically, the skin revealed a laminated thick orthokeratotic hyperkeratosis on the epidermis and superficial parts of hair follicles. The epidermis was irregular and moderately hyperplastic. Corneal epithelium had wide interrupted segments.

Discussion and Conclusion: To the best of our knowledge, the present case is first report of IF in a Holstein calf in Turkey. Molecular mechanisms underlying the onset of ichthyosis are largely unknown in cattle, but candidate genes have been proposed in people. Further studies will determine expressions of possible causative genes in the skin samples.



Poster Abstracts

P76: IMMUNOHISTOCHEMICAL EXPRESSION ANALYSIS OF THE PROAPOPTOTIC BCL-2-FAMILY MEMBER BAK IN NORMAL CANINE TISSUES AND LYMPHOMAS

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Introduction: Loss of expression of the pro-apoptotic Bcl-2-family member BAK may contribute to tumorigenesis by impairing apoptosis. We selected an antibody suitable for immunohistochemical detection of BAK in canine tissues and evaluated its expression in normal canine tissues and lymphomas.

Material & Methods: The polyclonal anti-human Bak NT antibody (Upstate) was selected out of 3 commercially available antibodies using immunohistochemistry and Western blotting with recombinant canine Bcl-2-family proteins. The antibody (1:100, overnight 4 °C; sections pretreatment 20 min 98 °C, pH 9.0) was used to evaluate tissue arrays with canine normal tissues and over sixty classified lymphoma cases using an immunoperoxidase method (intensity score 0-3).

Results: In non-neoplastic tissues, the strongest signals were detected in the cytoplasm of the epithelia of skin and intestine, of urothelium, distal kidney tubuli and adrenal gland cortex. Normal lymphnodes mostly appeared negative to weakly positive. Lymphomas showed the whole range of labelling intensities with over half of the cases showing a moderate or strong intensity and about one fourth displaying lack of reactivity.

Conclusions: BAK labelling of normal tissues was roughly comparable to that reported for human tissues. In lymphomas BAK upregulation may be relatively common, suggesting enhanced apoptotic signalling. In a considerable portion of tumors with very low levels, BAK may not significantly contribute to apoptosis.



Poster Abstracts

P77: CHONDROBLASTIC OSTEOSARCOMA OF THE HUMERUS IN A HORSE

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Introduction: Osteosarcomas are the most common bone tumor in humans, dogs and cats, comprising 80% of malignant bone tumors in dogs and 70% in cats (Meuten, 2002). In horses, osteosarcoma is rare, with the majority of cases occurring in the mandible of young horses (Jenner et al., 2003).

Materials and methods: A 16-year-old Belgian Warmblood gelding was referred to the equine clinic of the Faculty of Veterinary Medicine with a history of limping and muscular atrophy of the left front leg. On radiography there was evidence of an abnormal soft tissue density at the proximolateral aspect of the diaphysis of the left humerus. After necropsy the firm, expansive mass with central necrosis was submitted for histopathologic examination.

Results: Histopathologic examination revealed malignant osteoblasts, which varied from pleomorphic spindle shaped cells resembling fibroblasts to plump, oval to rounded cells with basophilic cytoplasm and eccentric, hyperchromatic nuclei. Osteoid and chondroid was present as irregular islands separated by the malignant osteoblasts. There was multifocal mineralization of the osteoid and mitotic figures were common. Central necrosis and a neutrophilic infiltration was present with evidence of destruction of the cortex and invasion of the bone marrow.

Conclusion: The histological result is consistent with a diagnosis of chondroblastic osteosarcoma. The presence of osteoid and chondroid matrices denotes this diagnosis.



Poster Abstracts

P78: A CASE OF PREPUTIAL PNST IN A HORSE: MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY.

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Introduction: The term peripheral nerve sheath tumours (PNSTs) has been used for neoplasms such as schwannomas and neurofibromas. In the equine species, and particularly in the horse, descriptions of skin and / or extracutaneous locations of PNSTs have been reported by several authors. The PNSTs in the horse involve eyelids, neck and axillary regions and they have been also described in stomach, cecum, shoulder regions, face, hock and intracranial site. Regarding the preputial location, bibliographic data show only the presence, but not the description, of a case of neurofibroma / sarcoma in penile and preputial region.

Material and Methods: A male, 15 y.o. Quarter horse showed several preputial neoformations. One of them was surgically removed, fixed in 10% buffered formalin and routinely processed. Histological sections were stained with Haematoxylin-Eosin and for S-100 and Vimentin immunohistochemistry reactions.

Results: All data were supportive of a diagnosis of PNST. To author's knowledge, this seems to be the first report of such neoplasm in horses supported also by immunohistochemical characterization.



Poster Abstracts

P79: SPONTANEOUS CANINE MAMMARY CARCINOMAS AS A MODEL OF HUMAN TRIPLE-NEGATIVE BREAST CANCER

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Introduction: The aim of this study was to establish the value of the human breast cancer immunophenotypic classification in dogs.

Materials and methods: 194 canine mammary carcinomas (CMCs) were evaluated. Recorded data included breed, age, cause and time to death, histological subtype, grade, emboli and presence of metastasis. Immunohistochemistry was performed for oestrogen and progesterone receptors, Her2, cytokeratins 5-6, epidermal growth factor receptor (EGF-R) and Ki67.

Results: Age at diagnosis was 10.8±2.1 years. Histology subtypes were simple tubulopapillary (53%) and solid (32%) CMCs. Grade II (49%) and III (42%) tumors predominated. Immunophenotypes included luminal A (11.9%), luminal B (5.1%), basal-like (59.3%) and non basal-like (23.7%) triple negative CMCs. No Her2-overexpressing CMCs as defined by immunohistochemistry were observed. Factors with prognostic significance included weight ($p=0.01$), histologic subtype ($p=0.001$), presence of emboli ($p<0.0001$) or lymph node metastasis ($p=0.02$), and the Ki67 index ($p=0.03$). Shorter specific survival existed for triple-negative carcinomas (median=224 days) when compared to luminal A CMCs (641 days) ($p=0.016$).

Conclusion: The molecular classification of human breast cancer identifies in dogs 4 subtypes and 83% of CMCs were of the triple-negative subtype and associated with a shorter specific survival.



Poster Abstracts

P80: IMMUNOPHENOTYPIC CLASSIFICATION OF FELINE MAMMARY CARCINOMAS

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Introduction: The aim of this study was to establish histological and immunohistochemical factors with prognostic significance in feline mammary carcinomas (FMCs) and to demonstrate the value of the human breast cancer immunophenotypic classification for FMCs.

Materials and methods: 122 FMCs treated by surgery were used, composed of 117 infiltrative and 5 *in situ* tumours. Recorded data included breed, age, neutering status, cause and time to death, tumor size, histological subtype, grade, mitotic index and presence of emboli. Immunohistochemistry was performed for estrogen receptors, Her2, basal cytokeratins, and Ki67.

Results: Age at diagnosis was 11±3 years. 50% of cases represented intact females. Cancer-related death before 1 year was noted in 62% of the cases. 57% of the tumors > 20mm in size had prognostic significance (p=0.035). Histologic subtypes included tubular/tubulopapillary (44%) and cribriform (39%) with Grade III (76%) and II (22%) observed. Median mitotic index was 35 per 10 high power fields (influencing prognosis, p=0.016). Emboli were observed in 48% of FMCs (associated with poor survival, p=0.03). 71% of FMCs were ER-negative (no prognostic significance, p=0.65). The only Her2-overexpressing FMCs as defined by immunohistochemistry was an *in situ* carcinoma.

Conclusion: Infiltrative FMCs are aggressive neoplasms, associated with a 50% cancer-related death rate at 2 years. They could be a model of invasive non estrogen-dependant human breast cancer.



Poster Abstracts

P81: EXTRAMEDULLARY HEMATOPOIESIS IN CANINE MAMMARY TUMOURS – TWO CASES

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Introduction: Mammary extramedullary hematopoiesis is generally rare in human and dogs. We reports two cases of extramedullary hematopoiesis (EMH) in mammary tumours collected in our institution over the last 12 years.

Materials and Methods: Samples were obtained from two female dogs (7-and 8-year-old) during surgery. Tissue samples were routinely processed. Histopathology (H-E method) and immunohistochemical staining (e.g. CK, Vim, p63, S100, vWF, CD68, myeloid/histiocyte antigen) were performed.

Results: In both cases histopathological and immunohistochemical examination revealed benign mixed tumors that included hematopoietic components (myeloid and erythroid cells and megakaryocytes) among areas of cartilage, bone with focal areas of calcification, and adipose cells.

Conclusion: EMH can be detected as an incidental finding associated with benign mammary tumours. Our cases confirm the extreme rarity of mammary EMH in dogs. These findings are similar to those described recently (Grandi, 2010).



Poster Abstracts

P82: EPITHELIAL-MESENCHYMAL TRANSITION IN CANINE MAMMARY TUMOURS: THE ROLE OF MYOEPITHELIAL CELLS.

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Introduction: In epithelial malignant tumours the Epithelial-Mesenchymal Transition (EMT) phenomenon is known. In the mammary gland, two cell types are present: luminal epithelial and basal myoepithelial cells, these latter hypothesized as precursors of mesenchyma. A panel of immunohistochemical markers of myoepithelial cells was applied to 4 different types of neoplasms with the aim of testing the role of myoepithelial cells in EMT in mammary tumours of the dog.

Materials and methods: Monoclonal and polyclonal antibodies anti-ER, p63, Vimentin (VIM), and α -Smooth Muscle Actin (α -SMA) were used on mammary gland neoplasms (5 benign and 3 malignant myoepitheliomas, 8 carcinomas in mixed tumours and 9 complex carcinomas) and an algorithm was built to characterize the resting and the motile phenotype of mioepithelial cells.

Results: ER labelled some basal (resting) and stellate (motile) myoepithelial cells. Myoepithelial markers, namely p63, VIM and α -SMA, stained basal myoepithelial cells. Stellate myoepithelium lost the labelling with p63, but maintained its positivity for VIM and α -SMA, which are also typical of EMT. This affinity, together with the motility increase of myoepithelium from resting basal to motile stellate cells, seem indicative of a transition from myoepithelial polarized immotile cells into highly migratory fibroblast-like cells.



Poster Abstracts

P83: INTRAOCULAR PRIMITIVE NEUROECTODERMAL TUMOURS IN TWO HORSES

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Introduction: Intraocular tumours of neuroepithelial origin are rare in horses. In the present study morphological and immunohistochemical features of two primary intraocular neoplasms of neuroectodermal origin are presented.

Material and Methods: Case 1: The posterior eye segment of a 1.5 yr. old male warmblood presented a whitish mass (5 x 3 x 2 cm), lifting the retina and occupying 40% of the vitreous body. Case 2: A 4 yr. old male Icelandic horse revealed a 3.5 x 2.5 x 2 cm wide and whitish neoplasm expanding the ciliary body and growing by expansion into the vitreous and infiltrating the iris.

Results: Histopathologically, tumour No. 1 consisted of densely arranged polygonal cells forming few rosette-like structures with subretinal and intravitreal spreading. Neoplasm No. 2 was composed of polygonal cells forming numerous rosette-like structures and a spindle cell component embedded in a myxomatous matrix. Several immunohistochemical markers (Vimentin, GFAP, S-100 and neurofilament) were applied.

Discussion: On base of the histopathological features a medulloepithelioma was diagnosed in case 1, a teratoid medulloepithelioma in case 2.



Poster Abstracts

P84: EVALUATION OF MALIGNANCY OF CANINE MAST CELL TUMOR USING COMPUTERED MORPHOMETRY

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Introduction: Mast cell tumors (MCT) are potentially malignant neoplastic processes and their histopathological grading often proves to be subjective. Consequently, computerized morphometric technique developed as one of the objective method of grading and predicting the behaviour of MCT.

Materials and methods: 18 canine cutaneous MCT were morphometrically analysed with regard to mean cellular area and perimeter, mean nuclear area and perimeter, nucleus/cytoplasm ratio and regularity factor, using May Grünwald Giemsa stained cytopathology smears. Lesions were graded as I, II or III according cellular morphology and degree of degranulation. The smears were analysed with Olympus BX41 microscope coupled to a computer equipped with Cell B analysis system.

Results: There were significant differences between cellular area of grade I and II and between grade I and III. There were no significant differences of cellular perimeter between grades. Values of the nuclear perimeter increased with increase in MCT's grade. The nucleus/cytoplasm ratio had higher values in grade III MCTs than in grade II. There were inexpressive differences between the values of regularity factor.

Conclusions: The study adds new data to cellular morphometry of MCT, correlating the values of selected parameters with the grade of the tumor. In combination with the rapid and cheap cytopathology technique, cellular morphometry becomes a useful tool for evaluation of tumor malignancy.



Poster Abstracts

P85: THE TRACHEAL NEUROENDOCRINE CARCINOMA IN A CAT – CASE REPORT

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Introduction: Neuroendocrine carcinoma is rare in the larynx and extremely rare in the trachea. Humans with laryngeal neuroendocrine carcinoma are mainly elderly male smokers.

Materials and Methods: A 7 year old, male, Main Coon cat presented with an intensifying dyspnoea of 6 months duration. Tracheoscopy showed a deformation of the tracheal mucous membrane. The segment of the trachea with the deformation was removed surgically and the specimen was fixed in 10% buffered formaldehyde and embedded in paraffin wax, sectioned and stained with H&E, periodic acid Schiff's (PAS) reaction and the silver method (Grimelius). Immunohistochemical methods were using with Monoclonal Mouse Anti-Human Cytokeratin (clon MNF116), Monoclonal Anti-Human Vimentin (clon 3B4), Polyclonal Rabbit Anti-Human Chromogranin A, Polyclonal Rabbit Anti-Human Calcitonin.

Results: Microscopic investigation showed round and fusiform cells with hyperchromatic nuclei, arranged in nests, sheets or gland-like patterns. Immunohistochemical staining for chromogranine A and cytokeratin was positive in some tumour cells and calcitonin expression was not observed. Some vimentin positive and argyrophilic cells were observed too.

Conclusion: The tumor was recognized as neuroendocrine carcinoma.



Poster Abstracts

P86: HEAT SHOCK PROTEIN EXPRESSION IN CANINE OSTEOSARCOMA

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Introduction: Abnormal levels of Heat Shock Proteins (HSPs) have been observed in many human neoplasms, demonstrating both prognostic and therapeutic implications. Since human and canine osteosarcoma (OSA) share several biological and molecular features, the aim of this study was to evaluate HSP expression in canine OSA model, in relation to histological grade and overall survival (OS), in order to investigate their potential prognostic and/or therapeutic value.

Materials and Methods: Immunohistochemical expression of Hsp27, Hsp72, Hsp73, Hsp90 was evaluated in canine OSA samples of different histological grades. A semi-quantitative method was used to analyse results.

Results: Hsp27, Hsp73 and Hsp90 showed a variably intense, cytoplasmic/nuclear immunoreactivity, not associated with histological type or grade. For Hsp72, immunosignal intensity and percentage of positive cells was highest ($\geq 75\%$) in grade III, whereas absent immunolabelling was associated with prolonged OS. Neoplastic emboli were inconstantly positive for Hsp27, faintly immunoreactive for Hsp72 and intensely immunolabelled by Hsp73 and Hsp90.

Conclusion: Results demonstrate expression of several HSPs in canine OSA. Absence of Hsp72 immunosignal appears to be associated with favourable prognosis, whereas widespread Hsp90 immunoreactivity detected in tumour cases and neoplastic emboli suggests that this protein can represent a potential molecular target for therapy of human and canine OSA.



Poster Abstracts

P87: HEAT SHOCK PROTEIN EXPRESSION IN CANINE PERIPHERAL NERVE SHEATH TUMOURS

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Introduction: Abnormal Heat Shock Proteins (HSPs) levels have been observed in many human neoplasms, demonstrating prognostic and therapeutic implications. Since information concerning HSP expression in peripheral nerve sheath tumours (PNST) are limited, the aim of this study was to evaluate their expression in canine PNST, in order to investigate their potential prognostic and/or therapeutic value.

Materials and Methods: Immunohistochemical expression of Hsp27, Hsp32, Hsp90 was evaluated in normal peripheral nerves, 4 benign PNST and 15 malignant PNST. A semi-quantitative method was used to analyse results.

Results: In normal tissue, HSPs were detectable in axons, epineurial fibroblasts and scattered Schwann cells. In benign PNST, all HSPs showed diffuse, moderate to intense, cytoplasmic and nuclear immunoreactivity, with prevalent nuclear signal for Hsp32. In malignant PNST, Hsp27 immunolabelling was reduced in both intensity and percentage of positive cells; Hsp32 exhibited high, predominant cytoplasmic positivity in the most of tumour samples, characterized by presence of scattered more intensely labelled tumour cells; Hsp90 showed intense and diffuse immunosignal in all cases.

Conclusion: Results demonstrate different expression patterns of Hsp27 and Hsp32 in benign and malignant PNST. High Hsp90 immunoreactivity detected in all tumour cases suggests that it could represent a therapeutic molecular target for these tumours, as recently hypothesized for the human counterpart.



Poster Abstracts

P88: CDV- AND CPIV-INFECTION RELATED DECREASED CORTACTIN EXPRESSION IN DH82 CELLS

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Introduction: DH82 cells are a macrophage/monocytic cell line, derived from a dog with a disseminated histiocytic sarcoma. Infection of canine histiocytic sarcoma cells (DH82) with canine distemper virus (CDV) leads to morphological and functional modifications suggesting a less malignant biological behaviour. The aim of this study was to evaluate the impact of viral infection on tumor cell growth using CDV and canine parainfluenza virus (CPiV). Furthermore, the influence of acute infection on the cortactin expression and distribution in DH82 was analyzed *in vitro*.

Materials and methods: For inverted microscopy and immunofluorescence, non-infected and freshly CDV-Ond-, CDV-R252- and CPiV-infected DH82 cells were examined for cytopathic effects for 10 dpi. Subsequently, the cortactin expression was analyzed using immunofluorescence.

Results: At 10 dpi, CDV-Ond-, CDV-R252- and CPiV-infected DH82 cells showed a diffuse, cytoplasmic expression of cortactin in most cells with a prominent, cell membrane-associated expression only in 1%, 15% and 31% of the cells, respectively. In contrast, non-infected DH82 cells displayed a prominent, membrane-associated cortactin expression in about 80% of the cells at 10 dpi.

Conclusion: Reduced cortactin expression along the cell membrane of CDV-Ond-, CDV-R252- and CPiV-infected DH82 cells was noted. This might lead to a reduced cellular migration and may therefore be associated with a less malignant behavior of canine histiocytic tumors.



Poster Abstracts

P89: TERATOMA IN A TURKEY

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Introduction: Teratoma is a tumor arising from totipotential germ cells that have undergone somatic differentiation and this gives rise to two or more of the embryonic layers in the tumour with a variety of tissues being present.

Material and methods: A 6-week-old male turkey presented with a large excrescence in the area of the left eye. Intra-surgical observations revealed that the excrescence was a tumor situated in front of the left eye-ball. Specimens were taken for microscopic examinations (H&E, PAS, alcian blue-PAS, Mallory trichrome and IHC stainings).

Results: The histopathological examination revealed the tumor included structures derived from all three germ cell layers: cartilaginous, osseous, hematopoietic, fibrous, nervous, glandular, keratinized epithelial and smooth muscle tissues. The presence of the keratinized epithelium as well as smooth muscles was confirmed using immunohistochemical methods designed for mammals. The proliferative activity of the tumor cells was confirmed using PCNA immunostaining.

Discussion: This is the first report of a primary, spontaneous and probably congenital teratoma in a farm turkey, localized in front of the left eye-ball. The unique location facilitated excision of the tumor and the bird survived.



Poster Abstracts

P90: EXPRESSION OF KI67, BCL-2 AND COX-2 IN CANINE CUTANEOUS MAST CELL TUMOURS: CORRELATIONS WITH GRADING AND PROGNOSIS

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Introduction: Histological grading and cell proliferation markers are typically used to predict post-surgical prognosis of cutaneous mast cell tumours (MCTs).

Material and methods: In the present study, sixty MCTs underwent histological evaluation, grading, immunohistochemistry and total RNA isolation by Real Time RT-PCR, for Ki-67, Bcl-2 and Cox-2.

Results: Ki-67 protein was significantly associated with tumour grade and prognosis. Bcl-2 mRNA was associated with the tumour grade and prognosis: the probability of dying for dogs with mRNA ≥ 0.22 was 5 times higher than dogs with a lower value.

The Cox-2 protein was expressed in 51 out of 60 MCTs (85%), and a significant increase in the gene expression was observed, but no association with tumour grade and prognosis was detected.

Discussion: These results confirm the prognostic role of Ki-67 protein, and suggest the possible role of Bcl-2 gene in MCTs progression. The Cox-2 mRNA up-regulation and the protein expression would suggest a role of Cox-2 in MCTs pathogenesis. Further investigations are required to evaluate the prognostic and therapeutic implications of Cox-2 expression in canine MCTs.



Poster Abstracts

P91: T-CELL-LYMPHOSARCOMA WITH INTRALESIONAL LEISHMANIA AMASTIGOTS IN A DOG

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Introduction: Samples of a 6-year-old mixed breed male dog with a history of intermittent fever and nodular swellings in the tongue and the left suprascapular muscle were sent to our institute for investigation.

Material and Methods: Formalin-fixed biopsies were processed for histology, paraffin-embedded and stained by routine techniques. Stains included hematoxylin and eosin (H&E) as well as Giemsa. Immunohistochemistry was performed with antibodies against leishmania species, CD3 and CD79a. Samples of both localizations were used for Direct PCR[®]. PCR was performed for the detection of leishmania species.

Results: Histologically the swellings were identified as a neoplasia composed of blastoid round cells. Within the tongue there was additionally ulceration and infiltration with neutrophils and macrophages. Occasionally macrophages contained amastigotes morphologically consistent with leishmania species.

PCR and immunohistochemistry confirmed the diagnosis of leishmania species.

Immunohistochemistry revealed that the neoplastic cells were positive for CD3 and negative for CD79a, classifying the tumor as T-cell-lymphosarcoma.

Discussion: Here we describe an interesting case of a T-cell-lymphoma with intralesional leishmania amastigots. Concurrent lymphoma and leishmania infections are describe in man and dog, but in the latter amastigots were not demonstrated within the neoplastic lesions.

Whether the coexistence of both entities is accidental or whether the parasitic infection was a promoting event in tumor genesis by stimulation of the cellular immune response remains a matter of speculation.



Poster Abstracts

P92: INTRAVENTRICULAR MENINGIOMA WITH CHOLESTEROL GRANULOMA IN THE CHOROID PLEXUS OF A CAT

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Introduction: A 12-year old cat presented to the clinic with neurologic signs. Neurologic and clinical examinations revealed moderate obtundation and mild decreased postural reactions on all limbs.

Magnetic resonance imaging (MRI) revealed an intraventricular, nonhomogeneous, well defined mass of mixed signal intensity and irregular contrast enhancing. The cat was euthanized and necropsy was performed.

Material and Methods: Formalin-fixed samples were processed for histology and stained by routine techniques. Immunohistochemistry was performed with antibodies against cytokeratin, S100-protein, vimentin, lysozyme, major histocompatibility complex (MHC) II, MAC387, glial fibrillary acidic protein (GFAP) and neuron-specific enolase (NSE).

Results: Replacing the choroid plexus of the lateral ventricle and occluding the third ventricle was a brownish, firm mass compressing adjacent neuronal tissue. Due to the obliteration of the mesencephalic aqueduct the cerebral ventricles and the olfactory recess were enlarged. Histologically the mass was composed of numerous microvacuolated macrophages, interspersed giant cells, and numerous acicular clefts adjacent to and intermixed into a proliferation of spindle cells in dense streams and whorls, focally infiltrating into the neuropil. Multifocal hemorrhages and mineralization were present. Immunohistochemistry revealed positive reactions of the macrophages and giant cells with the antibodies against MHC II, lysozyme, MAC387 and vimentin. The spindle cells were positive for vimentin and NSE. No portion of the mass was positive for cytokeratin, S100-protein or GFAP.

Discussion: Histology and immunohistochemistry identified the mass as meningioma with a cholesterol granuloma. Intraventricular meningiomas are common in cats, often containing cholesterol crystals. In this case an additional formation of a cholesterol granuloma, similar to the granulomas seen at the same location in old horses, was observed.



Poster Abstracts

P93: CANINE INTRAOCULAR HISTIOCYTIC SARCOMA

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Introduction: Disseminated histiocytic sarcoma is a common neoplasm and ocular involvement often occurs. However, primary intraocular histiocytic sarcoma is uncommon. We report the histopathological, immunohistochemical and ultrastructural characteristics of an intraocular histiocytic sarcoma.

Case History: An 8-year-old female golden retriever dog presented with an abnormality in the left eye. Because a tumor lesion was suspected by ultrasonography, enucleation was performed. Clinical observations revealed no other abnormalities however, one month after the operation, disseminated cutaneous masses are identified. These masses were also diagnosed as a histiocytic sarcoma.

Grossly, the left eye was swollen and approximately 2.5 cm in diameter. Tumor mass was mainly located in anterior and posterior uvea and was gray-white or black in color. Histologically, the large pleomorphic mononuclear cells, with multinucleated giant cells, expanded the iris, ciliary body and choroid. Cytoplasm was eosinophilic and varied from scant to abundant. Nuclei were extremely variable in size. Neoplastic cells often engulfed erythrocytes, melanin granules, neutrophils and mononuclear cells. These neoplastic cells were moderately immunoreactive with histiocyte markers (Iba-1, lysozyme and MHC-class II), but no immunoreactive with melanocyte markers (MelanA and S100). In electron microscopy, tumor cells had abundant cytoplasm containing primary lysosome, but immature melanosome was not seen.

Conclusion: The morphological, immunohistochemical and ultrastructural characters of this tumor were suggestive of a primary uveal histiocytic sarcoma.



Poster Abstracts

P94: ROLE OF COX-2 IN COMEDO-TYPE FORMATIONS IN A MOUSE MODEL XENOGRAFT OF CANINE MAMMARY COMEDOCARCINOMA.

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Introduction: Mammary comedocarcinoma is a histological type of mammary neoplasm, recently included in the histological classification of canine mammary tumors. Little is known about this type of canine mammary tumor. Similarly to human mammary comedocarcinoma, it is characterized by the presence of necrotic areas within the center of the neoplastic cell aggregates. The aim of this study was to investigate the formation and evolution of the canine mammary comedo-type formations, using a mouse model xenograft.

Materials and Methods: Samples of a canine mammary comedocarcinoma grade III were obtained immediately after surgery. Serial transplanted xenografts were established in BALB/c SCID female mice. Mice were sacrificed at 4,6,8,10 and 12 weeks after inoculation. At necropsy (n=19), tumor samples were taken for histopathology and immunohistochemistry. Immunohistochemistry of cytokeratin AE1/AE3, CK14, vimentin, actin, ER α , ER β , PR, AR, Her-2, COX-2 and Caspase-3 (apoptotic marker) were performed on the canine mammary tumour and the mice xenografts.

Results: Histology and immunohistochemistry of tumor xenografts were similar to canine primary mammary tumours: AE1/AE3+, CK14+, vim+, actin+, ER α +, ER β +, PR+, AR+, Her-2-. Comedo-type formations were firstly seen at 6 weeks of development of the neoplasms. All tumours were negative for COX-2 except some strongly stained cells typically located in the necrotic limit area. The presence of apoptosis was demonstrated by the staining of caspase 3.

Discussion and Conclusion: The comedo-type formation is a mixture of karyorrhexis and apoptosis in which COX-2 has an important role, probably via apoptosis.



Poster Abstracts

P95: NUCLEAR FACTOR- κ B SIGNATURE OF FELINE INJECTION SITE SARCOMA BY IMMUNOHISTOCHEMISTRY

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Introduction: The nuclear factor- κ B (NF- κ B) transcription factors consist of dimeric proteins of the Rel homology family known to be involved in inflammatory and stress responses. Inappropriate NF- κ B activation can stimulate cell transformation and growth. In addition NF- κ B seems to have a role in tumours with a high inflammatory component. The NF- κ B protein family is composed by different members: p50, p52, p65, cRel, RelB, that form various homodimers and heterodimers, Only specific combinations of NF- κ B family members are transcriptionally active. The aim of this study was to evaluate immunohistochemically the expression of the different NF- κ B transcription members in feline injection site sarcoma (FISS) (with inflammatory component) and in spontaneous feline soft tissue fibrosarcoma (FSTF) (without inflammatory component), in order to compare the presence of transcriptionally active NF- κ B dimers between the two tumours.

Material and Methods: Twenty-three cases of FISS and eleven cases of FSTF were analysed by immunohistochemistry. Antibodies were used for staining of p65, p50, p52 and RelB. Staining was evaluated semi quantitatively for percentage and intensity. The score for each antibody represented the product of percentage of positive tumour cells and intensity. Scores from 1 to 3 were considered as low expression and without functional activity, scores from 4 to 6 as intermediate expression, and scores ≥ 7 as high expression. Then for each case we looked for transcriptionally active combinations of NF- κ B family taking into account the fact that homodimers of p50 or p52 or the heterodimer p50/p52 are transcriptionally inactive. When transcriptionally active NF- κ B dimers were identified the tumour was considered positive for NF- κ B.

Results: We identified 20 of 23 (87%) FISS specimens with transcriptionally active NF- κ B dimers and 2 (18%) FSTF specimens with transcriptionally active NF- κ B.

Conclusions: This study show that NF- κ B is more often activated in FISS compared with FSTF. Concluding, NF- κ B transcription factor pathway seems to be important in cancer related inflammatory genesis and represents a phenotypic signature of feline injection site sarcoma and possibly offers a novel molecular target for treatment of FISS.



Poster Abstracts

P96: INHIBITION OF CELL PROLIFERATION IN CANINE MAMMARY CARCINOMA CELL LINE CMT-U27 TREATED WITH PROGESTERONE AND ANTIPROGESTINS

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Introduction: Antagonists of progesterone receptor (PR) RU486 or ZK299 have been proved to possess a PR-mediated antiproliferative effect in several human breast cancer cell lines.

Material and methods: CMT-U27 canine mammary carcinoma cells were incubated for 24, 48 and 72h either with 10^{-6} M P, RU486, ZK299 or vehicle or with 10^{-6} M RU486 or ZK299 before incubation with 10^{-6} M P for 24, 48 and 72h each. A WST-8 *in vitro* cell proliferation assay was performed in 96-wells plates by triplicate. Afterwards, cells were fixed, paraffin embedded and sectioned to analyse the immunohistochemical expression of PR (PR10A9 monoclonal antibody). The number of PR-positive cells was counted in three consecutive sections and expressed as the percentage of the total number of cells.

Results: Twenty four percent control cells expressed PR. RU486 and ZK299 inhibited cell proliferation both alone ($p=0.05$) and combined with P at 24h ($p=0.05$) without effect on PR expression level. In contrast, P decreased both cell proliferation at 48h ($p=0.05$) and PR expression (14%) at 24h ($p=0.05$).

Conclusion: Antiprogestins RU486 and ZK299 inhibited proliferation of CMT-U27 cells at 24h exclusively but the role of PR on this effect is not clear due to the low PR expression level.

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Poster Abstracts

P97: IMMUNOHISTOCHEMICAL PROFILE OF A GASTRIC DIFFUSE SIGNET-RING-CELL CARCINOMA IN A DOG

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Introduction: Immunohistochemistry was employed to establish the diagnosis in an atypical gastric tumour.

Materials and Methods: A 7-year-old male Caucasian sheepdog presented with a history of chronic vomiting, weight loss and abdominal pain. Clinical, post mortem, histopathological and immunohistochemical examinations were performed.

Results and Discussion: Serum creatine kinase levels were elevated. Abdominal ultrasound showed thickening of the gastric wall and pylorus. Despite treatment, the dog expired. On necropsy, the gastric fundus wall and pyloric antrum appeared diffusely thickened and firm. Histology revealed a mass composed of pleomorphic round or signet-ring-like cells that had diffusely infiltrated the muscular layer, occasionally forming acinar structures, and the gastric mucosa to a smaller degree. Tumour cells had PAS-positive, PAS-diastase resistant mucin and immunohistochemically were positive to pancytokeratin, and negative to vimentin, desmin, a-SMA, CD68, lysozyme, CLA, CD3, CD79a and CD138 (mesenchymal, muscle cell, macrophage, lymphocyte, T-cell, B-cell, and plasma cell markers respectively), confirming their presumed epithelial origin and differentiating them from macrophages and mesenchymal round cells. A diagnosis of gastric diffuse signet-ring-cell carcinoma was proposed. E-cadherin staining was absent, while beta-catenin staining was negative or cytoplasmic, indicating aberrant expression of both cell adhesion proteins, and therefore activation of the wnt/beta-catenin signaling pathway.



Poster Abstracts

P98: ENZOOTIC NASAL TUMOUR OF SHEEP IN CYPRUS AND GREECE

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Introduction: The enzootic nasal tumour (ENT) of sheep arises from the ethmoid turbinate following infection by ENTV-1, an exogenous retrovirus, and is usually classified as an adenoma or low grade adenocarcinoma.

Materials and Methods: 42 tumours were examined, 17 of which were from Cyprus and 25 from Greece, the first of which were initially diagnosed in 1996 and 1991 respectively. The tumours were examined grossly and microscopically and a selected number of tumour samples was analysed using PCR and sequencing.

Results: Although no metastases were observed, most Cypriot cases were locally aggressive carcinomas, with 14/17 presenting with exophthalmos, while the Greek cases presented a wider range of lesions but were overall of a lower grade, and two cases were considered as hyperplasia/dysplasia. The cases originated from two regions in Cyprus (Larnaca and Paphos) and from north Greece. PCR detected ENTV-1 proviral DNA within the tumours examined.

Conclusions: ENT of sheep is present and appears to have widespread geographical distribution in Cyprus and Greece. ENT cases from Cyprus appear to be of markedly higher grade.



Poster Abstracts

P99: TWO CASES OF CUTANEOUS TRANSMISSIBLE VENEREAL TUMOUR WITH INTERNAL METASTASES

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Introduction: Two cases of cutaneous Transmissible Venereal Tumour (TVT) with internal metastases are described.

Materials and Methods: Ultrasonography, cytology, and histopathology were employed to establish the diagnosis of TVT in a six-year-old intact male Siberian husky and an 18-year-old intact male Old English sheepdog cross with one and multiple cutaneous masses respectively.

Results: Fine needle aspirate (FNA) cytology from the masses revealed a homogenous population of round cells, characterised by large nuclei bearing one or two prominent nucleoli, multiple clear vacuoles frequently arranged in chains at the periphery of the cytoplasm, and a high mitotic rate, consistent with cutaneous TVT. Many amastigotes of *Leishmania infantum* were noticed free among the neoplastic cells in the second case. Hepatomegaly and splenomegaly were detected in both dogs. Cytology of ultrasound-guided FNAs or biopsy specimens imprints from both organs confirmed TVT metastasis in both cases. In the first case, total splenectomy was performed and liver biopsies were taken. In the second case, samples were collected from skin, spleen and liver tumour masses upon necropsy. Histopathology confirmed the diagnosis of TVT.

Discussion and Conclusion: The presence of multiple internal metastases in the absence of external genitalia lesions is a rare presentation of TVT.



Poster Abstracts

P100: PARAVERTEBRAL MALIGNANT PERIPHERAL NERVE SHEATH TUMOUR (MPNST) IN A HORSE

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Introduction: Peripheral nerve sheath tumours (PNSTs) are a heterogeneous group of neoplasms that include schwannomas and neurofibromas. While the latter are separate entities in human neuropathology, the more generic term PNSTs is preferred in veterinary medicine. In the horse, PNSTs are rare, and this represents the first report of a malignant PNST in the epaxial musculature of a horse.

Materials and Methods: A Lipizzaner showed intermittent ataxia, hindlimb weakness and soft tissue opacity cranial to the tuber sacrale, with lysis of L5/6. Post mortem examination including histology, immunohistochemistry (IHC) and transmission electron microscopy (TEM) was performed.

Results: A partially encapsulated, multilobulated, yellow-tan mass (~12x8cm) of moderately firm consistency, extending into the spinal canal was present in the left lumbosacral region. Histologically, a septated, infiltrative neoplasm consisting of pleomorphic cells with a spindle, stellate or multinucleated appearance was seen. Neoplastic cells exhibited strong vimentin, S100 protein and GFAP and moderate NGFR and myoglobin expression. They were negative for pancytokeratin, melan A and desmin. TEM showed pleomorphic infiltrative cells with long cytoplasmic processes.

Conclusion: A diagnosis of malignant PNST was made. The IHC reaction pattern is most consistent with a malignant Schwannoma rather than a malignant neurofibroma.



Poster Abstracts

P101: IMMUNOHISTOCHEMICAL EXPRESSION OF MELAN-A IN CANINE MELANIC TUMORS

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Introduction: We evaluated the expression of Melan A/MART-1 (melanoma antigen recognized by T cells) by comparing routine immunostaining of 12 clinical specimens from melanoma cases, 1 metastatic melanoma and 1 melanocytoma with staining for Melan A/MART-A as part of a diagnostic panel.

Results: Melan-A reactivity was demonstrated in 10 of 12 melanomas (83.3%). The intensity of staining was high in 6 (50%) cases, over moderate in 1 (8.3%), moderate in 3 (25%), weak in 0 (0%), and absent in 2 (16.6%). Staining was over moderate in the metastatic melanoma (59.27%) and in the melanocytoma (67.49%). All tumoural cell types demonstrated reactivity for Melan A. Epithelioid tumour cells had a tendency to stain strongly (3/5), mixed epithelioid and spindle cell types also had this tendency (2/3).

Discussion: Melan-A reactivity was observed in the majority of cases. The intensity of Melan-A staining was moderate to intense in melanoma, while it was over-moderate to moderate in the melanocytoma and the metastatic melanoma case. The nuclear stain was more prominent in melanoma cases and weak in melanocytoma. The staining in majority of the cases was heterogeneous. All tumoural cell types demonstrated reactivity for Melan A.



Poster Abstracts

P102: EFFECTS OF ANTIPROGESTAGENS RU486 AND ZK299 ON LIGAND-DEPENDENT PHOSPHORYLATION OF PROGESTERONE RECEPTOR IN CANINE MAMMARY CARCINOMA CELL LINE CMT-U27

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Introduction: Activation of PR by progesterone (P) in human breast cancer cell lines is associated to proliferation changes. The aim of this study was to determine whether antiprogestins affect PR activation by measuring the degree of PR phosphorylation.

Material and methods: CMT-U27 canine mammary carcinoma cells were incubated for 24h either with 10⁻⁶M P, RU486, ZK299 or medium alone or with 10⁻⁶M RU486 or ZK299 before incubation with 10⁻⁶M P for 24 each. Cells were fixed, paraffin embedded and sectioned to analyse the immunohistochemical expression of PR either constitutive (isoforms A and B, PR10A9 monoclonal antibody) or phosphorylated at serine 294 (pSer294 polyclonal antibody). The number of PR-positive cells was counted and expressed as the percentage of the total number of cells.

Results: Twenty six percent of control cells expressed PR. Basal phosphorylation was 14% and ligand-dependent phosphorylation increased to 98%. RU486 (13%) and, to a lesser extent, ZK299 (74%) reduced P-dependent PR phosphorylation. Whereas ZK299 alone failed to induce PR phosphorylation, RU486 had similar effects as P.

Conclusion: Antiprogestins RU486 and ZK299 reduced activation of P-induced PR. These findings suggest that antiprogestins may block the transactivation effects of ligand-dependent PR activation.

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Poster Abstracts

P103: ULTRASTRUCTURAL INVESTIGATION OF A “CRIME SCENE”: CANINE OSTEOSARCOMA CELLS KILLED BY 17-AAG (17-ALLYLAMINO- 17-DEMETHOXYGELDANAMYCIN) THROUGH AUTOPHAGY/APOPTOSIS/ NECROSIS

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Introduction: 17-AAG, an Hsp90 inhibitor, exerts cytotoxic effects on several human transformed cells and the aim of this study is to investigate the mechanism of cell death induced by 17-AAG on D22, a canine osteosarcoma (OSA) cell line, using transmission electron microscopy (TEM).

Materials and Methods: D22 cell line was treated with 1, 3, 5 μ M of 17-AAG at 24 and 48 hrs, fixed in 2.5% glutaraldehyde, embedded in epoxy resin and ultrathin sections, stained with uranyl acetate and lead citrate, were observed with a Zeiss EM900.

Results: 17-AAG-treated cells were pleomorphic, with variable number of lamellipodia, surface bubbles and blisters, cytoplasmic vacuoles, increased RER, mitochondrial degeneration, numerous lysosomes and free ribosomes. Morphological signs of mitochondrial autophagy (mitochondria-RER complexes, isolation membranes, autophagosomes) first appeared at 24 hrs with 1 μ M 17-AAG, while apoptosis was prevalent at 3 μ M (24 hrs) and necrosis at 5 μ M (24 hrs). Ultrastructural features of the three mechanisms of cell death appeared early after 48 hrs treatment.

Conclusions: Our data demonstrate that 17-AAG exhibits a time- and dose-dependent selective cytotoxicity for OSA cells inducing different types of cell death, including the recently discovered “mitophagy”, providing support for its potential therapeutic application in clinical settings.



Poster Abstracts

P104: ABDOMINAL TERATOMA IN A DOMESTIC DUCK (*ANAS PLATYRHYNCHOS DOMESTICUS*)

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Introduction: Teratomas are infrequent tumors in domestic fowl and have been rarely reported in ducks. The authors describe the histochemical (Masson trichrome, PAS, Alcian blue stains), immunohistochemical and ultrastructural features of a multilobulated, mottled, abdominal mass (40x20x15 cm) found in a female White Pekin duck submitted for necropsy.

Results: The mass was composed of mature and embryonic tissues deriving from endoderm (intestinal- and respiratory-type epithelium, goblet cells, solid sheets of undifferentiated epithelial cells, tubular structures resembling renal tubules and glomeruli, immature glandular-like epithelium surrounding islands of immature cartilage), mesoderm (mature and immature cartilage, bone, myxoid tissue, adipocytes, smooth and skeletal muscle cells) and ectoderm (cystic spaces lined by squamous epithelium containing laminated and globular keratin, feather follicles, neurons, astrocytes, ganglion-like cells, melanocytes, undifferentiated small round cells). Immunohistochemistry revealed cytokeratin, desmin, smooth muscle actin, NSE, GFAP, S100 immunoreactivity of the different epithelial, mesenchymal and neuroectodermal components. Interesting ultrastructural findings were filaments with irregular masses of Z-line material and fibrous long-spacing collagens (Luse bodies).

Conclusions: The morphological features of the tumor was consistent with a tridermic teratoma with a probable ovarian origin. Since clinical signs in this and other cases were minimal and the tumor is not usually diagnosed at an early stage of development, any surgical therapy often comes too late.



Poster Abstracts

P105: ADHESION MOLECULES EXPRESSION IN METASTASIC AND NON METASTASIC FELINE MAMMARY CARCINOMAS

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

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

Results and Conclusions: Our results revealed that expression of E-cadherin and beta-catenin are both significantly higher in carcinomas without metastasis. Metastatic carcinomas present loss of E-cadherin expression and only 14% of these neoplasms have a functional expression (coexpression with beta-catenin). Functional expression of E-cadherin was significantly associated with high expression of K18 and low expression of K5.



Poster Abstracts

P106: CORRELATION OF FOXP₃ POSITIVE REGULATORY T CELLS WITH PROGNOSTIC FACTORS IN CANINE MAMMARY CARCINOMAS

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Introduction: Regulatory T (T reg) cells play a crucial role in tumor progression by suppressing anti-tumor immunity, but are not well-documented in veterinary oncology. The transcriptional factor forkhead box P₃ (Foxp₃) is currently considered the most reliable marker of T reg cells. To identify the functional roles of T reg cells in tumor microenvironments, the numbers of T reg cells were analyzed and compared with histological prognostic factors and molecular biomarkers in canine mammary carcinoma (MC).

Materials & Methods: Thirty-seven archived formalin-fixed paraffin embedded tissues of MCs were used for analyzing Foxp₃+ T reg cells.

Results: Abundant T reg cells were associated with high histological grade and lymphatic infiltration. The numbers of T reg cells infiltrated within intratumoral areas markedly increased in tumors with poor prognostic factors such as high histological grade, lymphatic infiltration, and tumoral necrosis.

Conclusions: These suggest that T reg cells play an essential role in canine MC progression. Furthermore, T reg cell numbers in intratumoral compartments may provide a potential prognostic factor when assessing canine MCs, which may in turn lead to the development of new immunologic therapeutics.



Poster Abstracts

P107: SNAIL EXPRESSION CORRELATED WITH POOR PROGNOSTIC FACTORS IN CANINE MAMMARY CARCINOMA

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Introduction: Epithelial tumor cells acquire motility and move to other sites easily during Epithelial-mesenchymal transition (EMT). Snail, one of the hallmarks of EMT, has an important role in tumor invasion and metastasis.

Methods: 54 canine mammary tumour (11 adenomas and 43 carcinomas) samples were analyzed for immunohistochemical expression of Snail, E-cadherin, estrogen receptor (ER), human epidermal growth factor receptor-2 (HER-2), cytokeratin14 (CK14) and p63. Snail mRNA from seven samples (one normal mammary gland, two adenomas and four carcinomas) was evaluated by reverse-transcription polymerase chain reaction (RT-PCR).

Results: Snail expression had a significant correlation with histological grade and lymphatic invasion. However, there was no relationship between Snail expression and other prognostic factors such as age, histological type, molecular subtype and loss of E-cadherin. Snail mRNA was detected in all samples.

Conclusion: Snail protein expression levels are significantly higher in poorly-differentiated carcinomas than in well-differentiated carcinomas. EMT may therefore support canine tumor invasion and metastasis. Moreover, Snail could be a good prognostic factor in the assessment of canine mammary tumor progression.



Poster Abstracts

P108: IMMUNOHISTOCHEMICAL EXPRESSION ANALYSIS OF BAD IN CANINE NORMAL TISSUES AND LYMPHOMAS

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Introduction: Loss of expression of the pro-apoptotic Bcl-2-family member BAD may contribute to tumorigenesis by impairing apoptosis. We selected an antibody suited for immunohistochemical detection of BAD in canine tissues and evaluated its expression in canine normal tissues and lymphomas.

Materials and Methods: AntiBAD 1541-1 (Epitomics) was selected out of 5 commercially available antibodies by immunohistochemistry and Western blotting with recombinant canine Bcl-2-family proteins. The antibody (1:1500, 1h RT; sections pretreatment 20 min 98 °C, pH 9.0) was used to evaluate tissue arrays with triplicate cores of canine normal and of over 80 lymphoma tissues with an immunoperoxidase method (intensity score 0-3).

Results: In non-neoplastic tissues, a moderate to strong cytoplasmic signal was detected in respiratory epithelium, exocrine pancreas cells, renal tubular epithelium, spermatocytes and the cerebellar granular cell layer. Skeletal and smooth muscle cells and fibrous tissue were negative. All other tissues showed weak to moderate or inconsistent labelling. Lymphomas were, in general, slightly stronger labelled than non-neoplastic lymphatic tissues. A few lymphomas were completely devoid of labelling.

Conclusion: BAD labelling of normal tissues was comparable with human tissues, with some differences. Labelling of lymphomas pointed to generally elevated levels of apoptotic signaling compared to normal lymphatic tissues. A complete loss of BAD expression appears to occur with low frequency in canine lymphomas.



Poster Abstracts

P109: SPLENIC MYXOID LIPOSARCOMA WITH HEPATIC METASTASIS IN A DOG

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Introduction: Non-angiomatous, non-leukocytic sarcomas of the spleen are uncommon in the dog and include mainly fibrosarcoma and leiomyosarcoma. The sole prognostic parameters identified for these tumors are mitotic index (MI).

Materials and Methods: An 8 year old, mongrel, male dog was presented for persistent vomiting. A 15 cm splenic mass was observed at ultrasound. Histology, histochemistry and immunohistochemistry (IHC) were performed and follow up was collected.

Results: Grossly, the lesion was soft, poorly demarcated, whitish, and greasy on cut surface. Histology revealed a myxoid sarcoma with lipogenic features with no necrosis and low MI. Rare neoplastic emboli were identified after examination of multiple samples of the tumor. Lipogenic differentiation and myxoid component were demonstrated by Oil-red-O and Alcian-PAS staining. IHC confirmed the mesenchymal origin (vimentin positive) and excluded smooth muscle or endothelial differentiation (FVIII and α -actin negative). After two months the dog was diagnosed with disseminated hepatic metastases with microscopic features similar to primary neoplasm and areas of dedifferentiation.

Discussion: Despite a low MI the primary tumor showed an aggressive behavior in contrast to what is reported in literature. The examination of gross appearance, multiple sampling for histological examination and the use of histochemistry were pivotal for the specific diagnosis and should be routine procedures in the evaluation of canine sarcoma.



Poster Abstracts

P110: TUMOUR EPITHELIAL VIMENTIN EXPRESSION IN CANINE CUTANEOUS SQUAMOUS CELL CARCINOMA

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Introduction: Tumour epithelial vimentin expression is a marker of mesenchymal differentiation during the epithelial-to-mesenchymal transition (EMT) and may be a useful marker of carcinomas with more aggressive behaviour. The aim of this study was to determine vimentin expression pattern and cellular co-localization with β -catenin in canine cutaneous squamous cell carcinoma (SCC).

Materials and Methods: Vimentin expression was detected by immunohistochemistry in 26 cases of SCC. Co-localization with β -catenin was evaluated by immunofluorescence on 6 selected infiltrative poorly differentiated cases.

Results: Normal epidermis, well differentiated neoplastic cords and islands were negative, other than scattered cells, representing melanocytes and epidermal dendritic cells. In SCCs, the percentage of vimentin-immunolabelled neoplastic cells ranged from 0% to 50%, mainly located at the front of tumour invasion, among "basaloid" cells, showing absent/inconstant membrane and increased cytoplasmic β -catenin expression. In two cases of spindle cell SCC, neoplastic cells were strongly immunolabelled. Vimentin positive cells were present also within neoplastic emboli and lymph node metastasis. Few cells showed co-localization of vimentin and nuclear β -catenin.

Conclusion: Our results suggested that tumour epithelial vimentin expression could correlated with poor histological differentiation of canine SCC. Its expression in infiltrative cells showing aberrant subcellular localization of β -catenin suggests that these cells undergo EMT.



Poster Abstracts

P111: SURVIVIN EXPRESSION IN CANINE HAIR FOLLICLE TUMOURS

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Introduction: Despite the vast body of knowledge on survivin expression in skin tumours, no data are available concerning survivin expression in hair follicle neoplasms. The aims of this study was to evaluate survivin expression pattern in canine hair follicle tumours.

Materials and Methods: Survivin expression was investigated by immunohistochemistry in 4 normal canine skin samples, 30 hair follicle tumours (6 pilomatricomas, 8 infundibular keratinizing acanthomas-IKA, 6 trichoepitheliomas, 10 trichoblastomas) and 2 basal cell carcinomas (BCC). A semi-quantitative method was used to analyse results. Mitotic index was morphologically evaluated.

Results: Nuclear immunolabelling with positive mitosis have been observed among basal cells of normal epidermis, outer root sheath, hyperplastic epidermis overlying the tumours and neoplastic cords of IKA cases. Scattered positive matrical cells were present in the bulb of normal hair follicles. The highest number of positive cells (>50%) were present in pilomatricomas and trichoepitheliomas, among cells with matrical differentiation, and in some cases of trichoblastoma. Few or absent positive cells were observed in BCCs.

Conclusion: In accordance with previous study on human hair follicle, our results suggested that survivin could represent a key molecule in the maintenance of cellular subpopulations of canine hair follicle, as well as of tumours deriving from these cells: trichoepithelioma, trichoblastoma and pilomatrixoma.



Poster Abstracts

P112: HAPLO-INSUFFICIENCY OF TUMOR SUPPRESSOR *PTEN* PREDISPOSES TO HEMANGIOSARCOMA IN ZEBRAFISH

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Introduction: Pten is a tumor suppressor that attenuates Akt/PKB signalling. The zebrafish genome encodes two *pten* genes, *ptena* and *ptenb*.

Materials & Methods: 30 out of 296 *ptena*^{+/-}*ptenb*^{-/-} fish (10%) and 1 out of 42 *ptena*^{-/-}*ptenb*^{+/-} fish (2%) developed tumors.

Results: All except 4 of the tumors in *ptena*^{+/-}*ptenb*^{-/-} fish were located close to the retro-ocular vascular network and morphologically consistent with hemangiosarcoma. CD31 immunohistochemistry confirmed endothelial origin of neoplastic cells and marked PCNA staining indicated rapid cell division. Akt/PKB signaling was activated in tumor tissue as evidenced by increased phosphoAkt and phosphoGSK-3 immunoreactivity, concurrent with residual Pten expression.

Conclusions: Our results indicate that zebrafish with *pten* gene dose reduced to a single copy are predisposed to tumor development in spite of retention of some Pten expression, comparable to reported autosomal associated tumor diseases in man. The resulting tumor spectrum is dominated by hemangiosarcoma which is consistent with a role for Akt/PKB signaling in angiogenesis.



Poster Abstracts

P113: FIBROEPITHELIAL POLYPS OF THE VAGINA IN BITCHES: A HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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Materials and Methods: 12 dog vaginal lesions, removed at surgery, were examined histologically and by immunohistochemistry using antisera to vimentin, desmin, smooth muscle actin, calponin and receptor for oestrogen and progesterone.

Results: All cases had histological features consistent with a diagnosis of vaginal fibroepithelial polyps. The characteristic histological feature was an abundant oedematous stroma containing spindle-shaped and stellate cells. Immunohistochemical staining with vimentin and desmin was essentially similar; there was positive staining of a wide variety of cells, including portions of intrinsic smooth muscle, the walls of blood vessels and both spindle-shaped and stellate cells within the polyps.

Staining for smooth muscle actin and calponin was essentially similar; there was staining of portions of intrinsic smooth muscle and of the wall of blood vessels. There was no unequivocal staining for receptors for either oestrogen or progesterone.

Discussion: The histological features of canine vaginal fibroepithelial polyps resemble those in women (Hartmann et al, 1999; American Journal of Clinical Pathology); the immunohistochemical findings are also similar. In women they are thought to be reactive, inflammatory lesions; 'curative' treatment is currently achieved by surgical excision. There have been no known recurrences or reported adverse outcomes among the dogs reported here.



Poster Abstracts

P114: A CASE OF ALIMENTARY MALIGNANT LYMPHOMA IN A MARE

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Introduction: Lymphoid tumors in horse can occur in subcutaneous, alimentary, abdominal, splenic and multicentric forms. Alimentary form is usually difficult to diagnose because of normal peripheral and palpable lymph nodes on gross examination. Histopathologically, tumors are heterogeneous and it is difficult to classify equine lymphoma.

Materials and Methods: A 3-year-old, female, hot-blooded British horse was euthanized due to severe colic. The animal had chronic weight loss and hypoproteinemia. Necropsy of the animal revealed a thickened small intestinal wall and nodular proliferations over the liver serosa. Biochemical analysis of the sera was performed and formalin-fixed tissues were routinely processed. Sections were immunohistochemically stained with CD3, CD20, CD57, CD138, kappa- and lambda-light chains.

Results: The mare was hematologically normal except for hypoproteinemia and hypocalcemia. Histopathologically, the nodular lesions over the liver serosa were composed of neoplastic proliferations of lymphoid cells which stained positively for CD20, kappa- and lambda-light chains. A diagnosis of B-cell lymphoma was made.

Discussion: The neoplastic type of the case was determined through morphologic evaluation, immunophenotyping and the use of immunohistochemical markers. In cases with chronic weight loss and hypoproteinemia without other manifestations, alimentary form of lymphoma should be taken into consideration as a possible cause.



Poster Abstracts

P115: P53 AND KI67 PROTEIN EXPRESSION IN OCULAR SQUAMOUS CELL CARCINOMAS OF DAIRY CATTLE

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Introduction: Bovine ocular squamous cell carcinoma (OSCC) is a well-characterized tumor occurring in cattle. To obtain a better insight into the genesis and neoplastic transformation process of bovine OSCC, abnormal protein expression and proliferation index were assessed by the immunohistochemical evaluation of p53 and ki67.

Material and Method: The expression of p53 and ki67 proteins was investigated in 19 formalin-fixed, paraffin-embedded ocular squamous cell carcinomas of dairy cows by an immunohistochemical procedure in order to evaluate the proteins overexpression.

Results: Microscopically, in 6 cases out of 19 (31.5%) the tumors were carcinoma *in situ* (G0), 13 cases out of 19 (68.5%) of the tumors were invasive carcinoma. Their distribution being as follows: G1, 1 (7.6%); G2, 1 (7.6%); G3, 3 (23%); G4, 8 (61.8%). Anti-human p53 and ki67 protein mouse monoclonal antibodies known to be cross-reactive with p53 and ki67 protein of the animal species examined were used. 18 cases out of 19 (94.7%) were immunoreactive for p53(++). 14 of 19 tumors (73.68%) showed Ki67 expression(++).

Conclusion: As in human squamous cell carcinoma, p53 and ki67 overexpression is frequent in bovine OSCC, providing support for a possible role of the protein in the pathogenesis of this neoplasia.



Poster Abstracts

P116: SILYMARIN EFFECT ON COX₂ AND INOS CHANGES IN HEPG₂

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Introduction: It is established that cytochrome oxidase subunit II (Cox₂) and nitric oxide synthases (iNOS) have important roles in oxidative processes in inflammation, stresses and in controlling apoptosis induction in cancers. These enzymes increase in hepatic cancers. Silymarin has impacts on inflammatory mediators and immune system, it is considered of low toxicity and a relatively safe drug. This study designed to investigate Cox₂ and iNOS level in human liver carcinoma cell line (HEPG₂) after silymarin treatment.

Material and Method: At first HEPG₂ were cultured then silymarin doses were determined by MMT test in 0-200 µg/ml of silymarin. Three groups of cell lines were treated by silymarin 50, 75, and 100 µg/ml and HEPG₂ cell viability were measured after 12 and 24 hours. Cox₂ and iNOS also were measured according Cox₂ EIA Assay, Quantakine iNOS kit. Data were analyzed by Pearson correlation

Results: Viability of HEPG₂ showed significant decrease in treated groups in comparison with control. Cox₂ and iNOS decreased significantly after 24 hours.

Discussion and Conclusion: Silymarin could inhibit NF-KB replication factor in nucleus (Salive et al 1988). Kang et al (2006) showed that stimulating macrophages with LPS, and then treating by silymarin, can decrease gene expression of Cox₂ and iNOS significantly. Our data confirmed that this drug over 50µg/ml caused decreasing growth of HEPG₂ cells by decreasing the COX₂ and iNOS after 24 hr of treatment.



Poster Abstracts

P117: IMMUNOHISTOCHEMICAL IDENTIFICATION OF AN OLFACTORY NEUROBLASTOMA OR ESTHESIONEUROBLASTOMA IN A SPITZ DOG

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Introduction: Olfactory neuroblastoma is an uncommon malignant tumor originating in the olfactory epithelium in the roof of the nose. It is derived from cells of neuroepithelial origin which are committed to the neuronal line of differentiation.

Materials and Methods: A 12-year-old male Spitz dog presented for evaluation of recurrent convulsions and dyspnea. In spite of treatment to reduce the neurological signs, the clinical signs deteriorated and the animal was euthanized.

Results: At necropsy, there was a single irregular mass filling the posterior one-third of the right nasal cavity, passing caudally to and occupying the posterior part of the cranium along with the olfactory bulb. The mass was tan to gray and had areas of necrosis and hemorrhage. Histopathological examination revealed clusters of a uniform cell population separated by an arborising fibrovascular stroma. Tumor cells showed rosette formation with central lumina. Nuclei were hyperchromatic with prominent basophilic nucleoli and numerous mitotic figures. Immunostaining of tumor cells for synaptophysin was strongly positive and it was negative for vimentin, GFAP (Glial fibrillary acidic protein) and cytokeratin.

Discussion and Conclusion: Base on histopathological findings and immunohistochemistry, the tumor was diagnosed as an olfactory neuroblastoma or esthesioneuroblastoma.



Poster Abstracts

P118: P53 GENE ALTERATIONS IN BOVINE URINARY BLADDER TUMORS

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Introduction: P53 is a tumor suppressive gene which is frequently mutated in malignant tumors of animals and humans, especially in urinary bladder tumors of humans. Urinary bladder tumors occur in cattle with bovine enzootic hematuria (BEH).

Material and Method: This study evaluated P53 mutations in 15 samples of different bovine urinary bladder tumors by PCR-SSCP method. Fifteen paraffin embedded blocks were selected from different kinds of bovine urinary bladder tumors. DNA was extracted from the samples and PCR was done by using specified primers for 5 to 8 exons and after electrophoresis, the PCR products were assessed by SSCP method. Samples with changes in electrophoresis patterns were selected and sequenced.

Result: At this survey after sequencing, intronic alterations of P53 gene were observed in three samples of the urinary bladder tumors. In the limit of exons 5 to 8, there were not any changes in electrophoretic pattern, but in each side of designed primers for exon 6 there were alterations to parts of the introns 5 and 6. The samples including Hemangioma, Papilloma and Carcinoma *in situ*, with electrophoretic pattern changes showing nucleotide T deletion with number 9332 in intron 6 in comparison with the bovine genome.

Conclusion: Intronic mutations can predispose to the development of cancers, therefore further analysis of intronic mutations of P53 gene will be needed to determine P53 intronic mutations roles in development of urinary bladder tumors in cattle with BEH.



Poster Abstracts

P119: SALIVARY GLAND ACINIC CELL CARCINOMA IN DOG

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Introduction: Salivary gland tumors are rare in dogs. According to available documentaries, 72 cases of these tumors have been reported in dogs.

Materials and Methods: An 11-year old female terrier shpits dog with a unilateral swelling in the right regional zygomatic side, was taken to veterinary hospital. In radiography, the involvement of zygomatic Salivary gland, and osteolysis of right frontal sinus and nasal bone was observed. Under general anesthesia, the mass was removed and sample fixed in 10% buffer formalin solution and referred to pathology laboratory.

Results: in histopathology, the involvement of mucosal acini of zygomatic salivary gland with neoplastic cells, is seen. Acinar epithelial cells showed pleomorphism and Polymorphism with high mitotic figures. Vacuolated cells and clear cells in tumor mass is seen. According to the above observations salivary gland acinic cell carcinoma solid type was confirmed.

Discussion: Of 72 salivary gland tumor reported in dogs, 21 were acinic cell carcinomas. The site distribution was 10 in parotid gland, 3 in sublingual, 2 each in the mandibular gland an gum, and 1 each in lip, tongue and larynx.

Conclusion: This tumor is reported in Iran for the first time Because of its rare involvements salivary gland and zygomatic gland is very rare reported.



Poster Abstracts

P120: A REPORT OF FIBROBLASTIC MAXILLARY EQUINE OSTEOSARCOMA

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Osteosarcoma is the most frequent malignant bone tumor in domestic animals and humans, representing 80-85% of malignant bone tumors in dogs and about 70-75% in cats. Only a few cases of osteosarcoma have been reported in horses with the majority in the mandible of young horses. Maxillary osteosarcoma causes disruption of the bones with subsequent disruption of the dental arcade and interference with mastication. We describe a case of primitive fibrous maxillary osteosarcoma in a 16-year old Anglo-Arabian horse, hospitalized first for a clinical diagnosis of sinusitis. This case is also unusual in that generally maxillary fibrous osteosarcomas are low grade malignancies with minimal potential to metastasize, yet in this case the tumor had already spread to a regional lymph node by the time the horse was presented for examination, confirming the unpredictability of osteosarcoma.



Poster Abstracts

P121: AN UNCOMMON CASE OF EXTRASKELETAL CANINE OSTEOSARCOMA

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Extrasosseous osteosarcoma is a primary malignant mesenchymal tumor arising in soft tissues with no direct involvement of the skeletal system. It is much rarer than either soft tissue sarcoma or skeletal osteosarcoma, and produces osteoid, bone or chondroid material. Very few cases of primary intestinal osteosarcoma have been described in the literature in dogs and other species of animals. This report describes a case of a jejunal osteosarcoma in a 14 year old, male Cocker Spaniel dog. Clinical work up showed a tumor involving abdomen and small intestine. Histopathological and Immunohistochemical investigations support the diagnosis of primary intestinal canine osteosarcoma. The histologic differential diagnosis included osteosarcoma, undifferentiated sarcoma, malignant peripheral nerve sheath tumor, and Gastro Intestinal Stromal Tumor (GIST). After surgery the animal survived for other two months to die due to the metastatic progression of the tumor. This tumor was finally classified like an osteoblastic productive extrasosseous primary osteosarcoma of the jejunum.



Poster Abstracts

P122: H5N1 EXPERIMENTAL INFECTION IN BLACK HEADED GULLS (*CHROICOCEPHALUS RIDIBUNDUS*)

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Introduction: H5N1 virus infections have been associated with mortality in some gull species. It is known that *Chroicocephalus ridibundus* is susceptible to H5N1 virus infection but nothing more is known about the pathogenesis in this species. We have performed experimental infection in *Chroicocephalus ridibundus* to investigate the dynamics of H5N1 virus infection.

Materials and Methods: 16 gulls were inoculated with 1×10^4 TCID₅₀ H5N1 virus (A/turkey/Turkey 1/2005) into the trachea and oesophagus. Birds were monitored daily for clinical signs or death until day 15. Oropharyngeal and cloacal swabs were collected daily to detect viral shedding. Four birds were euthanized on 2, 4, 6, and 15 day post infection (dpi). Histopathological and immunohistochemical evaluation for influenza A virus nucleoprotein was performed on all tissues.

Results: Viral shedding titers indicated that all birds became productively infected. Spontaneous deaths and neurologic symptoms were evident from 4 to 7dpi.

From 7dpi onwards there was a decrease in viral shedding and general symptoms. Viral antigen was detected as soon as 2dpi in thymus and adrenal gland; from 4 to 7dpi was detected in CNS, pancreas and adrenal gland. At 15dpi there were no symptoms, no antigen in tissues and no viral shedding.

Conclusion: Our research provides new information about H5N1 pathogenesis in gulls



Poster Abstracts

P123: PULMONARY ADIASPIROMYCOSIS IN A CRESTED PORCUPINE (*HYSTRIX CRISTATA*) IN ITALY

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Introduction: Adiaspiromycosis is primarily a pulmonary disease (i.e. characterized by necro-granulomatous pneumonia) of small mammals, reported in several wild species, but never in crested porcupine (*Hystrix cristata*). It is rarely described in humans and, when so, it affects in particular immunodeficient patients. It is caused by dimorphic fungi belonging to the genus *Emmonsia*. *E. crescens* is the main species involved in European cases of adiaspiromycosis.

Materials and Methods: A crested porcupine (young male of 3.5Kg) was found dead during a heavy snowfall in the Winter of 2010-11 in the province of Bologna. It was necropsied and samples of lung tissue were fixed in 10% buffered formalin and routinely processed (Hemathoxylin & Eosin and Grocott's techniques).

Results and Discussion: Multiple fractures of pelvis and posterior legs with diffuse edema and hemorrhage were recorded. In the thorax, multifocal mild chronic adhesive pleuritis and disseminate, severe, granulomatous pneumonia were present. Microscopically, severe diffuse chronic interstitial pneumonia was observed with multifocal necro-granulomatous lesions surrounding cystic structures. These structures (about 100-300µm in diameter), generally empty or rarely filled with basophilic unstructured clod-like material, had an eosinophilic amorphous wall surrounded by a typically granulomatous infiltrate and a fibrous capsule. The walls of the cystic structures appeared black with Grocott's technique. The described characteristics were consistent with pulmonary adiaspiromycosis.



Poster Abstracts

P124: ELECTROCUTION LESIONS IN WILD BROWN HOWLER MONKEYS (*ALOUATTA GUARIBA CLAMITANS*) FROM SÃO PAULO CITY: IMPORTANCE FOR CONSERVATION OF WILD POPULATION

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Introduction: The brown howler monkey is the biggest New World Primate with arboreal habits that still inhabits remaining islets of green areas of São Paulo city. Its habitats and population is decreasing due to city growth, leading the species to an endangered status. The aim of the study was to determine the occurrence of electrocution as a factor that leads howlers to death in this city.

Materials & Methods: From June 2006 to December 2010, 46 howlers were necropsied and 14 (30,43%), 9 male and 5 female, showed gross lesions consistent with electrocution..

Results: Ten animals were euthanized. All animals had poor body condition, and had superficial to severe deep burns such as: fur scorched (5/14), acute blisters (1/14), necrotic and ulcerative cutaneous lesions (14/14), muscle necrosis (14/14), bone exposure and necrosis (4/14) and myiasis (5/14). Lesions were located at least in one of the forelimbs, hindlimbs, lips and tail, or at multiple sites.

Conclusions: This is the first time that electrical burns were recognized and pointed as a significant factor of death of wild howler monkeys and it indicates the necessity of the establishment of proper and specific conservation programs for this species in São Paulo city.

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Poster Abstracts

P125: MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL DESCRIPTION OF A SPLENIC HAEMANGIOMA IN A CAPTIVE EUROPEAN WOLF (*CANIS LUPUS*)

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Introduction: In wild animals, tumours are rare, particularly in wild carnivores, where few reports are available in literature. A splenic haemangioma in a 9-year old male captive European wolf (*Canis lupus*) from an Italian zoological garden is reported and histological and immunohistochemical features are described.

Materials and methods: The subject was humanely euthanized and at necropsy a splenic mass of 35x25x11cm in size was detected. Representative portions of the lesion were fixed in 10% buffered formalin and routinely processed for histopathological evaluation (H&E) and for histochemical studies (masson's trichrome, perls and toluidine blu). Immunoreactivity to vimentin, actin, vWF, CD117, VEGF-R, VEGF-C, CD44 and MIB-1 was investigated.

Results: Morphologically the tumour showed a cavernous pattern with large, dilated and massively engorged vascular spaces. Peripherially, a capillary component was present. The walls were occasionally thickened by adventitial fibrosis with scattered hemorrhages. Inflammatory cells, hemosiderin laden macrophages and hematin deposits were observed throughout the stroma. Cells lining neoplastic lacunae scored positive for vimentin and vascular markers, confirming the neoplasm's endothelial origin. No immunoreactivity to CD44 was noticed. The average of MIB-1 positive nuclei was 2-3/hpf and a moderate grade of mast cells infiltration also observed.



Poster Abstracts

P126: INCREASED INCIDENCE OF BRONCHOPNEUMONIA IN WILD CHAMOIS (*RUPICAPRA RUPICAPRA*) DUE TO AN EMERGING BACTERIOLOGICAL PATHOGEN

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Introduction: Chamois are a wild ruminant species, native to alpine spaces across Europe. In springtime 2010 (January-April) a sudden increase in fatalities in Chamois was reported in Lower Austria. In several areas approximately 80 km apart, hunters noted several dead animals.

Material and Methods: Within 4 months 19 chamois were submitted for necropsy at the Research institute of wildlife ecology, Vienna. Gross examination, histopathological, bacteriological and parasitological examinations were performed. In some cases additional virological analysis was performed.

Results: All animals were emaciated, showed a severe multifocal to coalescing suppurative bronchopneumonia, as well as a marked infestation with lung worms. The bacteriological results ranged from *Escherichia coli*, to *Pasteurella-like* microorganisms. Some double-infections occurred. The virological analyses were all negative. The *Pasteurella* like isolates/strains were subjected to further genotypic characterization (Random amplification of polymorphic DNA (RAPD), Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR and 16S rDNA sequence analysis).

Conclusion: *Pasteurella* has been reported to be an emerging pathogen in humans as well as in animals. It must be included in the list of differential diagnoses even in wild animal population, as it can cause severe die-offs.



Poster Abstracts

P127: ATYPICAL FORM OF SUBCUTANEOUS AVIAN TUBERCULOSIS IN AN EAGLE OWL (*BUBO BUBO*)

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Introduction: Avian tuberculosis is a worldwide disease which affects companion, captive exotic, wild and domestic birds. It is a slowly spreading, chronic bacterial infection most commonly caused by *Mycobacterium avium sp. avium*. The disease is more common in captive than in wild birds. This work describes a clinical case of an unusual form of avian tuberculosis in an eagle owl.

Material and Methods: Necropsy was performed on the animal and tissue samples were collected. Samples were stained with H&E and Acid fast techniques, and tested by PCR in tissue paraffin samples against the specified sequence IS901 of *Mycobacterium avium sp. avium*. Immunohistochemistry to detect mycobacteria was made.

Results: A large subcutaneous yellowish and caseous mass, involving the neck which surrounded the cervical vertebrae and trachea and compressed the oesophagus was observed. Histopathologic examination of lesions of the cervical region revealed a severe granulomatous inflammation in the subcutaneous tissue characterised by multifocal coalescent granulomas with a well-defined area of central necrosis surrounded by macrophages and giant cells and a fibrous capsule. Acid-fast bacilli and mycobacteria antigens were detected in lesions, which were also PCR positive. No significant lesions were observed in others organs.

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



Poster Abstracts

P128: CLINICAL AND PATHOLOGIC FINDINGS IN WILD PIGEONS (*COLUMBA LIVIA*) INFECTED WITH PIGEON PARAMYXOVIRUS-1

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Introduction: Pigeon paramyxovirus-1 (PPMV-1), a variant Newcastle Disease Virus, affects pigeons (*Columba livia*) and other birds, including poultry. Most pathology descriptions come from captive or experimentally infected pigeons. An ongoing outbreak of PPMV-1 in wild pigeons in Sweden provided an opportunity to describe clinical and pathologic changes seen in naturally infected, free-ranging birds.

Materials and Methods: From August 2010 to March 2011, 66 wild pigeons from 25 separate incidents were submitted for necropsy, microscopy and virologic testing.

Results: PPMV-1 was confirmed in each incident. Pigeons often were found dead. Those observed alive primarily showed neurologic disturbances. Thirst and diarrhea also were described. Gross lesions were absent or usually limited to pale, mottled kidneys. Microscopically, lesions typically were non-specific and/or subtle. Inflammation was lymphoplasmacytic. Interstitial nephritis with occasional necrosis was almost always seen. Pancreatitis and periportal hepatic infiltration were common. Meningoencephalitis was evident in 60% of cases. Rarely, lymphoplasmacytic infiltrates were seen in other organs. Most pigeons were in good condition, but 20% were emaciated and/or had concurrent infections.

Discussion: Pathology of PPMV-1 in wild pigeons is consistent with other reports. Although neurologic dysfunction often is observed, microscopic evidence of meningoencephalitis can be subtle or absent. This, coupled with the lack of pathognomonic lesions, highlights the need for virologic testing in suspected outbreaks.



Poster Abstracts

P129: BALANOPOSTHITIS IN EUROPEAN BISON FROM BIAŁOWIEŻA FOREST – PRELIMINARY INVESTIGATION

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Introduction: Some necrotic and inflammatory lesions of various intensity (slight inflammatory lesions to auto-amputation) were observed in the prepuce and penis region in the European bison. Investigations on the aetiology of this disease were carried out but did not reveal the primary ethiological factor. Jakob and others in 2000 proved that *Fusobacterium necrophorum* spp. plays a significant role in balanoposthitis.

Materials and Methods: 41 males of European bison aged 5 month – 16 years were examined post mortem. Samples were collected for bacteriological, parasitological and histopathological investigation.

Results: Anatomopathological examination shows focal inflammatory or necrotic-purulent lesions in 36 cases including 7 with auto-amputation of prepuce and part of the penis. Parasitological investigation shows numerous ticks on the skin including some on the edge of the prepuce orifice. Microscopic examinations observed infiltrations of mononuclear cells with eosinophils, neutrophils of varying intensity and mikrofilariae of *Onchocerca* spp. in the skin of the prepuce and penis. Bacteriological examination of prepuce and penis allowed the isolation of *Corynebacterium* spp., *Staphylococcus* spp. coagulase-negative, *Streptococcus* spp. alpha-hemolytic, *Arcanobacterium pyogenes*.

Conclusion: Research suggests the primary factors responsible for disease may be bloodsucking parasites and mikrofilariae of *Onchocerca* spp. with bacterial infections and environmental factors potentially secondary causes.

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Poster Abstracts

P130: SEVERE HEPATITIS DUE TO AN ALVEOLAR ECHINOCOCCOSIS (ECHINOCOCCUS MULTILOCULARIS) IN A GORILLA *G. GORILLA*

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

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

Results: The abdominal cavity contained abundant ascitic fluid. The liver had multiple, white and firm nodules, some of them with central cavities filled with purulent material (0, 5-20 cm in diameter) that replaced about 70% of the liver parenchyma. Histologically, nodules consisted of central necrosis infiltrated by macrophages, lymphocytes, multinucleated giant cells as well as eosinophils and neutrophils in a less number and surrounded by fibrous connective tissue. The necrotic areas contained remnants of a laminated membrane, calcareous corpuscles and in the periphery, alveolar cysts with rests of the germinal epithelium. Hidatid cysts with scolices were also observed, and the remaining liver parenchyma was atrophied and fibrosed.

Conclusions: Findings concluded that the demise of this animal was related to hydatid cyst of *Echinococcus multilocularis* infection but a differential diagnosis must be done with other parasitic infections such as *E. granulosus*, *E. vogeli* and *Cysticercus spp*, and also abscesses and tumors. An intense and unusual inflammatory response against the parasite could result in this atypical presentation of a fatal granulomatous and necrotizing hepatitis.



Poster Abstracts

P131: METASTATIC CHOLANGIOCARCINOMA IN A LLAMA (LAMA GLAMA)

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Introduction: Reports of bile duct carcinoma in llamas are not common. This study describes a primary hepatic neoplasm and it's multiple metastatic lesions found in llama during necropsy exam.

Material and methods: A 2-year-old female llama from a private zoological park had anorexia, ataxia, dyspnea, ascitis and emaciation and was euthanised and submitted to the Faculty of Veterinary Medicine Cluj-Napoca for necropsy. Gross, histopathological, and immunohistochemical analyses were performed.

Results: A firm, white multinodular mass, with a 25 cm diameter was found in the liver parenchyma. The same metastatic nodules were found in lymph nodes, lung, diaphragm and peritoneum. Histopathology identified a highly invasive growth of neoplastic cells with anarchic arrangement that invaded the surround hepatic parenchyma. Intrasinusoidal penetration of neoplastic cells and numerous bizarre mitoses were also present. A diagnosis of poorly differentiated cholangiocarcinoma was made. Immunohistochemically, neoplastic cells were positive for pan-cytokeratin and negative for TTF1, CK7, CK20 and CEA.

Conclusions: Cholangiocarcinoma is a very aggressive tumor found in the llamas with death occurring from the metastatic lesions and chronic liver failure.



Poster Abstracts

P132: CAUSES OF INFANT DEATH OF COMMON MARMOSET (*CALLITHRIX JACCHUS*), BLACK TUFTED EAR MARMOSET (*CALLITHRIX PENICILLATA*) AND HYBRID MARMOSET (*CALLITHRIX SPP*) BORN IN CAPTIVITY

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Introduction: Despite significant advances in neonatal veterinary medicine, establishing the cause of death of newborn captive marmosets, using a multidisciplinary approach is poorly documented. . The goal of this study was to determine and compare the frequency of main infectious agents associated with death of infants I, born in captivity at a commercial breeding center.

Materials & Methods: Twenty seven newborns marmoset, 10 male, 15 female and 2 undefined, died and were necropsied from October 2007 to October 2008. They included *C. jacchus* (n=9), *C. penicillata* (n=9) and *Callithrix spp.* (n=9) with deaths related to respiratory disorders, such as infectious pneumonia (3), congenital malformation (2), aspiration pneumonia (5), in respectively 1.57%, 3.64% and 36.36% of species ($p=0,0162$), due to gastrointestinal problems such as colitis (3) and enteritis (3), in 2.36%, 0.91% and 18,18% ($p=0,0106$) and, to cannibalism (4/27) 0%, 1.82% and 18.18% ($p=0,0180$).

Conclusions: The high and significant frequency of death of hybrid marmosets might indicate that marmoset colonies should avoid mixing species, and that the factors related to respiratory, digestive, and parents stress behavior disturbs should be established and controlled to reduce newborns' death.

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Poster Abstracts

P133: LOWER RESPIRATORY TRACT LESIONS IN WILD BROWN HOWLER MONKEYS (*ALOUATTA GUARIBA CLAMITANS*): PATHOLOGICAL FINDINGS AND SIGNIFICANCE

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Introduction: The respiratory system is one of the most commonly affected systems in nonhuman primate. However few cases have been described about natural diseases of wild brown howler monkeys, one of the biggest New World Primates. The aim of this study was to describe pathological findings of lower respiratory tract of wild brown howler monkeys.

Materials & Methods: Complete necropsy of 46 howler monkeys from São Paulo city was performed from June 2006 to December 2010.

Results: Gross post mortem findings were: circulatory disturbances (84,78%), fibrinous pleuritis (50,0%), pleuropneumonia (15,21%) and pneumonia (10,68%). Four animals had filarid parasites in the thorax. Microscopy of 32 cases revealed congestion (65,62%), edema (59,37%), reactive fibrous pleural plaques (59,37%), hemorrhage (53,12%), pleuritis (40,62%), emphysema (31,25%) and anthracosis (28,12%). Eight cases of reactive fibrous pleural plaques had no associated inflammation. Other findings include fibrosis, interstitial pneumonia and bronchopneumonia. Only three deaths could be directly attributed to the respiratory system. Sixteen animals were euthanized and 13 died from other causes.

Conclusions: These results indicate for the first time that lower respiratory tract diseases are important, but not the main, causes of death of wild brown howler monkeys.

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Poster Abstracts

P134: HIGH PREVALENCE OF RESPIRATORY MYCOPLASMAS IN AUSTRIAN BIRDS OF PREY

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Introduction: In various animal species mycoplasmas are predominately associated with respiratory and genital diseases. Several mycoplasma species have been isolated from the respiratory tract of birds of prey, however, a correlation with pathological changes has rarely been demonstrated.

Materials and Methods: Tissue sections of formalin-fixed and paraffin-embedded lung samples from 100 birds of prey (Falconiformes and Strigiformes), which had been submitted for pathological examination to the University of Veterinary Medicine in Vienna, were screened for the presence of mycoplasmas employing immunohistochemistry. Specific antibodies against *Mycoplasma (M.) aquilae sp. nov.*, *M. buteonis*, *M. falconis* and *M. gypis* were used.

Results: In 42 birds belonging to 17 different avian species one or two *Mycoplasma* species could be detected in pulmonary tissue sections. Mostly, mycoplasmas were present in small groups inside the parabronchi. However, no common histopathological changes could be associated with their presence.

Conclusion: Respiratory mycoplasmas are highly prevalent in the Austrian bird of prey population, but in most cases the presence of mycoplasmas is not associated with a distinct pathological pattern. A possible facultative pathogenic potential of mycoplasmas should be further investigated, since other bacteria and protozoa associated with severe inflammation have been reported previously in birds of prey.



Poster Abstracts

P135: OSSIFYING FIBROMA IN A ROE DEER (*CAPREOLUS CAPREOLUS*)

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Introduction: An emaciated adult roe deer (*Capreolus capreolus*) presenting a large mandibular mass, was shot by a game warden in Sissach, Switzerland.

Materials and Methods: The head of the roe deer was submitted to the Centre for Fish and Wildlife Health at the University of Bern for macroscopic and microscopic examination. Additionally, a computed tomography (CT) scan was performed at the Institute of Forensic Medicine in Bern.

Results: Grossly, the mass consisted of a 6 x 7 x 4 cm mandibular exophytic growth, associated with loss of incisor teeth. On cut section, a hard light tan core was rimmed by a thick layer of soft tissue. CT examination confirmed the mandibular origin of the mass. Histologically, the mass consisted of an unencapsulated fibro-osseous neoplasm. The bony portion was composed of multiple anastomosing and branching spicules rimmed by osteoblasts with no associated periosteal layer. Embedding the bony spicules were short anastomosing and branching streams and bundles of spindle cells. The overlying partially ulcerated mucosa, showed prominent rete ridges deepening into the submucosa.

Conclusion: The gross, histological and radiological features of the mass along with its anatomic location are consistent with an ossifying fibroma.



Poster Abstracts

P136: A CASE OF A METASTATIC THYROID CARCINOMA IN A BROWN BEAR (*URSUS ARCTOS*)

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Introduction: Thyroid neoplasia is relatively important in dogs and cats but it has been reported sporadically in other domestic animals, including cows and sheep. The objective of this work is to report the first case of a metastatic thyroid carcinoma in a Brown bear.

Materials and Methods: A cachectic, 20 years old, male Brown bear presented with a solid mass located in the ventral cervical region. Multiple nodules compatible with pulmonary metastases were also observed. .

Results: The 20x25 cm mass was attached to the trachea producing dorso-ventral pressure and it was mostly necrotic. Lung and mediastinum showed multiple nodules randomly distributed. A mass was also observed at the right adrenal. An anaplastic thyroid carcinoma was diagnosed in the thyroid and in the lung. However, metastases in the adrenal gland showed a follicular thyroid carcinoma pattern. Immunohistochemistry for cytokeratin was positive in the three organs, whereas results for thyroid transcription factor 1 (TTF-1) and thyroglobulin were only positive in the adrenal metastasis but not in thyroid and lung.

Discussion & Conclusion: This is the first reported case of a thyroid carcinoma in a Brown bear. The tumour pattern and the immunohistochemical profile found are relevant for final tumour diagnosis.



Poster Abstracts

P137: PATHOLOGICAL CHARACTERIZATION OF LARVA MIGRANS CAUSED BY *ALARIA ALATA* INFECTION AT THE EUROPEAN MINK

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Introduction: The alariosis is a parasitic disease caused by the infection with trematodes from *Alaria* genus, being a major reemerging parasitosis of the wildlife animals from Central and Eastern Europe. In this study we described the pathological lesions produced by *Alaria alata* mesocercariae at the European mink.

Materials and Methods: After the full detailed necropsy examination was performed, muscular and subcutaneous tissue samples were taken and processed following routine protocols for histological examination. For the detection and characterization of the parasite we used the artificial digestion technique.

Results: The mesocercariae were distributed throughout the muscles of the trunk, neck, hind and forelimbs, without a preferential localization in one of the muscular groups. Muscular and subcutaneous migration of *Alaria alata* mesocercariae causes mechanical damage and tissue necrosis, mononuclear infiltration and finally the appearance of the granulation tissue as a healing mechanism. The principal pathologic alterations are those of lymphohistiocytic polymyositis, panniculitis and the muscular fibroplasia secondary to the tissue destruction.

Conclusion: The polyphasic lesions that were identified in the muscle indicate an ongoing or repeated insult, the mesocercarial migration being followed by the mononuclear cell infiltrate and by the appearance of the granulation tissue which will lead to muscle and subcutaneous conjunctive tissue fibroplasia.



Poster Abstracts

P138: A CASE OF ANTHRAX IN A PUMA (*PUMA CONCOLOR*)

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Introduction: Anthrax is primarily a disease of herbivores but its occurrence in a wide range of omnivores, carnivores and other vertebrates are not uncommon. *Bacillus anthracis* is the causative agent of anthrax and the disease is characterized by sudden death with the invariable signs in wildlife and zoos.

Material and Methods: The case material was a five years-old male Puma from the zoo. Tissue specimens were fixed in 10% buffered formalin solution and after routine processing for histopathology, 5-6 micron paraffin sections stained with Haematoxylin-Eosin, Giemsa and Gram staining. *B. anthracis* was confirmed by PCR from paraffin blocks.

Results: At the necropsy, the spleen was markedly enlarged and dark-red in color, the liver and kidney were also congested. The mesenteric lymph nodes were large and edematous. Microscopically, necrosis of the lymphocytes in the spleen and lymph nodes were seen. Haemorrhages and bacteria were also observed. Degenerative and necrotic changes were detected at the kidney and liver. In PCR, 500 bp bands were seen.

Discussion and Conclusion: A Puma in the zoo died suddenly without signs of any illness. Although poisoning was strongly suspected as a cause of death, anthrax was observed. Unfortunately, Anthrax sporadically occurs in Turkey.



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ESTP

Speaker and Poster Abstracts



Speaker Abstracts

So1: PRE-CLINICAL SAFETY AND IMMUNOGENICITY OF VACCINATION WITH ENDOGENOUS RETROTRANSPOSABLE ELEMENT ANTIGENS

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Long Interspersed Nuclear Element-1 (L1) and Human Endogenous Retrovirus (HERV)-K are endogenous retrotransposable elements (ERE) thought to be repressed in healthy somatic tissues outside immune-privileged sites but are reported to be activated in the context of cancer or HIV infection, potentially making them useful targets for vaccines and immunotherapies. We set out to address the safety and immunogenicity of vaccination with these elements. Immunohistochemical analysis of tissue arrays comprising healthy human, non human primate and murine tissues together with literature searches identified the potential for ERE antigen tissue expression and therefore highlighted the risk for autoimmunity, enabling us to add safety endpoints to the immunisation studies in mice and non human primates and refine our preclinical safety strategy. Immunisation of mice with murine L1 Open Reading Frame-2 (L1O2) induced strong CD8 T cell responses in the absence of vaccine-related histopathological findings consistent with an autoimmune response. Immunisation of rhesus macaques with human L1O2 (92% conserved with macaque), Simian ERV (SERV)-K Gag and Env induced polyfunctional CD8 T cell responses to all antigens plus CD4 T cell and antibody responses to SERV-K Env. However, there were no hematological, clinical chemistry, urinalysis or histopathological findings related to vaccination. These studies provide the first evidence that immune responses can be induced safely against self or near-self retrotransposable elements in two relevant preclinical species without a risk of autoimmunity and warrant further investigation of these antigens as targets in cancer and HIV.

Speaker Abstracts

S02: REGENERATIVE MEDICINE: “NEW MAKES” FOR THE PHARMACEUTICAL INDUSTRY AND “NEW SHAKES” FOR TOXICOLOGICAL PATHOLOGY?

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Contemporary advances in human stem cell research have led to revolutionary changes in our understanding both of normal tissue homeostasis, as well as of many severe patho-physiological processes. Simultaneously these advances have spawned possibilities to treat and cure many human diseases by manipulating stem cell biology in various ways, as has been the case with bone marrow transplantation for many decades. Current medical indications range from serious degenerative diseases such as Parkinson's Disease and ALS, IDDM, myocardial infarction and emphysema, through to complete digit and limb regeneration. The therapeutic modalities being explored today range from pharmacological manipulation of resident stem cell populations, through to transplantation of stem cells and stem cell-derived progeny. Complex combinations of manipulations are also being considered such as transplantation of cellular material on three dimensional bio-matrices, impregnated with biological and pharmacological “fostering factors”. In exploring these emerging therapeutic modalities and several challenges are emerging for assessment of safety risks to patients. For instance, manipulation of stem cell biology in situ requires for very sensitive and specific examination of both positive and negative effects on often scarce populations of cells, against a very large background of differentiated progeny. Additionally, much of the pharmacological screening taking place today is being conducted “blind”, in that compounds able to affect stem cell fate are being discovered, in the absence of an understanding of the pharmacological target. Moving to the cell replacement therapies, transplantation of pluripotent stem cells, either of human embryonic origin or in the form of induced pluripotent stem cells, or cells committed to particular cell lineages, carry many risks not normally focused upon within the development of classical pharmaceutical development. These range from the induction of teratoma to the development of tissue-specific hyperplasia. Finally, introduction of complex therapeutic “devices” comprising of cells, matrix material and potent growth regulatory factors (proteins, miRNAs, chemicals etc) may produce complex, long-term changes to tissue architecture not normally seen in traditional therapeutic development programs. In each of the above scenarios, challenges will be made to the science of toxicologic pathology. So are we currently equipped to manage the safety issues arising within regenerative medicine? A debate will be developed not only to discuss areas where current practices may suffice, but also, importantly, those areas of science which need to be developed to meet the needs of the emerging and diverse medical approaches to tissue regeneration in the human body.

Speaker Abstracts

S03: PRECLINICAL DEVELOPMENT CONSIDERATIONS FOR CELLULAR THERAPY AND REGENERATIVE MEDICINE PRODUCTS

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There is great potential for cellular therapies and regenerative medicine products to treat unmet medical conditions requiring the repair, restoration, replacement, or regeneration of diseased or damaged tissues and/or organ systems. The pathway to advance these products from discovery to translation into the clinic includes *in vitro* and *in vivo* preclinical studies. Adequate information from proof-of-concept and toxicological studies are essential to support a scientific and regulatory decision that it is reasonably safe to conduct a first-in-human clinical trial with an investigational cellular therapy or regenerative medicine product. Well designed pivotal preclinical studies are based on a detailed understanding of the specific investigational product and the potential clinical risks associated with administration of the product. In addition, well designed preclinical studies may minimize the overall number of studies and number of animals necessary to adequately address the safety and potential efficacy of the investigational product. Therefore, sponsors should engage the FDA early in product development to discuss their preclinical study designs and product development plan.

This presentation will focus on the US FDA Center for Biologics Evaluation and Research (CBER) perspective of the preclinical evaluation of cellular therapy and regenerative medicine products to support a clinical trial under an Investigational New Drug (IND) application. Areas of discussion will include: 1) a brief overview of the FDA and the regulation of cellular therapy and regenerative medicine products, 2) preclinical study design considerations for these products, and 3) how preclinical studies help guide early phase clinical trial designs for these products.

Speaker Abstracts

So4: TRANSCRIPTOMIC APPROACH TO EXPLORE MODE OF ACTION OF LIVER CANCER: APPLICATIONS AND CHALLENGES

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Toxicogenomics is the application of toxicology, genetics, molecular biology and environmental health to describe the response of organisms to environmental stimuli. The field of toxicogenomics has developed over the past 15 years mainly due to advances in toxicology, molecular genetics and cell biology. Its prospective use to resolve crucial data gaps and data inconsistencies could improve risk assessments by providing additional data to increase the understanding of mechanisms and modes of action (MOA) and enhance the reliability of dose-response extrapolation. Thus, toxicogenomics holds promise for advancing the scientific basis of risk assessments. However, one of the current issues is how genomics/transcriptional data is being used to further describe a MOA for oncogenicity and, in turn, its potential uses in cancer risk assessment. This presentation outlines how toxicogenomics could be used on a case by case basis to add information to a MOA, addressing both the opportunities and challenges this technology holds. Some key aspects of generating, analyzing and understanding toxicogenomic datasets prior to its use in the risk assessment process and regulatory decisions will be discussed.

Speaker Abstracts

S05: PHD THESIS PRESENTATION: PROTEIN BIOMARKERS IN MALIGNANT MELANOMA: AN IMAGE ANALYSIS-BASED STUDY

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The thickness of a primary malignant melanoma tumor is still the most important prognostic indicator for the individual patient. However, protein research has indicated that combining results from several markers may help in identifying patients that are of increased risk of dying from malignant melanoma. The primary aim of the study was to study the protein expression of multiple proteins in malignant melanoma tumors employing tissue microarrays and immunohistochemistry to identify novel prognostic and diagnostic melanoma biomarkers. The immunohistochemistry staining was evaluated manually where the fraction of positive tumor cells was evaluated and the staining intensity. In addition, the manual data was analyzed with bioinformatics tools. For additional five markers, which have shown promising results in another study, an automated approach was utilized. Several markers, like GPR143 and MITF correlated with the established melanocyte marker Melan-A. In addition, a four-marker protein panel was identified that gave significant survival results independent of T-stage in multivariate analysis. The proteins included were RBM3, which has not been characterized previously in malignant melanoma, SOX10, MITF and Ki-67. Good prognosis was coupled to many cells staining for RBM3, strong SOX10 and MITF staining intensity and a few cells staining for Ki-67. An automated algorithm was used to test the previously identified five marker panel. This protein panel showed a trend towards significant results in multivariate analysis including T-stage in our cohort. We have identified a novel protein panel with very interesting results which need to be explored further, including analysis in other patient cohorts.

Speaker Abstracts

So6: APPLICATION OF TRANSCRIPTOMICS IN STUDIES AT THE US NATIONAL TOXICOLOGY PROGRAM

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To date, the “omics” technologies have provided massive amounts of biological information and fewer-than-expected applications in predictive profiling in toxicological pathology as well as human medicine. The analysis and reporting of transcriptomic data has evolved over the last decade from an observational science, primarily reporting lists of differentially expressed genes in normal and abnormal conditions, to more in-depth, hypothesis-driven approaches utilizing advanced bioinformatics such as Principal Component Analysis (PCA), biological networks, and pathway analyses. Over the past decade investigators at the US National Toxicology Program (NTP) and National Institute of Environmental Health Sciences (NIEHS) have incorporated transcriptomics research into a variety of studies including those involving hepatotoxicity, hepatocarcinogenesis, and cardiotoxicity. This presentation is an overview of the NTP and NIEHS experience and plans for transcriptomics research.

The rodent liver transcriptome has been studied by numerous researchers worldwide for over a decade and it has provided us insight into the complex biological processes involved in liver injury and repair and hepatocarcinogenesis. To date, hundreds of hepatotoxicants have been studied; many are known to cause cancer in the rodent; and some are human carcinogens. Gene expression data could be used as an early indicator of liver toxicity, because xenobiotic-mediated gene expression changes are often detectable before clinical chemistry, histopathology, clinical, or even ultrastructural changes. Liver injury also causes detectable changes in the transcriptome and microRNAs in blood.

Phenotypic anchoring of gene expression data to histological alterations have linked biological data to specific gene regulation and raised the awareness of the cellular heterogeneity of samples and biological plausibility of genomics results. Gene expression profiles and Principal Component Analysis (PCA) have been successfully applied to discriminate normal tissue from benign or malignant neoplasms, which not only supports published histopathological diagnostic criteria but also provides key molecular pathways in cancer development and progression.

Pathway analyses and networks help to group processes together to better understand mechanisms of toxicity. For example, the majority of hepatotoxicants studied have overwhelming responses in gene expression changes related to cell injury and degeneration, metabolism, DNA repair, and regeneration as the liver begins the healing process. Our challenge is to find the key specific gene changes and molecular events that play roles in chronic debilitating diseases such as cancer. There have been a number of studies that classify chemicals based on their status as either a nongenotoxic or genotoxic carcinogen in the rat or mouse, and then use these predictive profiles to classify chemicals with unknown carcinogenic activity. Network analyses can identify clusters of genes that initially appear unrelated to obviously pertinent to biological processes.



Speaker Abstracts

Studying gene expression profiles of neoplasms sheds light on the processes involved in spontaneous and chemically induced liver neoplasms. This is leading to the discovery of new oncogenes, tumor suppressor genes, biomarkers of disease development, and/or targets for cancer therapy. Studies are also identifying the many similarities in the molecular pathogenesis of cancer between rodents and humans. The new technologies will continue to contribute towards our better understanding of human risks of disease.

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Speaker Abstracts

So7: PROTEOMIC PROFILING OF LIVER FROM MALE F₃₄₄ RATS TREATED WITH HEPATOCARCINOGENS FOR 90 DAYS

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Rodent 2-year bioassays are commonly used to identify potential human cancer hazards of pharmaceutical and environmental agents. Because these assays are time consuming and costly, and there are tens of thousands of agents with unknown hazard potential, there is a considerable interest in establishing short-term, predictive approaches to assess the carcinogenic potential of chemicals. The objectives of the present study were to: 1) identify carcinogen-specific protein profiles in the liver of male rats exposed to hepatocarcinogens for 90 days, and 2) link histopathology with changes in protein levels. We utilized 2-D gel electrophoresis, quadrupole ion trap-matrix-assisted laser-desorption ionization mass spectrometer, microarray gene expression profiling and histopathology to analyze protein, gene and phenotypic alterations in the male rat liver after 90 days exposure to known rat hepatocarcinogens. The hepatocarcinogens were aflatoxin B₁ (AFB₁), N-nitrosodimethylamine (DMNA), methyleugenol (MEG) and l-amino-2,4-dibromo-anthraquinone (DBANQ), and the non-carcinogenic agents were acetaminophen (APAP), ascorbic acid/Vitamin C (VITC) and L-tryptophan (TRY). Seventy-one of approximately 4300 liver protein spots separated on 2D gel electrophoresis were significantly increased or decreased based on software and manual analyses, as a result of carcinogen or non-carcinogen treatment. Glutathione S-transferase Mu₁ (GSTM₁), Protease 28 subunit alpha (PA28 α) and aflatoxin aldehyde reductase (akr7a2) were identified as potential early proteomic biomarkers for chemical induced hepatic cancer. Thirteen proteins showed significant post-translational modifications following exposure to carcinogen or non-carcinogens. In particular, difference in fructose-1,6-bisphosphatase modifications across multiple carcinogens was observed. Various isoforms of fructose-1,6-bisphosphatase were identified and confirmed with dPC chip western analysis. The results from the present study suggest that liver carcinogens and non-carcinogens can dysregulate the characteristic groups of proteins in the rat liver after subchronic exposure and provide insight to the early cellular alterations leading to carcinogenesis. The correlation between protein and transcript indicate variable concordance depending on the chemical exposure. We also demonstrate that both post translational and post-transcriptional alterations play potential key roles in the protein modifications and liver response to agents that target liver.



Speaker Abstracts

So8: TERATOMA ASSAY IN THE ASSESSMENT OF NEW THERAPEUTIC APPROACHES OF HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent embryonal stem (ES) cells and human induced pluripotent (IPS) cells have emerged as key tools in both fundamental and applied research and they are expected to become cornerstones in future regenerative therapies. However, safety concerns have been raised about potential development of tumors and this has been further emphasized by the finding of specific chromosomal changes in both ES and IPS cells shared by spontaneous human germ cell tumors.

Since the first mouse ES cells were established the in vivo teratoma assay has been used to ascertain the pluripotency of these cells. With the development of their human counterparts, teratoma formation in immunodeficient mice have become the standard assay. This lecture will discuss different modalities for the establishment of the teratomas, including drawbacks and advantages. Different outcomes will be discussed and exemplified.

Speaker Abstracts

S09: BACKGROUND LESIONS OF SPRAGUE DAWLEY AND HAN WISTAR RATS AND THE NEW ZEALAND WHITE RABBIT

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Introduction: Pathological evaluation of tissues is not only concerned with the recognition of lesions caused by treatment or disease directly, but also with the identification of background lesions. Background lesions are generally congenital or hereditary findings, normal findings that are unique to the histology of an animal species, trauma, normal aging changes, normal physiologic or hormonal changes and some degenerative conditions.

Materials and Methods: This paper will focus on unusual background lesions in Sprague Dawley and Han Wistar rats and New Zealand White rabbits used in toxicological studies.

Results: Lesions in rats include eosinophilic inclusions in the respiratory epithelium of the nasal turbinates. Eosinophilic inclusions (also known as globules and droplets) are observed commonly in aging rats in the nasal epithelium (Renne et al 2003). The inclusions occur in the olfactory, respiratory epithelium and mucus glands. The inclusions are thought to be proteinaceous and the lesion may also be induced by irritant compounds (Renne et al 2003). Multinucleate hepatocytes occur spontaneously in older rats (Burek 1978). If these lesions are noted in increased numbers, these findings may be related to treatment.

Hyaline droplets in the kidney are eosinophilic, intracytoplasmic inclusions that generally occur in the cortical tubules. They are thought to represent liposomes

containing protein (Hard et al 1999). Large, eosinophilic, cytoplasmic inclusions are occasionally seen in untreated rat Clara cells (Kambara et al 2009).

The nucleus circularis is a focus of neurones in the neonatal rat brain which may

be confused with a treatment-related finding. The nucleus circularis, in the anterior hypothalamus, is a group of magnocellular elements arranged in a ring around a capillary bed. The entire nucleus is surrounded or encapsulated by myelinated fibers (Hatton 1976). The neonatal rat brain also contains focal areas of neuroblasts, which may be confused with gliosis. In addition, the neonatal rat brain may also display foci of extramedullary hemopoiesis, which is normal at day 12. Furthermore, the neonatal brain also displays the presence of cysts and large, bizarre mitotic figures. Ectopic granule cells are also visible in the neonatal rat brain.

Lesions in New Zealand White rabbits include large, eosinophilic histiocytes which are seen commonly within the thymus, lymph nodes and follicles of the epithelium-associated lymphoid tissue (e.g. bronchiolar and gut-associated lymphoid tissue). These histiocytes may have particulate debris within the cytoplasmic vacuoles (Salamon 2007). In the thymus the histiocytes are usually situated in the medullary area of the peripheral lobules. Duplication of the gallbladder and cholecystitis have been reported in rabbits (Gupta 1975). The interstitial gland in the rabbit ovary is made up of abundant interstitial tissue in the female rabbit is referred to as the ovarian interstitial gland (Mori & Matsumoto 1973).

Discussion: Although standardized terminology is encouraged, there are an infinite variety of findings and very often several terms exist for the same lesion.

Speaker Abstracts

S09: BACKGROUND LESIONS OF SPRAGUE DAWLEY AND HAN WISTAR RATS AND THE NEW ZEALAND WHITE RABBIT.

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Speaker Abstracts

S10: MICE IN TOXICITY STUDIES: INBRED VERSUS OUTBRED STRAINS AND CHALLENGES OF BACKGROUND FINDINGS

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Many toxicologists use outbred mice in their toxicity and carcinogenicity studies. The many years of using outbred mice, such as the CD-1, has permitted development of large databases of background lesions that can be useful in interpreting study outcomes. Because outbred mice are genetically undefined, their use in toxicology screening studies represents an uncontrolled variable in a situation where the experimental goal is to test one variable, the test agent. Consequently, it can be argued that one cannot tell if an observed response is due to genetic or non-genetic causes. Proponents for toxicity testing in an outbred stock consider that the genetic heterogeneity in their random-bred mice reflects genetic heterogeneity in human populations and that at least a few mice will respond to the test agent with a relevant signal if that agent has human health consequences. Geneticists have argued for several years that the genetically undefined outbred mouse is the wrong animal model for routine toxicity studies and a better strategy would be to use a small selection of inbred strains without having to increase the number of test animals. Since inbred mice of a given sex are isogenic, i.e., genetically identical, the toxicologist has control of an important test variable. It is further argued that the genetic spectrum provided by multiple inbred strains is a realistic representation of the type of genetic diversity seen in human populations, with sensitive as well as resistant individuals being represented. The National Toxicology Program has used the F1 hybrid B6C3F1 mouse for toxicity and carcinogenicity testing for over 40 years. Much like inbred mice, the B6C3F1 mouse is isogenic. However, the B6C3F1 mouse represents only a single genetic set, and while useful because of its genetic uniformity, it has proven to be a liability in toxicity testing because of a genetically related exaggerated liver tumor background incidence and response to test agents. Two recent efforts to potentially strengthen the relevance of toxicity testing involve using a 'mouse model of the human population' for translational clinical research approach and the establishment of hundreds of recombinant inbred mice in a 'Collaborative Cross' effort based on derivation of new inbred strains from 8 genetically diverse inbred mouse strains. While these latter approaches are not yet ready for immediate application in routine toxicity testing, they provide opportunities to better identify disease risk factors in susceptible human populations and to help delineate mode of action for toxicants, including carcinogens.

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Use of Inbred Mice in Toxicity Testing (with reference citations) - <http://www.isogenic.info/index.html>

Collaborative Cross - <http://csbio.unc.edu/CCstatus>

Poster Abstracts

TP01: BRUNNER'S GLAND LESIONS IN RATS INDUCED BY A VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-2 INHIBITOR

Akira Inomata, Kyoko Nakano, Satoru Hosokawa, Yasuhiro Fujikawa, Jiro Sonoda, Kazuhiro Hayakawa, Etsuko Ota, Yoshikazu Taketa, Yuki Seki, Aya Goto, Yvonne Van Gessel, Sandeep Akare, David Hutto, Akiyoshi Suganuma, Toyohiko Aoki, Kazuo Tsukidate

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Vascular endothelial growth factor (VEGF) receptor-2 inhibitors for cancer have been reported to cause proliferative changes in the duodenal mucosa in rats. A diagnosis of adenosis, an unusual proliferative duodenal lesion characterized by epithelial hyperplasia with transmural invasion, was recently reported in a rat chronic toxicity study of a VEGF receptor inhibitor and affected the clinical trials (J Toxicol Pathol, 2010).

We have also experienced similar duodenal changes in rat 4-, 13-, and 26-week toxicity studies of E7080, a VEGF receptor-2 tyrosine kinase inhibitor. At 4-weeks, the change was first noted and characterized by neutrophil infiltration around Brunner's glands, which in severe cases extended into the duodenal mucosa and/or muscular layers. At 13-weeks, the Brunner's gland inflammation was still present with secondary regenerative hyperplasia of duodenal and pyloric epithelium. The Brunner's glands were atrophic with flattened epithelial cells, and occasionally replaced by regenerative duodenal crypt epithelium. At 26-weeks, the inflammatory change was more advanced and chronic. Almost all Brunner's glands were replaced with crypt epithelial cells forming cystic dilatation in the submucosa/muscular layer, as if down-growth of duodenal epithelium had occurred.

Considering the pathogenesis of the duodenal lesion by VEGF receptor inhibitors, the results of our longitudinal histologic examination of the duodenum revealed that the duodenal lesion is reactive hyperplasia subsequent to inflammation in the Brunner's gland. This notion differs from that of adenosis in previous publications, which was interpreted as a proliferative change with down-growth, with potential of malignancy. Full evaluation of the Brunner's gland is needed in preclinical toxicity studies of VEGF receptor inhibitors for understanding the precise pathogenesis of the duodenal lesions.

Poster Abstracts

TP02: ACUTE NEURONAL CELL DEATH, AND LONG-TERM COGNITIVE IMPAIRMENTS IN RATS EXPOSED TO THE ENVIRONMENTAL TOXIN BMAA (BETA-N-METHYLAMINO-L-ALANINE) DURING THE NEONATAL PERIOD

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Background: Cyanobacteria (blue-green algae) are ubiquitous organisms that exist in terrestrial and aquatic environments all over the world. Most cyanobacteria can produce the toxin β -N-methylamino-L-alanine (BMAA). Dietary exposure to BMAA has been suggested to be involved in the etiology of amyotrophic lateral sclerosis/Parkinsonism-dementia complex in Guam. However, the neurotoxic potency of the glutamatergic BMAA is relatively low in adult rodents and the role of BMAA in human disease is controversial.

The so called “brain growth spurt” is a sensitive period in development and appears in humans from the last trimester of gestation until about 3 years of age. The corresponding phase in rodents is a short period that peaks around postnatal day 10. Our previous studies revealed a high uptake of BMAA in the hippocampus and striatum of neonatal rodents, in contrast to a low brain uptake in adult rodents. Furthermore neonatal rats treated with BMAA displayed acute but transient motor disturbances and failed to show habituation at juvenile age.

Aim: The aim of this study was to identify early morphological changes in the neonatal brain and to investigate the long-term effects of neonatal BMAA exposure on learning and memory mechanisms.

Materials and Methods: Neonatal male Wistar rats were given s.c injections of 50, 200 or 600 mg/kg BMAA hydrochloride, or vehicle, on postnatal days 9-10. A subgroup of pups was sacrificed 24 h after the second injection and the brains fixed in formalin and processed for histopathological examination. The remainder of the animals was subjected to behavioral testing at 13 weeks of age, including the open field test, the object recognition test, the water maze test, the elevated plus maze test, and the radial arm maze test.

Results: Histopathological examination revealed early neuronal cell death as determined by TUNEL staining in the hippocampus 24 hours after a high dose (600 mg/kg) of BMAA. In addition, there was a low degree of neuronal cell death in the retrosplenial and cingulate cortices, areas that are also important for cognitive function. BMAA administration at lower doses (50-200 mg/kg) did not induce any early or late morphological changes, but caused deficits in spatial learning and memory. We did not detect any impairment of recognition memory, suggesting that neonatal exposure to BMAA affects the neuronal systems that are important for spatial tasks. These results indicate that BMAA is a developmental neurotoxin and that the potential risk to human health should be considered if this neurotoxin is biomagnified in food chains.

Poster Abstracts

TP03: A SYSTEMATIC APPROACH FOR THE EVALUATION OF HEART VALVES IN RATS AND MICE

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Numerous examples of compounds inducing heart valve lesions have been encountered in the past, leading to preclinical or clinical attrition. For the same reason several drugs have been withdrawn from the market such as the anorectic agents fenfluramine and phentermine and the ergot-derivative pergolide for treatment of Parkinson's disease. Although valvulopathies represent a serious risk for patient, little attention is paid to the histopathological assessment of heart valves in routine toxicological studies. Frequently, no or only small parts of one or two heart valves are present on the standard longitudinal section despite the vital role of the valves in proper heart function.

We developed a technique for trimming and step sectioning of the heart in order to obtain all four heart valves for histopathological evaluation. Commonly seen histological artifacts of the heart valves are presented such as apparent increase in cellularity due to tangential sectioning, increased basophilia or eosinophilia or overlay with erythrocytes.

Poster Abstracts

TP04: SEMI-AUTOMATED IMAGE-ANALYSIS FOR QUANTIFICATION OF KI67, TBR2 AND DOUBLECORTIN IMMUNOSTAINING AS TISSUE-BASED BIOMARKERS OF NEUROGENESIS IN A “RUNNING WHEEL” MOUSE MODEL

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Introduction: The aim of the study was to assess semi-automated image analysis of doublecortin (DCX) as a suitable tool to monitor the efficacy of possible neurogenesis-inducing compounds in in vivo screens. DCX and T-brain gene-2 (Tbr2) are established markers of neurogenesis and are expressed by intermediate progenitor cells (Tbr2), migrating neuroblasts and immature neurons (DCX). Ki67 is a well-established proliferation marker and is expressed by all proliferating cells during phase G1 through M of the cell cycle.

Materials and Methods: Neurogenesis was induced by volunteered exercise in a running wheel for 14 days in 6-week old female C57/BL6J mice housed in rat cages while control animals were housed in mouse cages without running wheel. “Runners” and controls (12 animals each) were housed in 4 cages with 3 animals per cage. Necropsy was performed and the right hemisphere was fixed for 24 hrs in 10% formalin. The fixed brain was cut with help of a brain matrix and coronal sections of equivalent regions of the caudal and rostral hippocampus were then embedded in paraffin with 6 animals per block (3 controls, 3 runners). 4 slides per block were cut with a leave-out of 10 slides in between. Consecutive slides were stained with anti-Ki67 (rabbit monoclonal, Ventana[®]), anti-Tbr2 (rabbit polyclonal, Abcam[®]) and anti-DCX (rabbit polyclonal, Abcam[®]) primary antibody on the Ventana Discovery[®] XT and scanned with Aperio ScanScope[®]. Semi-automated image-analysis of immunostaining was performed with the Definiens Tissue Studio[™] software on the basis of manually annotated regions of interest and compared to evaluation by a pathologist. For DCX, semi-automated image analysis comprised quantification of stained area, whereas evaluation by a pathologist was based on semi-quantitative grading. For Ki67 and Tbr2, positive nuclei were counted with both methods and positivity indices were established with semi-automated analysis

Results: There was an overall good correlation between semi-automated analysis and evaluation by a pathologist. Especially DCX-expression analysis as stained area per nucleus correlated well with the grading by the pathologist. Semi-automated counting of stained nuclei for Ki67- and Tbr2-IHC was well correlated with “manual” counting as well. There was a clear increase in DCX immunoreactivity in “Runners” compared to controls ($p < 0.0001$). Mean Ki67 positivity index of nuclei was doubled in the runners as compared to controls. However, extensive variability between animals and between the different slides from the same animal were noted and the overall number of stained nuclei was very low in controls as well as in “Runners”. Therefore no statistically significant difference was demonstrated ($p = 0.06$). Tbr2 immunostaining was increased in the “Runners” ($p = 0.01$), but, as for Ki67, there was great variability between animals and different slides from the same animal.

Discussion: The value of DCX as a tissue-based biomarker of neurogenesis was confirmed. For Tbr2 the variability was too high and the overall number of stained nuclei was too low as to make it a reliable biomarker for screening tests. This also applied for Ki67. Additionally for Ki67 no statistically significant difference could be demonstrated between the two groups evaluated. Semi-automated image analysis of the three markers proved to be robust and reliable as it correlated well with the evaluation results of the pathologist. Furthermore it facilitated quantification of immunostaining instead of semi-quantitative grading in case of DCX. For Ki67 and Tbr2, positivity indices for the nuclei were easily accessible.

Conclusion: DCX is a suitable tissue-based biomarker of neurogenesis and semi-automated image analysis of DCX immunostaining facilitates rapid quantification and thus high-throughput in vivo screening of possibly neurogenesis-inducing compounds.

Poster Abstracts

TP05: IN VITRO VISUALIZATION OF HYALURONIC ACID DERMAL FILLER INJECTION IN HUMAN SKIN: COMPARISON BETWEEN THREE DIFFERENT FILLERS

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BACKGROUND: Dermal filler properties differ both between and among classes. Hyaluronic acid (HA) based fillers have varied life spans ranging from weeks to months depending upon their degree of reticulation. Highly reticulated HA fillers are more resistant to in situ degradation and show clinical efficacy for up to 1 year. The aim of this work was to compare the diffusion patterns of three fillers only differing by their degree of reticulation using an ex vivo human skin model.

METHODS: Frozen human skin samples from 3 different donors were used after thawing. Each skin sample was injected intradermally in triplicate with Emervel[®] Touch, Emervel[®] Classic or Emervel[®] Deep (100 µL) and kept in organo-culture conditions for 24 hours. Dermal thickness was evaluated by quantitative high-frequency (20 MHz) ultrasonography before and after filler injection. In addition, histopathology evaluation, including image analysis, was performed on skin samples at T0 and T24h after specific staining for HA.

RESULTS: Using ultrasonography, the three fillers were clearly visible within the dermis and induced a very reproducible skin thickness increase (about 1.8-fold, CV<5%) immediately after injection. The lowest increase was observed with Emervel[®] Touch (lowest reticulation), although it did not reach statistical significance. Image analysis, and specifically the 'texture' parameter, showed statistically significant differences between Emervel[®] Touch (lowest reticulation) and the two other fillers ($p < 0.001$).

CONCLUSION: A rapid *in vitro* excised human skin model, combining dermo-ultrasonography and histopathology with image analysis, is capable of clearly differentiating a range of fillers which differ only in their degree of reticulation.

Poster Abstracts

TPo6: HUMAN NAIL HISTOLOGY DOCUMENTATION: TECHNICAL IMPROVEMENT AND MORPHOLOGY REVIEW

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The nail unit is a challenge for histology technique due to its mechanical properties and in particular due to the adjacent situation of very hard tissues (bone and nail plate) and very delicate structures (bed epithelium and its vascularised dermis). The aim of this histology documentation study was to define the more adequate technique for the nail unit in the context of the research program on nail diseases treatment.

The first samples of cadaver human finger were received frozen and put thereafter in buffered formaldehyde at 10%. After 8 days of fixation, the samples were cut using a saw with diamond-tipped wire. And then let 2 days more in fixative to obtain adequate fixation. Decalcifying was performed either with TBD2 for 48 hours or formic acid at 10% with ion-exchanger resin (RAF) for 24 hours before dehydration/ impregnation with paraffin. Four μm -thick sections were obtained from paraffin blocks after immersion in a cooled solution of 'Mollifex[®]' for approximately 10 minutes.

Further assays were performed with cryostat to evaluate a possible refinement of the technique with frozen cadaver human finger samples: no improvement was seen and therefore, this technique was not considered adequate for histology purposes.

According to the pilot phase, the paraffin technique with formic acid at 10% with ion-exchanger resin (RAF) was selected and used for additional samples fixed in formaldehyde at 10%.

All sections were stained with hematoxylin and eosin and special stains were used to complete the documentation: Masson's trichrome stain to better discriminate the collagen fibers and PAS/alcian blue to allow evaluation of glycosaminoglycan contents in dermis.

The proposed methodology was successfully used for a good visualisation of all nail structures. This technique will be a useful tool to support research program on nail diseases.

Poster Abstracts

TP07: THIRTEEN-WEEK REPEAT ORAL TOXICITY STUDY OF ZINC OXIDE NANOPARTICLES IN SPRAGUE-DAWLEY RATS

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This study was conducted to evaluate the toxicity of zinc oxide nanoparticles (ZnO-NP) after oral administration in a 13-week repeat dose study in Sprague-Dawley (SD) rats.

In a single dose toxicity study, the approximate lethal dose was greater than 2147 mg/kg in males and females. In a 14-day dose-range-finding study, body weight changes were significantly decreased with treatment at 1073.5 mg/kg of ZnO-NP to male rats. Erythrocytes, hemoglobin, hematocrit and MCV values of males were significantly decreased at doses of 1073.5 and 2147 mg/kg. ALP values in both male and females were significantly increased at doses of 1073.5 and 2147 mg/kg. Based on above preliminary results, we allocated 54 male and 54 female SD rats into groups receiving doses of either 0, 67.1, 134.2, 268.4 or 536.8 mg/kg/day for 13 weeks by oral gavage. Biodistribution of ZnO-NP in tissues was also measured.

Piloerection was observed in 20% of males given 536.8 mg/kg of ZnO-NP. In ZnO-NP-treated male animals, body weight changes were significantly decreased during treatment at 536.8 mg/kg. In males, hematological parameters including hemoglobin, hematocrit, MCV and MCHC were significantly decreased at the 536.8 mg/kg dose. However, platelet values were significantly increased. In males, ALP was significantly increased at the 536.8 mg/kg dose. Effects in females were milder than in males. Histopathological findings included chronic pancreatitis in the 536.8 mg/kg ZnO-NP-treated group in both males and females. With respect to biodistribution, ZnO-NP was significantly increased in a dose-dependent manner in blood, urine, feces, liver and kidney of both sexes.

These data suggest that the no observed adverse effect level (NOAEL) of ZnO-NP in SD rats after oral administration is 268.4 mg/kg in a 13-week repeat dose toxicity study.

Poster Abstracts

TPo8: SPONTANEOUS HEMATOPOIETIC TUMORS IN CONTROL SPRAGUE DAWLEY RATS: SIX CASES

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The spectrum of tumors of the hematopoietic systems in laboratory rats occur with strain-related differences. The Fischer 344 rat strain is typically characterized by a high incidence of Large Granular Lymphocyte (LGL) Lymphoma/Leukemia, with reported incidences exceeding 50% in several studies. Conversely in the Sprague-Dawley rat strain, hematopoietic tumors occur at lower frequency compared with neoplasms in other organs, such as mammary gland and pituitary. The most common hematopoietic tumor in Sprague Dawley rats is Histiocytic Sarcoma, followed by Lymphoblastic Lymphoma, LGL Lymphoma/Leukemia and Granulocytic Leukemia.

We report a retrospective survey on spontaneous hematopoietic tumors in control Sprague Dawley rats used in non-clinical safety studies at our institution. The survey included one 2-year carcinogenicity bioassay and seven sub-chronic and chronic repeat-dose studies ranging from 2 to 26 weeks in duration.

In the 2-year carcinogenicity bioassay hematopoietic tumors in animals from control groups were diagnosed in 3/50 males and 2/50 females. Tumors in males included Lymphoma (one case) and Granulocytic Leukemia (two cases). Two Histiocytic Sarcomas were diagnosed in females. The Lymphoma was observed in a male at the final sacrifice and was assessed as incidental. Conversely both Granulocytic Leukemias and both Histiocytic Sarcomas were observed in animals either sacrificed moribund or found dead preterm and were confirmed as fatal and contributory causes of death. In animals bearing Histiocytic Sarcoma and Granulocytic Leukemia, moderate to marked intracytoplasmic accumulation of hyaline droplets in cortical renal tubules was observed. Hyaline droplet nephropathy is actually reported to occur in association with Histiocytic Sarcoma and Myeloid Leukemia in rodents.

In the seven sub-chronic and chronic repeated dose studies surveyed, histopathologic findings were reviewed for 80 males and 80 females (only animals with evaluation of all protocol required tissues were considered). A single Lymphoma (likely a LGL Lymphoma/Leukemia type) was diagnosed in one male from a 13-week study sacrificed at the end of the dosing period.

The results of this survey confirm that hematopoietic tumors in control Sprague Dawley rats are most commonly observed in aged animals (older than 12 months) in the context of 2 year carcinogenicity bioassays. Nevertheless hematopoietic tumors may be occasionally observed in younger animals in the context of 90-day chronic studies.

Poster Abstracts

TP09: TRANSITIONAL GENE EXPRESSION PROFILING OF OVARIAN FOLLICLE IN RATS TREATED WITH INDOMETHACIN AND RU486

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Background: Single dosage of RU486 (RU, progesterone receptor antagonist) or indomethacin (IND, dual inhibitor of cyclooxygenase 1 and 2) can induce unruptured luteinized cyst (ULC) in rats when they are administered on the proestrus day. Trapped cumulus-oocyte complex is found in these ULCs, but the morphology of follicular epithelium is different, i.e., theca cells and granulosa cells in IND-treated rats are larger than in RU-treated rats, and the borderline between theca cell layer and granulosa cell layer is distinct in RU-treated rats whereas it's blurry in IND-treated rats. Previously we determined 4-patterns transitional gene expression profile of ovarian follicles in rats during ovulation; pattern 1, transient up- or down- regulation at 22:00 on the proestrus day, pattern 2, up- or down- regulation only at 10:00 on the estrus day, pattern 3, continuous up-regulation to 10:00 on the estrus day, pattern 4, up- or down- regulated at 22:00 on the proestrus day and maintained the same level at 10:00 on the estrus day (*in press*). In the present study, to characterize the difference between RU-treated and IND-treated rats in the same method, we used Laser Micro Dissection (LMD) technique to collect ovarian follicle samples from RU-treated, IND-treated and untreated rats at 10:00 and 22:00 on the proestrus day and 10:00 on the estrus day, and profiled the transitional changes in gene expression during ovulation.

Materials and Methods: 21 female Crl:CD(SD) rats (eight weeks old) were used. Estrous phases of each animal was determined by vaginal smears for five consecutive days. RU (100 mg/kg) and IND (4 mg/kg) were orally administered at 10:00 and 15:00 on the proestrus day respectively, and ovaries were removed at 22:00 on the proestrus day and 10:00 on the estrus day, frozen with embedded in OCT compound and stored at -70 °C until use. Ovaries were also collected from untreated rats at 10:00 and 22:00 on the proestrus day, and 10:00 on the estrus day as control groups. Each group consisted of three animals. Graafian follicle at 10:00 and periovulatory follicle on the proestrus day, and postovulatory follicle were collected by LMD from each group of ovary, and total RNA was isolated from these follicles to be subjected to GeneChip analysis.

Results and Discussion: Comparing these profiles of untreated rats with those of RU-treated and IND-treated rats, hundreds of genes which showed statistically significant changes were identified and some genes functionally related to ovulation were found among them. Expression of sulfotransferase 1B1 (pattern 1, up-regulated in control rats) was increased, endothelin-2 (pattern 2, up-regulated in control rats), a disintegrin and metalloproteinase with thrombospondin motif 1 and 9 (pattern 4, up-regulated in control rats) were down-regulated in RU-treated rats, whereas none of these were changed in IND-treated rats. To analyze the difference of these profiles further, would make it possible to find genes or pathway which play important roles in the ovulatory impairment.

Poster Abstracts

TP10: NEUROPATHY IN AN EXTENDED ONE-GENERATION REPRODUCTIVE TOXICITY TEST WITH LEAD ACETATE

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Lead acetate (0, 100, 800 and 1700 ppm) was provided in drinking water to adult (Fo) Wistar rats throughout pre-mating, gestation, and lactation, and to the F1 generation up to adulthood. Progeny (10/sex/group/interval) were sacrificed at 21 and 70 days of age for examination of central and peripheral nervous system tissues. The rats were perfusion-fixed *in situ*, using 10% neutral buffered formalin. The parents were sacrificed at the end of the study and similar tissues were preserved.

The brain was sectioned at several levels, so that representative functional areas could be fully evaluated. The levels included rostral brain (behind olfactory lobes), rostral brain (across the caudate putamen), mid brain to include the dentate gyrus, corpus callosum and thalamic nuclei, caudal brain across the cranial colliculi and cerebellum with cerebellar peduncles and pons. Each brain was sectioned in a similar manner, for possible future morphometric assessment, to demonstrate any lead effects, and as a reference for future studies of this nature.

In addition representative sections of the spinal cord (cervical enlargement, mid-thoracic and lumbar enlargement), trigeminal nerve cranial and ganglion, sciatic, tibial and sural peripheral nerves were taken.

Evaluation of the proximal sciatic, sural and tibial nerves from both hind limbs of all animals revealed an increased incidence and severity of nerve fibre degeneration in the high-dose parents (Fo) and 70-day pups compared to controls. There was no evidence of these changes in the 21-day pups.

These findings demonstrate that chronic exposure to lead may result in peripheral neuropathy similar to that observed in humans (Bleecker et al, 2005), suggesting that the rat may be a relevant model.

Poster Abstracts

TP11: PRECLINICAL SAFETY ASSESSMENT OF MEDI-551, AN ANTI-HUMAN CD19 MONOCLONAL ANTIBODY DIRECTED AGAINST B LYMPHOCYTES

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MEDI-551 is a humanized, afucosylated, IgG1 antibody with high affinity for the human CD19 antigen on B lymphocytes. CD19 is a B-cell restricted transmembrane protein and a positive regulator of B cell signaling in complex with CD21. MEDI-551 kills the target B-cell population through enhanced ADCC (Antibody-Dependent Cellular Cytotoxicity) with NK and macrophage effector cells. The preclinical safety characteristics of this molecule have been assessed through tissue cross-reactivity studies, and one, three, and six-month parenteral toxicology studies conducted in transgenic (Tg) mice heterozygous for human CD19. In these mice, the expression of human CD19 is restricted to B cells and follows the expected expression along the B lineage. A GLP compliant TCR (Tissue Cross Reactivity) study was conducted using normal human, Fisher-344 rat and hu-CD19 transgenic mouse tissues at MEDI-551 concentrations of 0.5 and 5 µg/mL. Membranous staining of B cells within the spleen, lymph nodes, tonsil and gut was found in both the human and Tg mouse tissues, but not the rat. Additional staining of cytoplasm and cytoplasmic granules was found in multiple cell types in all three species and was unrelated to known CD19 expression. To support the development of this molecule in multiple indications, progressively longer toxicology studies were conducted in hu-CD19 transgenic mice, which were the only model with affinity for MEDI-551. The three studies were of similar design, 19 or more animals/sex/group with slightly different doses, lengths of administration, and recovery periods of 35 to 37 weeks in duration. Ten animals/sex/group were necropsied at the end of dosing, and the remainder after recovery. B220+ lymphocytes were monitored by flow cytometry and the recovery necropsy was scheduled to occur when these cell levels in MEDI-551-dosed groups were >25% of concurrent control counts. The one-month study used iv doses of 0.675, 3.71, or 36.6 mg/kg, the three-month study 0.5, 3, or 30 mg/kg, and the six-month study two dose groups of 3 or 30 mg/kg. All three studies included a control group which received an equivalent volume of vehicle. All three studies showed no test article-related effects on clinical signs, ophthalmology findings, body weight, or food consumption during the dosing phase. Hematology evaluations demonstrated decreases in total leukocyte counts and absolute lymphocytes, associated with dramatic decreases by Day 30 in B220+ lymphocytes in peripheral whole blood, spleen, and bone marrow. Additional changes related to MEDI-551 were found in the spleen and mesenteric lymph nodes after ≥ one month of dosing, and also in the mediastinal lymph nodes at ≥ three months. Spleens in exposed animals were small at end-of-dosing necropsy with weight reductions of up to 50%. There was decreased size and cellularity of the white pulp compartments in the spleen due to decrease or absence of B-cell regions, and a minimal to severe reduction in the size and cellularity of the B cell follicles in the cortex of the mesenteric and mediastinal lymph nodes. After six months of administration with weekly doses of MEDI-551, there was no difference in the severity of these changes between animals receiving either 3 or 30 mg/kg, but after 36 weeks of recovery, 90% of the animals had spleens and lymph nodes equivalent to controls. Overall, repeated weekly dosing with MEDI-551 at doses up to 30 mg/kg for up to 6 months resulted in safety characteristics that support continued development.

Poster Abstracts

TP12: SEARCH FOR *IN VIVO* RAPID SCREENING MARKERS OF RENAL CARCINOGENS BASED ON THE ANALYSIS OF INDUCTION MECHANISM OF KARYOMEGALY

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By exposure to renal carcinogens in experimental animals, bizarre nuclear enlargement, known as karyomegaly, often appears in the proximal tubular epithelial cells from the early stage. Recent studies have shown aberrant expression of cell cycle-related molecules in the karyomegalic cell area, and this aberration has been suggested to be an initial step for induction of carcinogenic responses. To search for rapid screening markers of renal carcinogens, we focused on karyomegaly and examined phenotype changes in the target cell area by induction of karyomegaly applying microarray analysis on gene expression. Male F344 rats were treated either with karyomegaly-inducing renal carcinogen [ferric nitrilotriacetate, ochratoxin A (OTA) or monuron], karyomegaly-inducing non-renal carcinogen [p-nitrobenzoic acid (PNBA)], renal carcinogen without inducing potential of karyomegaly [tris(2-chloroethyl) phosphate or potassium bromate], or non-carcinogenic renal toxicant without inducing potential of karyomegaly (acetaminophen), for 28 days at doses to induce karyomegaly, carcinogenicity or toxicity. Microarray analysis of the outer stripe of the outer medulla (OSOM), known as a proximal tubular area to show higher proliferation activity than other tubular areas as a target of some renal carcinogens inducing karyomegaly, was performed to obtain gene clusters showing fluctuations in mRNA expression by OTA but unaltered with PNBA as compared with the untreated controls.

Immunohistochemical analysis of candidate molecules selected by microarray analysis as well as cell cycle-related molecules and cell proliferation markers was performed in the OSOM. TUNEL-assay was also performed in this area. Microarray analysis revealed up-regulation of *Mcm3* (proliferation marker) and genes for apoptosis and G2/M checkpoint. Immunohistochemically, cell proliferation markers (Ki-67 and minichromosome maintenance complex component 3) could distinguish renal carcinogens with or without inducing potential of karyomegaly from non-carcinogens. Increase of apoptotic cells was also characteristically observed with renal carcinogens. Among cell cycle-related molecules, topoisomerase II alpha, playing a role from the late S to G2/M phase, responded to most of renal carcinogens, whereas phospho-histone H3 and aurora B as M phase molecules did not respond specifically to renal carcinogens.

These results suggest that renal carcinogens commonly show acceleration of cell cycle to result in high proliferation activity in the proximal tubular epithelia of the OSOM after 28-day of treatment. Also, aberration of cell cycle probably at the late S-phase occurs simultaneously during proliferation to result in increase of apoptosis. In conclusion, we found here that measurement of cell proliferation activity, apoptotic cell index and distribution of topoisomerase II alpha-immunoreactive cells in the OSOM may provide a valuable tool for rapid prediction of renal carcinogens irrespective of the karyomegaly-inducing potential.



Poster Abstracts

TP13: MODIFICATION EFFECTS OF LIVER TUMOR PROMOTION/LIVER INITIATION IN COMBINED ADMINISTRATION OF OMEPRAZOLE (OPZ) AND B-NAPHTHOFLAVONE(BNF) IN RATS

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Introduction: Omeprazole (OPZ) and β -naphthoflavone (BNF) are CYP1A inducers and have a reactive oxygen species-mediated liver tumor promoting effect. In this study, we investigated modification effects of 1) liver tumor promotion and 2) liver initiation of combined administration of OPZ and BNF in rats.

Methods: 1) Male rats were subjected to partial hepatectomy (PH), and given oral doses of 138 or 276 mg/kg OPZ, 0.125% or 0.25% BNF in diet or 138 mg/kg OPZ+0.125% BNF for 6 weeks after an i.p. injection of N-diethylnitrosamine. 2) Male rats were subjected to PH after treatment with the same doses of OPZ or BNF as Experiment 1 for a week. MeIQx was orally administered to rats 12h after PH. Then the rats were fed basal diet for 2weeks, followed by diet containing 0.015% 2-acetylaminofluorene for the next 10 days with a single oral dose of CCL4 3 weeks after PH.

Result: 1) In treated groups, the number/area of GST-P-positive foci significantly increased. Especially in OPZ+BNF group, the GST-P positive foci were significantly higher than the OPZ or BNF alone groups. Real-time RT-PCR analysis showed that the expression of Ahr gene battery such as Cyp1a1, and Cyp1a2, and NRF2 gene battery such as Afar and Yc2 significantly up-regulated in treated groups, and the up-regulation of these genes was much higher in OPZ+BNF group. 2) Initiation activity was not enhanced in treated groups except for 0.125% BNF alone group.

Conclusion: Combined administration of OPZ and BNF resulted in synergistic effects in the liver tumor promotion, but not in the liver initiation in rats.

Poster Abstracts

TP14: DEVELOPMENT EXPOSURE TO MANGANESE INDUCES SUSTAINED ABERRATION OF NEUROGENESIS AND GLIAL PROLIFERATION IN MICE

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Chronic exposure to excess manganese produces neurodegenerative disorder affecting the basal ganglia characterized by reactive gliosis and expression of neuroinflammatory genes, which then causes injury of neurons that is associated with parkinsonian-like motor deficits, termed manganism. In the present study, to elucidate whether exogenously administered manganese targets developing nervous system causing permanent disruption of normal growth, oral doses of manganese chloride (0, 32, 160, or 800 ppm in diet) were given to maternal mice, and male offspring were immunohistochemically examined in the hippocampal dentate gyrus at the end of lactational exposure on postnatal day (PND) 21 and also on the adult stage at PND 77. Brain level of manganese as examined in the cerebellum was increased on both PND 21 and PND 77 in offspring of all manganese-exposed groups. Immunohistochemically, sustained increases in reelin-expressing but NeuN-lacking population suggestive of immature GABAergic interneurons and NeuN-expressing postmitotic neurons were observed in the hilar region at 160 and 800 ppm until the adult stage. This result suggests a sustained aberration of neurogenesis similarly to our previous study regarding developmental hypothyroidism using anti-thyroid agents in rats. While manganese caused hypothyroidism in offspring examined at 800 ppm, magnitude of the hypothyroid effect was rather mild, in contrast to the sustained effect on GABAergic interneurons. In the neuroblast-producing subgranular zone (SGZ) of the dentate gyrus, increases in both cell proliferation and apoptosis of progenitor cells and a decrease of Dpysl3-positive cells suggestive of immature granular cells were observed with manganese as examined at 800 ppm on weaning. At the adult stage, cell proliferation and apoptosis were rather reduced from the prepubertal stage (weaning), showing no fluctuations by manganese-exposure; however, population of Dpysl3-positive cells was slightly increased by manganese as examined at 800 ppm. These results suggest that manganese targets early postmitotic immature granular cells causing compensatory proliferation of earlier progenitor cells during exposure to result in excess production of immature granular cells. Sustained increase of reelin-producing immature interneurons in the hilus may be the reflection of sustained aberration of neurogenesis for correct positioning of excess population of granular cells. Also, GFAP-positive astrocytes were increased in the hilus as examined at 800 ppm on both weaning and adult stage. These results suggest that developmental manganese exposure induces sustained aberration of neurogenesis and following neuronal migration and stimulates glial cell proliferation in mice.

Poster Abstracts

TP15: PULMONARY PHOSPHOLIPIDOSIS IN GÖTTINGEN MINIPIGS

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Phospholipidosis is a well described lipid storage disorder often encountered in pre-clinical studies with cationic amphophilic drugs (CADs). Most reports are from rat studies but phospholipidosis is also described in other species like mice, hamsters, guinea pigs, rabbits and dogs.

However, even though the Göttingen minipig is now used extensively in the pre-clinical testing of new pharmaceuticals there are, to our knowledge, no literature on phospholipidosis in this species.

The lesions presented here are from a regulatory 4-week toxicity study in minipigs with a compound that can be characterized as a CAD. The minipigs were necropsied according to protocol after 4 weeks of dosing. There were no gross findings. At the histological examination, focal accumulation of alveolar macrophages with a foamy cytoplasm were observed in the lung in both sexes in the high dose group and in one male in the intermediate dose group. The minimal number of alveolar macrophages observed in the control group did not have a foamy cytoplasm. The appearance of these findings were consistent with the description of pulmonary phospholipidosis in other species and considered related to treatment and the cationic amphophilic nature of the drug.

Poster Abstracts

TP16: OUTBREAK OF ULCERATIVE VULVITIS IN A SHEEP FLOCK CAUSED BY OVINE HERPESVIRUS TYPE 2

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This is a report of an outbreak of a recurrent ulcerative vulvitis and balanitis in a sheep flock of 16 animals in the mountains of Switzerland. All adult animals showed swollen, reddened vulvas with fresh vesicles or ruptured vesicles with purulent inflammation. The ram had multifocal ulcerations on his penis. A biopsy sample was taken from one animal and sent in for histological investigation. The histological lesion consisted of an acute ulcerative and neutrophilic and eosinophilic dermatitis without intranuclear inclusions. In the same animal ovine herpesvirus type 2 was detected by PCR in the skin lesions.

In addition by immunohistochemistry, the antigen could be seen within the keratinocytes adjacent to the ulcerations. This report describes for the first time an ulcerative vulvitis and balanitis in the sheep with intralesionally demonstration of the ovine herpesvirus type 2 by immunohistochemistry. The authors suggest the ovine herpesvirus type 2 as a possible etiology of ovine ulcerative vulvitis and balanoposthitis in the sheep.

Poster Abstracts

TP16: PATHOPHYSIOLOGY OF UROMODULIN-ASSOCIATED KIDNEY DISEASE: WHAT WE CAN LEARN FROM UMOD-MUTANT MICE

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Aims: Uromodulin-associated kidney disease (UAKD) is a heritable renal disease in humans caused by mutations in the uromodulin (*UMOD*) gene. Clinical symptoms of UAKD are very heterogeneous and can consist of hyperuricemia, gout, impaired urine concentrating ability, and inconstantly progressive renal failure. Histological alterations of the kidneys can comprise tubulointerstitial nephritis, tubular and glomerular cysts, and interstitial fibrosis. The pathophysiology of UAKD is mostly unknown. The objective of this study was to analyze the renal phenotype of two recently established mutant mouse lines carrying two different *Umod* mutations.

Methods: ENU-mouse mutagenesis, mutation identification, metabolic cage analyses, Western Blot, histopathology with IHC, CLSM, stereology, and TEM

Results: Mutants of both *Umod* mutant mouse lines exhibited strong reductions of urinary uromodulin excretion, urine osmolality, and fractional excretion of uric acid. Both *Umod* mutations lead to maturation defect and strong retention of uromodulin in the endoplasmic reticulum (ER) of thick ascending limb cells (TALH), associated with ER hyperplasia. In aged mice, histological kidney alterations included interstitial fibrosis, lympho-plasmacellular infiltrates, and occasionally glomerulo- and tubulocystic changes. Further, an association of different *Umod* mutations and allelic status with differences in onset and progression of the disease was present.

Conclusions: We demonstrated that *Umod* mutations act as gain-of-toxic function mutations leading to TALH dysfunction and impaired kidney function. Our two *Umod* mutant mouse lines represent valuable models for UAKD in humans.

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Poster Abstracts

TP17: PTAQUILOSIDE FROM BRACKEN (*P. AQUILINUM*) INDUCES A B-CELL LYMPHOPROLIFERATIVE MALIGNANCY AND UROTHELIAL DYSPLASIA IN MICE

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Introduction: Bracken (*Pteridium aquilinum*) has long been known to cause cancer in farm animals, especially of the urinary bladder, and is a suspected human carcinogen (IACR group 2B). In mice, bracken administration was found to induce leukaemia. Ptaquiloside, a norsesquiterpene glycoside found in bracken, is considered bracken's main carcinogenic compound. Increased cell proliferation and activation of the WNT pathway are involved in human and rodent bladder carcinogenesis. WNT activation results in β -catenin nuclear translocation from the cell membrane, where it associates with E-cadherin to mediate intercellular adhesion, and in activation of proliferation-related genes.

Materials and Methods: An improved method for ptaquiloside isolation from bracken was adopted with minor modifications. 12 male CD-1 mice were administered 0.5 mg ptaquiloside i.p. weekly for 15 weeks, followed by another 15 weeks incubation period. 12 control mice were administered saline.

Two exposed animals died during the experimental work. On necropsy, blood and tissue samples (brain, eyes, thymus, heart, lungs, liver, digestive system, spleen, bladder, kidney, adrenal gland, urinary bladder, sexual accessory glands, testes, muscle, skin and femur) were collected for histological analysis. Leukograms were prepared from blood smears. Flow cytometry was used to assess blood T-(CD3⁺) and B-(CD19⁺)-lymphocytes, bone marrow granulocytic (CD11b⁺/Ly-6G⁻, CD11b⁺/Ly-6G⁺) and B-lymphocytic (CD19⁺/IgM⁻, CD19⁺/IgM⁺) populations and thymic T-lineage cell (CD4⁺, CD8⁺, CD4/CD8⁺) populations. Lymphoproliferative and urothelial lesions were studied immunohistochemically for antibodies against CD45 and CD3 and also Ki-67, β -catenin and E-cadherin, respectively. Results- 10/10 surviving exposed mice developed a B-cell lymphoproliferative malignancy with B-lymphocytic (CD45⁺/CD3⁻) renal (10/10) and hepatic (2/10) invasion, splenic white pulp hyperplasia (10/10) together with a significant ($p < 0.05$) increase in circulating B-lymphocytes and dysplastic lymphoid cells and neutropenia. No bone marrow changes were detected. Thymic cell numbers were increased when compared with controls mice, but proportions were maintained. 8/10 exposed mice had urinary bladder urothelial dysplasia, with increased ($p < 0.05$) Ki-67 labelling index but identical E-cadherin and β -catenin expression to control animals. No lesions were detected on any of the 12 control mice administered saline.

Conclusions: Results show that ptaquiloside induces a B-cell lymphoproliferative malignancy and bladder pre-neoplastic changes in mice. Increased cell proliferation is a feature of ptaquiloside-induced urothelial dysplasia but there is no evidence of WNT activation or of reduced intercellular adhesiveness through loss of E-cadherin.



Poster Abstracts

TP18: ISOLATION OF CARCINOGENIC AND CYANOGENIC BRACKEN (*PTERIDIUM AQUILINUM*) CONSTITUENTS FROM MAINLAND PORTUGAL SPECIMENS.

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Introduction: Bracken (*Pteridium aquilinum*) is known to cause cancer in animals and is a suspected human carcinogen. Ptaquiloside, a norsesquiterpene glycoside, is considered bracken's main carcinogen. Many other compounds with varied bioactivity have also been identified, depending on the geographical location, time of the year and part of the plant considered. Toxicological studies of ptaquiloside are hampered by the compound's instability and its difficult isolation procedure. This work aims to isolate relevant bracken constituents for toxicological testing.

Materials and Methods: 10 Kg (10% dry weight) bracken crosiers were collected in April 2010 in two bracken-infested pasture areas on the Lima valley, Arcos de Valdevez, Portugal (41° 49' 12" N, 8° 24' 11" W). Two samples (A and B) were collected from site 1. Sample C was collected from site 2. An improved method designed for ptaquiloside isolation from bracken was adopted, with several modifications. For sample A, the previously published method was mostly followed, except that it was frozen until use, instead of air-dried, and chromatographed with CHCl₃ before ethyl acetate (EtOAc). All fractions were controlled by ¹H and ¹³C nuclear magnetic resonance (NMR, 400 MHz). Fractions containing ptaquiloside were separated using two consecutive reverse-phase column chromatographic steps on octadecyl-silane silica gel with CH₃OH/H₂O (20/80 and 40/60) and CH₃OH, instead of HPLC, to obtain pure ptaquiloside. Samples B and C suffered an additional processing modification: the initial batch resin adsorption step was replaced by adsorption onto a resin (Amberlite XAD-2) column. CHCl₃ fractions from sample B were submitted to semi-preparative HPLC-DAD on a C₁₈ column at 4 ml/min (CH₃OH/H₂O 50/50). The first two EtOAc/CH₃OH fractions were also gathered and separated (CH₃OH/H₂O 20/80). Each compound's identity was confirmed by means of its previously reported ¹H and ¹³C NMR (APT, DEPT, HMBC and HSQC) and mass spectrometry data. Results- The ptaquiloside yield was 10mg for sample A and 100 mg for sample B and only vestiges were present in sample C. CHCl₃ fractions from sample B yielded 41.2 mg pterisin B (retention time 12min). The EtOAc/CH₃OH fractions yielded prunasin 67.4 mg (retention time 8 min).

Conclusions: Two toxic bracken constituents were isolated from mainland Portugal samples: the carcinogen ptaquiloside and the cyanogenic glycoside prunasin, partly responsible for acute bracken toxicity. Column adsorption methods were shown to be more efficient for ptaquiloside isolation than the previously proposed batch strategy.

Poster Abstracts

TP19: PULMONARY DEFENSE MECHANISMS AND THEIR POTENTIAL ROLE IN THE PREVENTION OF THIOUREA-INDUCED LUNG TOXICITY: HISTOPATHOLOGICAL AND ULTRASTRUCTURAL ASSESSMENT

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Thiourea and its derivatives have a wide range of uses, varying from the textile and dyeing industry to the production of pharmaceuticals (thyrostatic, antiseptic, antituberculous and antiretroviral agents) and pesticides. Based on preclinical toxicity studies conducted in laboratory animals, the major concern for human health associated with the exposure to thioureas is the chronic inhibition of thyroid gland function. At high dosages thiourea-containing molecules cause rapid insurgence of massive pleural effusion and severe pulmonary oedema in rodents. This type of pulmonary injury closely mimics the clinical picture found in human patients with adult respiratory distress syndrome (ARDS), which is characterized by leakage of fluid into the pulmonary interstitium and alveoli. Interestingly, however, rats become rapidly tolerant to the fatal effect of thioureas when exposed to a prior, small non-lethal dose of this class of molecules, an event known as “tachyphylaxis”. The mechanism underlying this resistance has been associated with the induction of “pulmonary cell hyperplasia”, a feature which has so far not been thoroughly investigated. The present study utilizes a proprietary small phenylthiourea derivative to further characterise the metabolic and morphologic features of the acute adverse effects of thioureas in the lung and to elucidate the mechanisms of early pulmonary defense. For this purpose, we have conducted an oral (gavage) toxicity study and examined the lungs of Wistar rats given a lethal dose. This was followed by a tolerance study conducted in order to assess the time course of the resistance related to the administration of the compound and the associated morphological alterations. Dosage groups included: vehicle-control; tolerance-inducing low dose alone; low dose plus high lethal dose challenge after 3 hours; and high lethal dose alone. The animals were euthanased at 3, 6 and 24 hours; 7 and 14 days after dosing and fully worked up morphologically (gross and light microscopical examination including immunohistology and transmission electron microscopy). In addition, in order to clarify the metabolism and induction of thiourea-dependent chemical stress associated with tolerance, pulmonary and hepatic glutathione levels and expression levels of pulmonary flavin containing monooxygenases (FMOs) isoforms from all groups were compared. Acute lethal toxicity was associated with rapid onset of severe respiratory clinical signs, which correlated with marked pulmonary oedema and severe hydrothorax. Light microscopy showed the presence of large amounts of eosinophilic, homogeneous or faintly granular fibrin-negative material within the alveolar lumen, the dilated lymphatic vessels and in the perivascular interstitial tissue. Transmission electron microscopy suggested that the oedema results from increased vascular permeability due to direct injury to endothelial cells. Preliminary results from the tolerance study show that rats are protected from the lethal pulmonary injury of thiourea derivatives when pretreated with a small dose and microscopic changes in the lungs are dominated by the presence of macrophages and type II pneumocytes rather than alveolar and interstitial oedema. These results indicate an initial, underlying pneumocyte loss leading to subsequent type II pneumocyte proliferation.

Poster Abstracts

TP20: A STEREOLOGY ASSESSMENT OF PRIMATE PANCREAS

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A baseline stereological evaluation of untreated primate pancreas was conducted to determine α -cells and β -cells counts and β -cell volume. Data collected from this study could subsequently provide valuable information for diabetes research and other pharmaceutical applications. Consecutive sectional pancreatic tissue sections were cut and processed from 4 control primates. Tissue were stained for glucagon (α -cells) or insulin (β -cells) using immunohistochemical methods. For the estimation of β -cell volume, a zero dimensional point grid probe was used to analyze each tissue section. With the determined number of β -cell hits, the Cavalieri equation was used to calculate approximate volume. For α -cell and β -cell count, a physical disector was used for each consecutive section. Using a formula to acquire numerical density, multiplied by the pancreas reference volume yielded an estimated numerical count of cells. In the estimation of β -cell volume, a mean volume of 6.7%, and a range of 4.2%-8.2% per pancreas was established. In the calculation of α -cell count, a mean of 1,504,249, and a range of 1,204,257-2,040,134 was obtained. This stereological approach demonstrates an initial representation of the quantification of cells in the pancreas and the variation of α -cell and the β -cell quantities and volume.

Poster Abstracts

TP21: VIRTUAL IMAGING PEER REVIEW (VIPER) – A CASE STUDY

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Histopathology peer review is a vital part of preclinical toxicology studies and has typically been conducted by on-site pathologist evaluation. With emerging digital pathology technologies, successful remote peer review may now be achievable. For many years pathologists have utilized photographic and digital images, both high magnification and whole slide tissue sections, for ad-hoc peer reviews and alternate opinions. This case study documents a peer review process utilizing entirely digitized tissue sections and remote access of the images. A previously peer-reviewed and closed study was used to study remote peer reviews processes. Whole tissue sets from pre-determined animals in control and high dose groups and target tissues from other dose groups were scanned and housed on a dedicated server. Individual animal data and a link to the images were provided to three veterinary pathologists, with extensive peer review experience, who accessed and evaluated tissue images from remote locations (other than the physical server location). The evaluation was conducted to see if the peer review pathologist could identify previously diagnosed lesions and assess the primary pathologist's evaluation. Information was provided by the pathologists on the virtual and remote peer review process and experience. This information will enable hardware and software vendors, and fellow veterinary pathologists to understand the pros and cons of remote peer review.

Poster Abstracts

TP22: RESULTS OF A SUBCHRONIC 90 DAYS STUDY ON ACESULFAME K

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The inadequacy of scientific information regarding the safety of artificial sweeteners, with particular regard to their potential carcinogenic effects, motivated the Ramazzini Institute to launch a project of lifespan carcinogenicity bioassays on Aspartame (APM), Sucralose and Acesulfame-k (Ace K). In order to define the experimental design of the long-term carcinogenicity bioassay on Ace K, one 90-day subchronic study on Sprague-Dawley rats was performed starting treatment at 6 weeks of age. The results of this experiment are reported here. The experiment was performed following OECD guidelines with a GLP process. Ace K (purity 99.95%) was administered in drinking water to groups of 10 males and 10 females Sprague Dawley rats, 6 weeks of age at the start, at doses of 30,000; 15,000; 7,500; 0 ppm. The treatment lasted 90 days, after which time all surviving animals were sacrificed. Blood samples were drawn for hematochemical analysis prior to performing complete necropsy and the weight of the organs was measured. No differences were observed during the biophase among treated and untreated animals. Organs and tissues of all animals of the different groups were examined. Non-neoplastic lesions of the liver and kidneys were observed in treated animals versus control, in both males and females, and in particular in the group treated at the higher dose. These lesions consisted in hypertrophic hepatocytes with presence of basophilic granulation. Kidneys inflammatory and degenerative lesions such as hyalinization and dilatation of tubules and pelvis were also observed. Sporadic lesions of this type were also observed in animals of all other treated groups. In our experimental conditions, Ace K showed mild toxic effects of the liver and kidneys, in particular at the dose of 30,000 ppm. Further investigations, in particular carcinogenicity bioassays, are needed in order to evaluate the potential carcinogenic risks related to long-term exposure. This is especially urgent given the growing consumption of Ace K blended with sucralose, possibly due to the current uncertainty about the safety of Aspartame.

Poster Abstracts

TP23: THE REPRODUCTIVE SYSTEM OF THE FEMALE HAMSTER: ANATOMIC POINTS TO CONSIDER WHEN READING A REGULATORY TOXICITY STUDY

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Little is known and published about the female Syrian hamster reproductive system (*Mesocricetus auratus*). The anatomy, macroscopic and microscopic appearances of the female reproductive tract are significantly different from the other rodents. Female hamsters also have particularities that can interfere with the reading of regulatory toxicity studies.

We present a visual guide of the major specific structures that one can observe in routine toxicologic pathology. In addition, we will provide some stage-specific examples of the Hamster peculiarities.

Note: "All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals"

Poster Abstracts

TP24: CHARACTERIZATION OF SKELETAL MUSCLE TOXICITY FOLLOWING CLOFIBRATE ADMINISTRATION IN RATS

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The characterization of biomarkers to monitor skeletal muscle toxicity in the rat in preclinical toxicity studies can be important to continue the development of a novel compound. In this investigation skeletal muscle toxicity in Sprague Dawley Rats was induced with clofibrate at different dose levels for 7 and 28 days, respectively. Standard clinical assays including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatine kinase enzyme activity (CK) were compared to novel skeletal muscle and cardiac muscle biomarkers including fatty acid binding protein 3 (Fabp3), myosin light chain 3 (Myl3), muscular isoform of creatine kinase immunoreactivity (CK_{MM}), parvalbumin (Prv), troponin I 1/2 (skeletal muscle), troponin T2 (cardiac) and troponin I3 (cardiac). The biomarker elevations were correlated to histopathological findings detected in several muscles of the rat, including m. soleus, m. gastrocnemius, m. triceps, m. quadriceps, and diaphragm. Clofibrate predominantly induced skeletal muscle toxicity of type I fibers. Useful biomarkers for skeletal muscle toxicity were AST, Fabp3, Myl3, (CK_{MM}) and Troponin I1/2. Measurements of CK enzyme activity by a standard clinical assay were not useful for monitoring clofibrate-induced skeletal muscle toxicity in the rat at the doses used in this study.

Poster Abstracts

TP25: VALIDATION OF A SIMPLE MORPHOMETRIC TECHNIQUE TO QUANTIFY ARTERIAL SMOOTH MUSCLE HYPERPLASIA IN A RAT MODEL OF PULMONARY ARTERIAL HYPERTENSION

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Background: Pulmonary arterial hypertension (PAH) is a syndrome characterised by a progressive increase in pulmonary vascular resistance leading to right ventricular overload and eventually to right ventricular failure and death. Increased vascular resistance is caused by vasoconstriction of pulmonary arterioles, smooth muscle cell and endothelial cell proliferation, thrombosis and eventually inflammation. New approaches for treatment are urgently needed for PAH is still considered a disease with a poor prognosis. Rats that are chronically exposed to hypoxia are a well established animal model for PAH. While the primary readouts of this model are the arterial and ventricular blood pressure and heart weight, a quantitative assessment of the distal muscularization of pulmonary arterioles could assist in the discrimination of potential drug candidates.

Objective: To validate a simple morphometrical technique to quantify the muscularization of pulmonary arterioles in a rat model of hypoxia-induced PAH.

Material and Methods: Rats were exposed to normobaric hypoxia ($10\pm 1\%$ oxygen) for 3 weeks (24 h per day). Controls were exposed to normoxia. At termination, blood pressure was recorded and hearts were collected and weighed. Lungs were fixed with an ethanol-glacial acetic acid-formaldehyde solution and were embedded in paraffin. Sections were stained with an immunohistochemical technique using antibodies against anti-hemophilic factor in endothelial cells and actin in smooth muscle cells. 200 blood vessels were evaluated in each section; each was classified according to its diameter (<20 , $20-40$, $40-60$, $>60\mu\text{m}$) and muscularization (non-muscular, partially-muscular, fully-muscular). Two independent experiments were evaluated.

Results: For both experiments, intraacinar arteries with a diameter $\leq 20\mu\text{m}$ showed a clear difference in the degree of muscularization. Partially-muscularized arteries decreased from 61% to 26% (experiment B: 60% to 38%), and fully-muscularized arteries increased from 14% to 69% (experiment B: 34% to 56%).

Conclusions: The degree of muscularization of intraacinar pulmonary arteries can easily be identified with a double-immunohistochemical staining technique using antibodies against the endothelial cell-derived blood clotting factor VIII and smooth muscle actin. Light microscopical evaluation and classification of $\leq 20\mu\text{m}$ diameter arteries allows determination of the degree of arterial muscularization and a clear distinction between normoxia- and hypoxia-exposed animals. The method was validated in two independent experiments. The technique allows discrimination of different drug candidates according to their efficacy, and it can most likely be used for alternative rat models such as monocrotaline-induced PAH as well.



Poster Abstracts

TP26: IMMUNE SYSTEM PATHOLOGY: WHEN IS A FINDING ADVERSE?

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During the past ten years there has been publication of best practice guidelines for evaluation and reporting of immune system (IS) pathology findings within toxicology studies. In addition ICH S8 has proposed a weight of evidence approach using pathology and other findings in deciding if functional tests of the IS are warranted. However one area where there is currently little guidance is in the assessment of adversity of hematological or morphological pathology findings encountered in the IS. This reflects a gap in our understanding of the correlation of this type of finding with a degree of impaired function of the IS. Decisions on adversity may impact clinical doses and subsequent development of a compound, particularly when translatable biomarkers are not available.

This poster will discuss various factors which may be considered in reaching an adverse/ non-adverse decision and provide illustrative examples.

Poster Abstracts

TP27: SPONTANEOUS MEDULLOBLASTOMA IN A CYNOMOLGUS MONKEY (MACACA FASCICULARIS)

Mukaratirwa Sydney, Petrina Rogerson, Ana Blanco, Alys Bradley

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Introduction: Medulloblastomas are infrequent primitive neuroectodermal tumors in animals, mainly seen in dogs and calves. Here we report a spontaneous case of medulloblastoma with glial and neuronal differentiation in a cynomolgus monkey.

Materials and Methods: A 3½-year old male cynomolgus monkey from a control dose group was maintained for a 26-week study. The animal showed signs of intermittent facial paralysis during the study until terminal necropsy. While trimming the brain, a 15 x 20 mm mass replacing the medulla was seen. Tissues were processed and stained with hematoxylin and eosin. Additionally, sections from the medulla were processed for streptavidin-biotin peroxidase immunohistochemical evaluation for S-100, neuron-specific enolase, glial fibrillary acidic protein, neurofilament, vimentin, and synaptophysin.

Results: Histologically, the mass was nonencapsulated and invasive with two different morphologic appearances. The predominant area consisted of densely packed undifferentiated, polygonal cells arranged in vague sheets. The second pattern consisted of less densely cellular areas composed of cells with glial and neuronal differentiation separated by eosinophilic fibrillar material. Immunohistochemical staining confirmed the presence of primitive undifferentiated neuroectodermal cells and cells with glial or neuronal differentiation.

Discussion and Conclusion: On the basis of histopathology and immunohistochemical findings, a diagnosis of medulloblastoma with glial and neuronal differentiation was made. In nonhuman primates, there is one report of a medulloblastoma in a baboon. This is the first report of spontaneous medulloblastoma in a cynomolgus monkey.

Poster Abstracts

TP28: THE BEAGLE BRAIN ATLAS PROJECT

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Beagle dogs are commonly used in preclinical research and neuroscience as the preferred canine model for safety studies and a deep knowledge of the gross and microscopic anatomy of the beagle brain is mandatory for anatomic pathologists to assess the safety of innovative medicinal and industrial chemicals, or to investigate new mechanisms of action.

Very little literature regarding the anatomy of the beagle brain was available to the scientific community in 2008, as most books were out of print or incomplete, and web-based resources only scant. Hence the decision to build an up-to-date reference atlas in this breed with modern tools and illustrations: this 3 year project is now completed and published*.

The poster describes all the technical different steps of the Beagle brain atlas project, including methods for perfusion fixation, orientation of the specimens, histotechnology, realization of the plates, integration of a stereotaxic grid, and labelling of major structures.

Representative plates stained with Weil-myelin Iron Hematoxylin and Nissl (thionine) stains are shown.

*Financial support by the ESTP

Poster Abstracts

TP29: CHARACTERIZATION OF RENAL CRYSTALLINE DEPOSITS IN RAT TOXICITY STUDIES USING MALDI IMAGING MASS SPECTROMETRY

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Tissue imaging using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) provides the ability to analyze the distribution of endogenous or drug related molecules and to correlate these images to histological defined areas. Han Wistar rats were administered a test compound for 1 week. Han Wistar rats administered the vehicle served as controls. Clinical pathology indicated renal damage (increased plasma levels of urea, creatinine and potassium) in the high dose rats. Pathology revealed pale discoloration of the kidneys and increased kidney weight in the high dose. The histopathological examination of kidneys showed marked tubular degeneration/regeneration, multiple pyogranuloma (accumulations of neutrophils/macrophages surrounding amorphous/crystalline material within tubules in the cortex and medulla), inflammation and hemorrhage in the renal pelvis, free crystals in the renal pelvis and transitional cell hyperplasia. Crystals were also detected in the urine and were analysed by NMR. Kidney sections were analyzed by MALDI imaging MS. The analyses were especially aimed at determining if the kidney toxicity was caused by precipitation of bisulfonamide, a metabolite to the administered test compound, since it was suspected to have poor solubility. MALDI MS images highlighted the spots where bisulfonamide (m/z 234,98) was accumulated which correlated well to crystal localization. In addition, this analysis showed several other ions present in treated animals but not in control animals. One of the ions at m/z 439.1 corresponded to the test compound.

NMR and LC/MS analysis of crystals dissected directly from the tissue confirmed that bisulphonamide is the major component of the crystals. The results propose precipitation of bisulphonamide as a possible cause to the kidney toxicity seen in this study.

The results from the present study show that MALDI imaging mass spectrometry is a powerful tool to determine the chemical nature of crystalline deposits in tissues.



Poster Abstracts

TP30: THE NATURE OF GRAFT ASSISTED HEALING RESPONSE OF FULL THICKNESS SKIN WOUND IN A RABBIT MODEL

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Regenerative medicine offers new approaches for treating wounds and the use of tissue engineering scaffolds are increasingly used as grafts. These grafts are expected to complement wound healing responses by preferentially stimulating regeneration to scarring. Many cellular and molecular signals regulating wound healing has been described and full thickness skin wound model is a convenient system for studying these healing process.

We had isolated extracellular matrix from the submucosa of small intestine, cholecyst (gall bladder) and urinary bladder with the prospect of using them as skin substitutes or as scaffolds for developing engineered skin constructs. This study examined the role of these potential skin grafts on the wound healing process in rabbit skin wound model by immuno-histo-morphological studies.

The study showed the overall healing response judged by routine histomorphological parameters like the extent of re-epithelialisation, granulation tissue formation and neo-collagen deposition comparable to the skin wound treated with an acellular graft available in market. However, the number of proliferating cells, the distribution of myofibroblasts and stem like cells were varied among the samples collected from wounds treated with the source of the graft material. The cholecyst-derived graft preferentially stimulated regeneration over fibrosis suggesting that it has potential clinical applications.

Poster Abstracts

TP31: LARYNGEAL LESIONS INDUCED IN WISTAR WU RATS BY INHALATION OF NANOSCALED TITANIUM DIOXIDE (TiO₂ P25)

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Titanium dioxide nanoparticles are widely used in industry, e.g. for production of dyes, lacquers, sunscreens and textiles. Human exposure may be by inhalation, skin absorption or ingestion. Upon inhalation, these very small particles may deposit in all regions of the respiratory tract, cross the air-blood barrier by penetration of cell membranes or translocation through cells and distribute to other organs via the bloodstream.

For investigation of lung effects, a 3-week nose-only inhalation study (6 h daily exposure) with nanoscaled TiO₂ (Aeroxide TiO₂ P25 [hydrophilic, mean size: 21 nm, 20% Rutile, 80% Anatase], Evonik Degussa GmbH, Germany) was performed in female Wistar WU rats [strain: CrI:WI(WU)] at aerosol concentrations of 2 and 10 mg/m³ TiO₂ P25, followed by a recovery period of 3 and 28 days, respectively. In an additional study, rats were investigated immediately following a 24-hour exposure to 10 mg/m³ of TiO₂ P25. The occurrence of laryngeal lesions in rats exposed to 10 mg/m³ TiO₂ nanoparticles was unexpected and to our knowledge has not been reported in the literature so far.

Acute erosions/ulcerations developed between the caudal base of epiglottis and the anterior ventral pouch as soon as 24 hours post exposure. A subsequent focal laryngeal inflammation with subepithelial formation of multinucleated giant cells (macrophages) in response to deposits of nanoparticles persisted until the end of the recovery period. Focal minimal to slight epithelial alteration and squamous cell metaplasia were further (adaptive) changes observed in the TiO₂-exposed larynges.

The present results show that the base of the epiglottis as the most sensitive region in the rodent larynx may be the target site for toxicological damage not only for many chemicals but also for aerosolized, highly diffusible nanoparticles.

Poster Abstracts

TP32: AN IMMUNOHISTO-MORPHOLOGICAL STUDY OF THE HEALING RESPONSE OF MUSCLE TO IMPLANTED POLYETHYLENE-TEREPHTHALATE IN A RABBIT MODEL

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The nature of tissue reaction induced by biomaterials implanted at various sites of the body of laboratory animals is a good indicator of biocompatibility. Hence, an assessment of local effects by histopathology has become an essential pre-requisite for safety evaluation of potential biomaterials and fabricated devices. Although standard procedures are available for the assessment, in the light of recent developments in cellular and molecular biology, there is an increased awareness for understanding the nature of cellular and molecular modulations occurring at implant niche. With this in view, in addition to the routine parameters commonly considered during *in vivo* evaluation of biocompatibility, this study evaluated the nature of tissue reaction by immunohistochemistry in rabbit muscle around implanted Polyethylene terephthalate fabric which is used for fabricating biomedical devices.

PCNA-positive cells were largely fibroblasts and cells of inflammation rather than rhabdomyocytes or satellite cells discounting the possibility of stem cells activation in this model. However, the injured rhabdomyocytes showed an indication of change in phenotype to a myofibroblast morphology. Different sub-populations of foreign body giant cells were detected depending on PCNA-immunopositivity. Immunohistochemistry to alpha smooth muscle actin and vimentin suggested a significant role for myofibroblast in the healing reaction. It is proposed that identification of specific cell types participating in tissue reaction and detection of molecules participating cell-matrix interaction might be useful for the assessment of safety evaluation of biomaterials and biomedical devices.

Poster Abstracts

TP33: OCT 4, NANOG, EPCAM AND CD44 EXPRESSION IN MOUSE HEPATIC LESIONS INDUCED BY DIETHYLNITROSAMINE

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Expressions of Oct 4, Nanog, EpCAM and CD44 were investigated in mouse hepatic lesions induced by diethylnitrosamine (DEN). Male 14-day-old mice were treated with DEN (10 mg/kg of body weight) and were killed at 6 h and at 36 weeks after DEN treatment for immunohistochemical investigation of four stem cell markers expression in early hepatic tissues and hepatic tumors, respectively. Using BioMark array (Fluidigm), Oct 4, Nanog, EpCAM and CD44 mRNA expressions were also examined in early hepatic tissues at 6 h, 1 day, 2 day, 3 day, 7 day and 28 day after treatment of DEN. Proliferation and DNA damage were also assessed. Oct4 expression was not detected in DEN-induced tumors and in early liver tissues treated with DEN, even though its expression was detected in embryo tissues used as positive control. Nanog expression was not detected in DEN-induced tumors, while its expression was detected in small round cells, but not in hepatocytes in early liver tissues treated with DEN. However, positive cells showed only small population of cells in the liver. EpCAM expression was detected in DEN-induced tumor cells, showing slight increased expression compared to surrounding normal tissue. And almost of EpCAM positive tumor cells showed cytoplasmic staining. And its expression was also detected in some normal cells around tumor tissues. In early liver tissues treated with DEN, its expression was detected in hepatocytes, bile ductular cells and surrounding round cells, showing cytoplasmic staining. CD44 expression was weakly detected in DEN-induced tumor cells and slight strongly detected in some hepatocytes surrounding tumor cells. However, this expression was not detected in early liver tissue treated with DEN. Many cells showed high proliferative potential detected by proliferating cell nuclear antigen (PCNA) staining, and DNA damages detected by TUNNEL staining were increased in early lesions of DEN-treated mice. Oct 4 mRNA expression was under detection level at all time points and Nanog mRNA expression showed some alteration, but no significant difference among the group. EpCAM mRNA expression showed there were significant increases at DEN-treated groups at day 1, 7 and 28 compared to saline-treated group at 6 h ($p < 0.01$). CD44 mRNA expression showed somewhat alteration, but no significant difference among the groups. Interestingly, EpCAM expression was detected in DEN-induced tumors and in several types of cells including hepatocytes, bile ductular cells in early liver tissues, and its mRNA expression showed significant difference among the groups at some time points.

Poster Abstracts

TP34: UNEXPECTED BRAIN LESIONS IN LACTATING SPRAGUE-DAWLEY RATS IN A TWO-GENERATION INHALATION REPRODUCTIVE TOXICITY STUDY WITH PENTAFLUOROPROPANE

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The study presented was conducted following the reproductive study guideline OECD Guideline 416 Two-Generation Reproduction Toxicity Study. Sprague Dawley rats were exposed to 2,000, 10,000 and 50,000 ppm pentafluoropropane. There was an unexpected mortality of lactating dams in the medium and high dose group beginning at day 10 of lactation. Statistically significant histopathological alterations were observed in the cerebellum of 9/30 females of the Fo-generation and in 10/27 females of the F1-generation of the high dose group. In contrast, there were no brain lesions found in males or non-pregnant females of all dose groups. Neuronal necrosis and degeneration in the cerebellar cortex were observed as the most severe finding. Furthermore, spongiosis in different degrees was diagnosed in 7/30 females of the Fo-generation and in 9/30 females of the F1-generation. Acute hemorrhages – in particular perivascular – occurred in 5/30 females of the Fo- and in 5/30 females of the F1-generation indicating a disturbed vascular integrity. The main lesions found in the cerebrum were glial scars in the corpus callosum which were found in 2/30 females of the Fo-generation of the high dose group. A significant increased incidence of myocardial fibrosis and mononuclear cell infiltration in males – indicating a cardiomyopathy – were only seen in the Fo-generation of the high dose group. Moreover, females of the F1-generation of the high dose group showed an increased incidence of minimal myocardial fibrosis. In summary, histopathology revealed that the brain, particularly the cerebellum, and to a minor degree the heart turned out to be the toxicological target organs of the substance. Presumably substance-related energy deprivation may be responsible for the observed changes.

Poster Abstracts

TP35: EVALUATION OF MIR-122 AND OTHER BIOMARKERS IN ACUTE LIVER INJURY IN RATS

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The detection of drug-induced hepatotoxicity remains an important safety issue in drug development. Several informative biomarker candidates have been suggested in published literature to show utility besides ALT. Recently, a specific microRNA species, mir-122, has shown potential for predicting liver injury in addition to the protein markers being evaluated so far. The objective of this study was to measure mir-122 along with a selected toolkit of qualified and candidate protein markers in specific settings of acute liver toxicity to determine the value of mir-122 as a diagnostic marker for liver injury. Therefore, rats were exposed to 3 well-established liver toxicants (acetaminophen, allyl alcohol, α -naphthyl isothiocyanate), a liver-enzyme inducer (phenobarbital) or a cardiotoxicant (doxorubicin). Circulating levels of mir-122 and several protein candidate markers (GLDH, SDH, α -GST, L-FABP, hemopexin, haptoglobin, Gc-globulin) were determined and compared to ALT and histopathology. Mir-122 exhibited a pronounced increase in serum with different compounds causing liver necrosis and not with phenobarbital or doxorubicin. The change in mir-122 paralleled that of other markers and the histopathology of liver injury. Furthermore, the changes in mir-122 were detected at an early time point. Therefore, these findings suggest the potential use of circulating mir-122 as a specific and sensitive biomarker of liver injury in rats.

Poster Abstracts

TP36: COMMON BACKGROUND LESIONS IN YOUNG SYRIAN GOLDEN HAMSTERS

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Incidences of spontaneous lesions in young adult Syrian golden hamsters aged upto 38 weeks are presented in this poster. Data were collected from 326 animals (163 of each sex) used as controls in 17 short term toxicity studies, conducted at Huntingdon Life Sciences, UK, during the period 1986 – 2011. Based on their age, animals were divided into three groups (8 -10 weeks, 11-20 weeks and 21-38 weeks). Incidences of the commoner background lesions were compared between the various age groups to assess their relationship to the age of animal. The most commonly observed background lesions were in the kidneys, liver and stomach in all age groups. Changes in the gall bladder were recorded in animals between 11-20 and 21-38 weeks of age. In the kidneys basophilic tubules and nephrocalcinosis were seen in all age groups, without any sign of progression with age. The changes were generally of a low severity in all groups.

In the liver, inflammatory changes were seen in all age groups. The infiltrates were predominantly lymphoplasmacytic and often concentrated around centrilobular veins, sometimes with lymphoid follicle development. Inflammation and choleliths were observed in the gall bladder in animals between 11-20 and 21-38 weeks of age. Inflammation was generally neutrophilic in nature. Choleliths, where seen, were generally not associated with inflammatory cell infiltration or epithelial changes. Mineralisation in the stomach was seen in all age groups, with no evidence of progression with age. Erosions and ulceration of the glandular mucosa were seen in animals between 11-20 weeks and 21-38 weeks with a low incidence. Special stains, performed on some of these lesions, revealed no organisms although similar lesions are described in the literature as being associated with *Helicobacter*.

Incidence of neoplastic findings was very low with only one tumour recorded in 326 animals. This was a nephroblastoma of the kidney, seen as an incidental finding in a female at the end of a 13-week study. The background lesions described here are similar to those described in the literature, but with information here about age of onset. Since hamsters are increasingly being employed as an alternative animal model to rats or mice, familiarity with the background pathology of this species, and with the chronological appearance of these lesions, is essential for accurate interpretation of treatment-related changes.

Poster Abstracts

TP37: SAFETY EVALUATION OF A CHOLECYST-DERIVED TISSUE ENGINEERING SCAFFOLDS INTENDED FOR REGENERATIVE MEDICAL APPLICATION

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Extracellular matrix isolates from various mammalian organs and tissues (e.g.: porcine small intestinal submucosa) have found excellent applications in regenerative medicine, especially as tissue grafts which promote cell growth, regeneration and morphogenesis. Over 25 products are now available in market for therapeutic grafting and over one million patients have received such beneficial grafts. The steps involved in the safety evaluation of such products poses challenges for the toxicologic pathologists. We isolated the extracellular matrix from the submucosa of porcine gall bladder (GBS) and compared its characteristics with extracellular matrices isolated from the submucosa of porcine small intestine (SIS) and urinary bladder (UBS) as well as with a reference clinical product (CSIS, small intestinal sub mucosa isolates marketed by M/s Cook Biomedicals™).

This presentation summarizes the strategy adopted for the safety evaluation of GBS. The GBS was rich in collagen. The protein content was similar to those scaffolds originating from other organs but contained a larger quantity of natural biomolecules like glycosaminoglycans and elastin. Prototype of a skin graft was used for functional evaluation in a rabbit full thickness skin-wound model. Results of *in vitro* Cytotoxicity (ISO 10993-Part 5), *in vivo* biocompatibility (ISO 10993-Part 6), test for pyrogenicity (USP 23/NF21<85>) and test for sterility on the finished product (USP 31/NF26<71>) were similar to the reference product and tissue engineering isolates from similar sources.

Poster Abstracts

TP38: FOCAL CEREBELLAR SPONGIOSIS IN A SPRAGUE DAWLEY RAT

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Background changes in the brain of rats are uncommon in toxicology studies. Here we report the occurrence of bilateral neuronal degeneration/necrosis of Purkinje neurons and spongiosis of the surrounding neuropile in the cerebellum of a 14 week old Sprague Dawley rat. Other findings in this animal included a decreased body weight, macroscopic observations (small size liver, epididymides, prostate, seminal vesicle and testes) and associated microscopic changes (marked bilateral and diffuse seminiferous tubule degeneration in the testes and moderate cellular debris and multinucleated spermatid giant cells in the epididymides lumen). Other microscopic changes present in this animal were considered to be part of the common background pathology of SD rats (vacuolation and cardiomyopathy in the heart and collecting ducts/tubules casts and dilation in the kidney) or treatment related (cellularity changes in lymphoid organs and minimal decrease in the primary spongiosa of the femur). The cause of this cerebellar neuronal degeneration/necrosis and spongiosis is unknown.

A fluorochrome staining method (Fluoro-jade C - FJC) was used to confirm neuronal degeneration/necrosis on formalin fixed paraffin embedded brain sections. In our hands, FJC provided sensitive and specific labeling of degenerating neurons, which complement the H&E observations of distinctive necrotic neurons (shrunken eosinophilic neurons with slender condensed nuclei). Dual labeling with FJC and GFAP which was carried out to address the possibility of non-specific labeling of GFAP positive cells proved technically challenging. Non-specific FJC labeling of GFAP positive cells (astrocytes) was not observed and fluorescence of erythrocytes did not interfere with the identification of positively stained neurons.

In conclusion we confirmed that similarly to Fluoro-jade B the most recent FJC fluorochrome is suitable for sensitive and specific labeling of degenerating neurons and for dual labeling in routine toxicology studies tissue (non-perfused immersion fixed paraffin embedded tissue).

Poster Abstracts

TP39: SPONTANEOUS IRON PIGMENT OVERLOAD IN THE LIVER OF HAN WISTAR AND SPRAGUE DAWLEY RATS

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In a review of the toxicological studies performed in our laboratories during the period 2006-2011 for carcinogenicity studies and 2010-2011 for short term studies, including 4 weeks and 13 weeks studies, - we occasionally observed significant pigment deposition in the periportal areas of the liver in control Han Wistar and Sprague Dawley rats of different ages and both sexes. The pigment was identified as iron pigment and a diagnosis of spontaneous iron overload was made. Iron pigment accumulated in both Kupffer cells (or macrophages) and hepatocytes.

Preliminary results from our survey showed no significant differences in the incidence of iron pigment overload between Sprague-Dawley and Han Wistar Rats in short term studies, and no sex difference. Data from long term studies showed a slight increase in the incidence of iron pigment overload in Han Wistar rats with female rats of this strain having incidences of more than three times that of males. In rats, most of the iron accumulates in the liver in the form of ferritin or as hemosiderin which is an insoluble end stage degradation product of ferritin. The periportal hepatocytes are the first affected followed by deposition in hepatocytes throughout the lobule and also in bile ducts epithelial cells, Kupffer cells and sinusoidal cells.

There is little compiled data on spontaneous iron overloading in the liver in rats used for toxicity studies, and no reports of this finding in Han Wistar rats have been published, to the best of our knowledge. The purpose of this report is to present and discuss the incidence and pathology of this background lesion, observed in rats used as untreated controls in toxicity studies. This is in view of the importance of having a well characterized control data base, for better assessment of potential treatment related effects that may alter the incidence or severity of a spontaneous finding.

Poster Abstracts

TP40: HYALINE GLOMERULOPATHY IN A CD-1 MOUSE

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Eosinophilic hyaline material within the glomeruli of the kidney presents not an unusual finding which is in most cases composed of amyloid. In addition, immune-mediated or diabetic glomerulopathies are frequently reported in mice. Although other causes for this hyaline alteration exist, in most of these cases the pathogenesis can not be elucidated.

The presented case is one of few CD1 mice maintained under specific pathogen free conditions of a laboratory animal house and killed in a moribund condition. This animal was 25 weeks old and during necropsy the thoracic cavity contained 0.5 ml fluid. In addition, the kidneys were bilaterally pale and showed an irregular pitted surface with few cysts. On the microscopic level, there were multifocal moderate interstitial and perivascular mononuclear cell infiltrates in the kidneys. Many tubuli were dilated with an eosinophilic homogeneous material in the lumen. Other tubuli exhibited signs of regeneration and degeneration of the epithelial cells with some degenerated cells within the tubular lumen. In addition, some tubular epithelial cells as well as some surrounding macrophages showed brown pigments in the cytoplasm which were Berlin blue positive. Furthermore, the glomeruli showed slight hypercellularity, synechia as well as fibrosis of the surrounding Bowman's capsule. In addition to these changes the glomeruli were multifocally segmentally expanded by a homogenous eosinophilic material. This material was PAS positive but without a positive signal after Congo-red staining in polarized light. The remaining organs were without any significant pathological changes.

The lesions in the kidneys of this mouse in comparison to the findings of other mice affected by a hyaline glomerulopathy will be presented.

Poster Abstracts

TP₄₁: EFFECT OF A CHELATING AGENT (DMSA) AND VITAMIN C ON NICKEL-INDUCED TESTICULAR TOXICITY IN RATS: TOXICOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL STUDY

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The present work aimed to study the effect of nickel toxicity induced by exposure to nickel chloride (NiCl₂) on testes of albino rats, and to investigate the effect of a chelating agent (DMSA) and an antioxidant (vitamin C) on such toxicity.

The results of the present study showed that subcutaneous administration of 4.4mg/kg/day NiCl₂ for 5 weeks resulted in significant increase of nickel and malondialdehyde (MDA) levels and significant reduction of reduced glutathion (GSH) and superoxide dismutase (SOD) activity in the testicular tissues. Histological examination of testes showed multiple degenerative changes and malformation of spermatozoa. The present study revealed that when vitamin C was administered concomitantly with NiCl₂ for five weeks, it caused a significant effect in preserving the histological and ultrastructural picture of the testis and more or less normalization of the antioxidant defense system. The nickel level was also significantly decreased but did not reach the control level. On the other hand, when DMSA was administered at a dose of 30mg/kgm/day in three divided doses orally for five days after daily subcutaneous injection of 4.4mg/kg/day NiCl₂ for 5 weeks, this caused a significant lowering of nickel level reaching the control values with mild improvement of the antioxidant defense system and lesser improvement of the histological and ultrastructural impairment.

Poster Abstracts

TP42: CHANGES OF LIPID PROFILES, GLUCOSE AND HAEMOGRAM AFTER ADMINISTRATION OF RUTA GRAVEOLENSE EXTRACT IN DIABETIC RATS

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Diabetes mellitus is a disease that affects about 6.6% of population in Iran. Many people in the world use therapeutic herbal medicine for many reasons. In the present study, the effects of *Ruta graveolense* extract on blood levels of glucose, lipids and haematological parameters have been studied. For this purpose thirty adult male Wistar rats weighing 200-300g were divided randomly into six groups (A, B, C, D, E, F) and housed in single cages. The control group (A) was injected with normal saline. Diabetes was induced by injection of streptozocine (60mg/kg, i.p.) in the other five groups. Group C received glibenclamide (10 mg/kg) orally and groups D, E and F received hydroalcoholic extract of *Ruta graveolense* (10, 20 and 30 mg/kg, i.p.) for ten days respectively. Blood samples were taken by heart puncture and the level of glucose and lipids were measured. Haematological parameters including complete blood count (CBC) was also determined by using automated cell counter. Results showed that administration of *Ruta graveolense* extract caused a significant decrease in the levels of cholesterol and LDL-c ($P < 0.05$) in a dose-dependent manner, whereas no significant changes were seen in glucose, triglycerides, VLDL-c and HDL-c values in diabetic rats. It appears that *Ruta graveolense* extract have significant effects on total cholesterol and LDL-c in diabetic rats, although further work is needed to elucidate the extent and mechanism of these changes.

Poster Abstracts

TP43: CHALLENGES IN SAFETY EVALUATION OF BIOMATERIALS, BIOMEDICAL DEVICES AND ENGINEERED TISSUE CONSTRUCTS

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Biomedical devices are made of biomaterials which are essentially non-viable materials intended for specific application and unlike a drug, they may not directly interact with the metabolic and biochemical pathways of cells. Biocompatibility evaluation is the corner stone of safety evaluation of candidate biomaterials and fabricated devices; essentially a histopathological assessment of tissue response to implanted materials/devices in a selected animal model. So much so, the toxicologic pathologist plays a pivotal role in biocompatibility evaluation of candidate biomaterials and biomedical devices. Material characteristics and surface topography are important determinant of biocompatibility. Although robust standards are available for the testing of biomaterials, they may need substantial modification for evaluating new generation specific products containing added drugs, biomimetic components and cell-signaling molecules. Medical devices designed for tissue engineering applications may have functional cells of xenogenic origin. Thus, the toxicologic pathologists face several challenges in the assessment of biocompatibility of products designed for diverse clinical applications.

Toxicologic pathologists associated with preclinical safety evaluation of biomaterials, devices and tissue constructs require a basic understanding of material sciences, bioengineering and regenerative medicine before assessing the nature of the tissue response to implanted material. Safety evaluation of materials is emerging as a sub speciality of toxicologic pathology.

Poster Abstracts

TP44: IMMUNE RESPONSES OF PROGENY OF HENS KEPT ON AFLATOXIN CONTAMINATED FEED

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Introduction: The present experimental study was conducted to determine the immunological responses of progeny of hens kept on aflatoxin (AFB₁) contaminated rations with or without vitamin E (VE).

Material and methods: Layer breeder hens were divided into 12 groups and fed rations containing different possible combinations of AFB₁ (0.00, 0.01, 0.05, 2.5, 5.0 and 10.0 µg/kg) along with VE (0.0 and 100 mg/kg). Hens were artificially inseminated with semen of males kept on basal ration. Experimental feeds were offered for three weeks. Fertile eggs were incubated to obtain the progeny chicks from AFB₁ intoxicated hens.

Results & discussion: Body weights of chicks from AFB₁ fed hens were significantly lower than controls. Antibody titers against sheep red blood cells (SRBC) were significantly lower in the chicks from the hens fed higher doses of AFB₁. Lymphoproliferative response to phytohemagglutinin-P (PHA-P) of the chicks was significantly lower in progeny of the hens fed 0.50 µg/kg or higher levels of AFB₁. Peritoneal macrophages of the chicks fed AFB₁ showed significantly low phagocytic potential and nitrite production when challenged with LPS.

Significant ameliorative effects of vitamin E (VE) were found upon AFB₁- induced damage to RBC engulfment and Nitrite production by peritoneal macrophages and anti-oxidant potential as determined by an azo-bis compound (2-2'-azobis (2-amidinopropane) hydrochloride).

Poster Abstracts

TP45: THIOREDOXIN REDOX SYSTEM PRESERVES THE CELL PROLIFERATING ACTIVITY OF LIVER CELL COMPONENTS DURING LIVER REGENERATION WITH AN ASSOCIATION WITH AP-1 TRANSCRIPTION FACTOR

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The process of liver regeneration (LR) after injury is a very complex and well-orchestrated phenomenon. It is successfully achieved by the participation of a large number of genes regulating cell proliferation and apoptosis. Thioredoxin (Trx) activity is regulated by Trx reductase (TrxR) enzyme and functions as a cell proliferating promoter and an anti-apoptotic factor in cells. In addition, this redox system could function for buffering the cytotoxic free radicals produced during the process of liver injury and regeneration. In the present study, we investigated the roles of Trx and the cooperative relation with AP-1 transcription factor in LR after partial hepatectomy (PH), using Trx transgenic (Tg) and hetero-knockout mice (hKO) as well as wild-type mice (WT). For the purposes, in addition to the measurement of liver volume, cell proliferating activity (PCNA and Ki-67) and TrxR activity at multiple time points of LR, we performed quantitative analyses of DNA synthesis and mRNA and proteins of Trx and AP-1 family proteins during LR in the WT and Trx hKO mice or C3H mice. LR was completed by the accurate regulation of cell proliferating activity, represented by the reserving, active and retardation phases after PH. Trx mRNA and protein levels were dynamically responsive depending on the regenerating phases. The significant implication of Trx for LR was further supported by the liver cell loss-dependent increase of TrxR activity. Among the AP-1 proteins, c-fos was up-regulated during LR, corresponding to the expression of Trx mRNA and protein. Transgene of Trx enhanced the cell proliferating activity of liver cell components during LR. The results all together demonstrated that Trx redox system plays a significant role in the LR process by preserving the proliferating activity of liver cell components with an association with AP-1 transcription factor.

Poster Abstracts

TP46: HISTOLOGICAL EXAMINATION OF THE CORNEA IN BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) ASSAY

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The bovine corneal opacity and permeability (BCOP) assay is one of the alternative methods to the Draize test. The evaluations of this assay are focused on the opacity related with corneal injury and permeability reflecting the increase of stromal injury. In this study, we try to evaluate the histological changes of the cornea in BCOP assay in order to assess the relationship between histological changes and BCOP scores. According to the OECD guideline, BCOP was performed three times for each test chemicals. The histological evaluation was performed on the superficial epithelial layer, Bowman's membrane and stroma and endothelia. There were degeneration and necrosis and desquamation of the epithelia. In the stroma, focal necrosis and degeneration of keratocytes were seen. Histological study showed the high relationship with the result of opacity and permeability. This results show that histological examination of the cornea after BCOP assay will be very effective to evaluate the ocular toxicity of the chemicals.

Poster Abstracts

TP47: TOXICOLOGICAL AND PATHOLOGICAL STUDY OF THE PUBLIC HEALTH INSECTICIDE COMMERCIAL PREPARATION TERMINATOR- 2.5% WP (DELTAMETHRIN) PRODUCED BY VAPCO

Nabil Hailat

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Deltamethrin is in the chemical class of pyrethroids and is considered a broad-spectrum insecticide. It is effective against insects via ingestion and direct contact. Pyrethroids, in general, interfere with normal production and conduction of nerve signals in the nervous system. Pyrethroids act on nerve membranes by delaying the closing of the activation gate for the sodium ion channel. Reported LD₅₀ values for deltamethrin in rats range from 30 mg/kg (with an oily vehicle) to greater than 5000 mg/kg (in an aqueous vehicle). The substance used to administer deltamethrin can influence the LD₅₀ for the oral route most likely by affecting absorption. The objectives of this study are to a- To determine the Median Lethal Dose (LD₅₀) of TERMINATOR-2.5% WP (DELTAMETHRIN), a public health insecticide, produced by VAPCO, in rats (acute toxicity), b- To study in rats the repeated oral dose toxicity of TERMINATOR-2.5% WP and to present the clinical, body and organ weights, and pathological signs and findings, c- To investigate the dermal toxicity of the TERMINATOR-2.5% WP in rats and to report the clinical, body and organ weights, and pathological signs and findings.

Three experiments were conducted in this study. At the end of the experimental period rats were slaughtered and blood samples were collected into EDTA treated tubes for blood profile and into plain tubes for sera analysis. In experiment one, twelve Wistar rats, 150-200g b.wt, were employed. They were divided into two groups of study of six animals each, all of which were females. Animals of group 1 were kept as untreated control. Those of group 2 were divided further into two groups of 3 rats each, given a single dose (5-6 ml) of TERMINATOR-2.5% WP by oral gavages at a dose of 300 and 500 mg/kg of the prepared product while the control received distilled water. Animals were observed for toxicity signs and mortality for a period of 48hrs. The mortality was recorded in each of the groups. The LD₅₀ of TERMINATOR-2.5% WP was found to be not less than 500mg/kg bwt which is similar to what is reported in the literature (200 to 5000 mg/kg body weight).

In experiment two, two groups of rats, each of six animals were employed. Animals of group 1 (A) were kept as control. Those of groups 2 (B) were given 3-4 ml of TERMINATOR-2.5% WP orally using a gavage needle at the dose of 1:250 of the LD₅₀ (2 mg/kg bwt) daily for a period of fourteen days. The animals were normal with no significant difference between the mean of their weights, their internal organs, RBC, WBC, Hb and PCV compared to the control. Also no significant changes were seen in the histopathological examination of the liver, lungs, heart and kidneys, and the liver function enzymes.

In the third experiment, ten rats were used. An electrical clipper was used to shave the back and the flank region of each animal. Animals were then divided into two groups. Those of group A were kept as control. Rats of group B were painted on the shaved area with 5 ml of TERMINATOR-2.5% WP (2 mg/kg bwt) respectively using a piece of sponge, daily for a period of 14 days. When Terminator applied on the skin of rats, by painting daily, for 2-3 minutes, at the dose of 2mg/kg (2-3ml) for a period of 14 days produced some deleterious effects on the skin at day 3 until day 11 and disappeared. All treated rats had erythema and showed some unease/pain or discomfort when we applied the test samples such as the rats trying to avoid the application. Ulcers were not observed in any of the treated rats. There was no histopathological or chemical change seen in the treated group.

Thus, TERMINATOR 2.5% WP, in this study, was found to be more than 500mg/kg bwt in rats and is considered slightly toxic (500mg-5g/kg- Low toxicity).

Poster Abstracts

TP48: HISTOLOGICAL CHANGES IN THE LIVER OF REARED SPOTTED SCAT (*SCATOPHAGUS ARGUS L.*) AFTER EXPOSURE TO MERCURY

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Introduction: The important environmental pollutants are those that tend to accumulate in organisms, those that are persistent because of their chemical stability or poor biodegradability, and those that are readily soluble and therefore environmentally mobile.

Materials and Methods: The toxic effects of Mercury (Hg), on the histology of the liver of reared Spotted scat (*Scatophagus argus L.*) was investigated. The experiment was conducted to identify the effect of metal concentrations and exposure period on degrees and nature of histological changes in the liver of exposed fish. Selected fish were exposed to 10, 20 and 30 $\mu\text{g.L}^{-1}$ concentrations of Mercury over short- term exposure period.

Results: Similar histological changes occurred in the livers of specimens exposed to 10, 20 and 30 $\mu\text{g.L}^{-1}$ concentrations, indicating a definite toxic response to all metal concentrations. These histological changes included hyalinization, hepatocyte vacuolation, cellular swelling, necrosis, vacuolar degeneration and congestion of blood vessels. The intensity of these histological changes was, however, influenced by the extent of the exposure period.

Conclusion: Conclusively, metals are stored in different sites in animals depending on the metal and on the animal species. To check the continual introduction of these metals into the food chain, a more cautious application of insecticides and pesticides should be employed and effluents from industries must be treated before disposal. Exposure period, however, influence the degree of histological changes. It was apparent that most histological changes became more conspicuous after 24, 48 and 96 hours exposure periods, indicating a short-term histological response.

Abstracts for INHAND

INHAND: INTERNATIONAL HARMONIZATION OF NOMENCLATURE AND DIAGNOSTIC CRITERIA FOR LESIONS IN RATS AND MICE – AN UPDATE

Global Executive Steering Committee: *Charlotte M. Keenan, Peter C. Mann, John L. Vahle, Julia F. Baker, Alys E. Bradley, Dawn G. Goodman, Takanori Harada, Ronald Herbert, Wolfgang Kaufmann, Rupert Kellner, Thomas Nolte, Susanne Rittinghausen and Takuji Tanaka*

Harmonization of nomenclature and diagnostic criteria in toxicologic pathology, especially for rats and mice, has been a goal of pathologists working in the profession for many years. In the latter part of the twentieth century, several initiatives were undertaken by the STP in the United States and by the RITA data base group (Registry of Industrial Toxicology Animal—data) in Europe. Their efforts resulted in a number of internationally recognized publications: SSND: Guides for Toxicologic Pathology and the WHO/IARC International Classification of Rodent Tumors. Beginning in 2005, the STP and European Society of Toxicologic Pathology (ESTP), in conjunction with RITA, developed a collaborative process to review, update, and harmonize existing nomenclature documents and databases - INHAND: International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice. In 2006, the British Society of Toxicologic Pathology (BSTP) and the Japanese Society of Toxicologic Pathology (JSTP) joined the initiative, so that the project has become truly global.

The objective is to produce publications for each organ system that provide a standardized nomenclature and differential diagnosis for classifying microscopic lesions observed in laboratory rats and mice in toxicity and carcinogenicity studies.

There is a Global Executive Steering Committee (GESC) with representation from major societies of toxicologic pathology. Fifteen organ system working groups (OWG) are defined by GESC with each group composed of expert toxicologic pathologists from each of the participating societies and responsible for developing preferred nomenclature and diagnostic criteria. Review of draft manuscripts is accomplished through the use of goRENI website (www.goreni.org), hosted by the Fraunhofer Institute. The OWGs have made significant progress, with two major systems published (respiratory and hepatobiliary), two more systems ready for publication (urinary and mammary gland) and several more systems ready for review by society members (CNS, Integument, Soft Tissue). Manuscripts are published in major toxicologic pathology journals and available on societies of toxicologic pathology websites and goRENI.



Abstracts for INHAND

INHAND UPDATE 01 – LIVER

Bhanu Singh, DuPont Haskell Global Centers, Newark, DE, USA

Karin Küttler, BASF SE, Ludwigshafen, Germany

R. R. Maronpot, Maronpot Consulting LLC, Raleigh, NC, USA

The INHAND (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) is a joint effort to standardize nomenclature and diagnostic criteria for both proliferative and non-proliferative lesions in laboratory rats and mice used in regulatory as well as research studies. The working group for the liver was comprised of representatives from the European, British, Japanese, and North American Societies of Toxicologic Pathology. In the process of establishing nomenclature and diagnostic criteria, the working group dealt with several debatable issues. For example, whether to use the diagnosis *fatty change* versus *cytoplasmic vacuolization* in the liver based solely on an H&E-stained slide was discussed. Similarly, whether to use the term *inflammation* or *cellular infiltrate* generated much discussion among the working group. As expected, opinions differed as to the most appropriate diagnostic terms for some proliferative lesions. There was considerable debate on diagnostic criteria distinguishing *cholangiofibrosis* and *cholangiocarcinoma*, as well as *Ito cell hyperplasia* and *Ito cell tumor*. The question was raised as to whether proliferative Ito cell lesions should actually be diagnosed as *stellate cell hyperplasia* and *stellate cell tumor*. Although an exhaustive discussion of the issues that were considered by the INHAND liver working group is not possible, Dr. Bhanu Singh and Dr. Karin Küttler will discuss selected non-proliferative and proliferative liver lesions, respectively, that should be of interest to the ESTP membership. During the presentation electronic audience voting on the selected cases will be available. Following voting, audience discussion of the case presentations will be encouraged.

The work of the INHAND liver working group is available in a published Toxicologic Pathology Supplement (Toxicologic Pathology 18: 5S-81S (2010)) and electronically on the internet (<http://goreni.org>). In the event you wish to establish dialogue with members of the INHAND Liver Working Group, their names and affiliations are provided here:

Bob Thoolen, Global Pathology Support, The Hague, The Netherlands; Robert R. Maronpot, Maronpot Consulting LLC, Raleigh, North Carolina, USA; Takanori Harada, The Institute of Environmental Toxicology, Joso-shi, Ibaraki, Japan; Abraham Nyska, Haharuv 18, Timrat, Israel; Colin Rousseaux, Wakefield QC, Canada; Thomas Nolte, Boehringer Ingelheim Pharma GmbH & Co., Biberach an der Riss, Germany; David E. Malarkey, National Toxicology Program, Cellular and Molecular Pathology Branch, Research Triangle Park, North Carolina, USA; Wolfgang Kaufmann, Merck KGaA, Darmstadt, Germany; Karin Küttler, BASF SE, Germany; Ulrich Deschl, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach/Riss, Germany; Dai Nakae, Tokyo Metropolitan Institute of Public Health, Shinjuku, Tokyo, Japan; Richard Gregson, Charles River Laboratories, Pathology Department, Senneville, QC, Canada; Michael P. Vinlove, Pathology Associates, Charles River, Frederick, Maryland, USA; Amy E. Brix, Experimental Pathology Laboratories Inc., Research Triangle Park, North Carolina, USA; Bhanu Singh, DuPont Haskell Global Centers for Health and Environmental Science, Newark, Delaware, USA; Fiorella Belpoggi, Ramazzini Institute, Bentivoglio (BO), Italy; Jerrold M. Ward, Global VetPathology, Montgomery Village, Maryland, USA

Abstracts for INHAND

INHAND UPDATE 02 – NERVOUS SYSTEM

PROLIFERATIVE LESIONS OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM IN RODENTS

Wolfgang Kaufmann

Merck KGaA, Darmstadt, Germany

Proliferative lesions in laboratory rodents may occur spontaneously as age-related lesion or as induced lesion in long-term (18 – 24 months) carcinogenicity studies. In general, spontaneous proliferative (neoplastic) lesions are rare in rats and mice.

In this interactive session several proliferative lesions taken from rats or mice will be shown and discussed with the audience and related to possible differential diagnoses under consideration of the Final Draft of the International Harmonized Nomenclature (International Harmonization of Nomenclature and Diagnostic criteria – INHAND) of the CNS and PNS, prepared by an international organ working group under supervision of STP, ESTP, BSTP and JSTP.

PROPOSED INHAND TERMINOLOGY OF PROLIFERATIVE LESIONS OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM

Neuronal

Medulloblastoma, malignant
Neuromyoblastoma, malignant

Glial / Schwann cell

Astrocytoma, malignant
• high grade

Glioma, mixed, malignant

- low grade
- high grade

Oligodendroglioma, malignant

- low grade
- high grade

Schwannoma, benign

Schwannoma, malignant

Meninges

Hyperplasia, granular cell
Granular cell tumor, benign
Granular cell tumor, malignant
Meningioangiomatosis
Meningioma, benign
Meningioma, malignant

Ependyma

Ependymoma, benign
Ependymoma, malignant

Choroid plexus

Carcinoma
Papilloma

Other lineages

Hamartoma, lipomatous
Reticulosis, malignant

Abstracts for INHAND

INHAND UPDATE 02 – NERVOUS SYSTEM

NON-PROLIFERATIVE LESIONS OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM IN RODENTS

Alys Bradley

Charles River Laboratories, Tranent, Scotland

Many non-proliferative lesions in laboratory rodents occur spontaneously in a low incidence or as induced lesion due to various infectious or neurotoxic agents in acute, subacute, subchronic or chronic studies. Neurotoxic lesions may be induced by one single or multiple treatments. They may occur as diffuse or multifocal lesions or may impact selectively specific morphological and functional units of the central and peripheral nervous system.

In this interactive session several non-proliferative lesions taken from rats or mice will be shown and discussed with the audience and related to possible differential diagnoses under consideration of the Final Draft of the International Harmonized Nomenclature (International Harmonization of Nomenclature and Diagnostic criteria – INHAND) of the CNS and PNS, prepared by an international organ working group under supervision of STP, ESTP, BSTP and JSTP.

PROPOSED INHAND TERMINOLOGY OF NON-PROLIFERATIVE LESIONS OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM

Neuron

Cell Body

- Cell loss, neuronal
- Chromatolysis
- Heterotopia, neuronal
- Necrosis, neuronal
- Neuronophagia
- Vacuolation, neuronal

Axon

- Atrophy, axonal
- Degeneration, axonal
- Dystrophy, axonal

Glia

Cell Body

- Alzheimer Type II astrocytes
- Astrocytic swelling / vacuolation
- Astrocytosis
- Gliosis NOS
- Microgliosis
- Satellitosis

Myelin

- Demyelination
- Intramyelinic edema

Choroid Plexus

- Vacuolation

Vascular

- Arteritis
- Infarct
- Thrombus

General

- Cholesterol clefts
- Epidermoid cyst
- Hemorrhage
- Hydrocephalus
- Inflammation
- Infiltrate, inflammatory cell
- Lipofuscin accumulation
- Mineralization
- Syringomyelia / Hydromyelia

Case Presentations

CASE 1: FRANCK CHANUT

Discovery and Regulatory Pathology, GlaxoSmithKline, Ware, Herts, SG12 oDP

• Species, breed	• Syrian Golden Hamster
• Sex	• Female
• Age	• 10 weeks
• Study type	• 14-day oral toxicity study
• Treatment	• None – Control animal
• Clinical findings	• None
• Organ(s)	• Stomach
• Gross finding(s)	• None
• Staining	• H&E

Case Presentations

CASE 2: VATANSEVER ALPER AND AKKOC AHMET

Department of Pathology, Veterinary Medicine, Uludag University, Bursa, Turkey

• Species, breed	• Mixed Breed
• Sex	• Male
• Age	• 1 Year
• Study type	• Tumor Pathology
• Treatment	• Surgically removed
• Clinical findings	• Mass which covers entire pinna
• Organ(s)	• Skin/Ear
• Gross finding(s)	• The mass, with nodular surface, covered the entire pinna of the right ear. During the palpation of the mass, it was felt that the mass has a hard structure and its cut surface was white.
• Staining	• Hematoxylin-Eosin, Anti Desmin, Anti Vimentin, Anti S100 immunohistochemistry

Case Presentations

CASE 3: MANUELA STOLTE

Sanofi-Aventis Deutschland GmbH, Preclinical Safety, Mainzer Landstraße 500, 65795 Hattersheim, Germany

• Species, breed	• SD rat, Charles River Germany
• Sex	• Male
• Age	• Approx. 26 month
• Study type	• 2 years carcinogenicity study
• Treatment	• Subcutaneous injection, control group
• Clinical findings	• Skin : palpable masses
• Organ(s)	• Skin/subcutaneous tissue, kidney, heart
• Gross finding(s)	• Raised firm, light brown foci/ areas
• Staining	• H.E., IHC

Case Presentations

CASE 4: EGZI AKDESIR

Department of Pathology, Veterinary Medicine, Uludag University, Bursa, Turkey

• Species, breed	• Dog, German Shepherd Dog
• Sex	• Male
• Age	• 10
• Study type	• Case report of a rare tumor
• Treatment	• Surgical removal and chemotherapy (vincristin, doxorubicin)
• Clinical findings	• Dysphagia, weight-loss, oral discomfort
• Organ(s)	• Tongue
• Gross finding(s)	• Lingual mass: Size of 2.5 x 5.5 x 5.5 cm. Heterogeneous structure on the cut-surface, fibro-elastic texture
• Staining	• H&E, PAS, Mallory's triple stain (Crossman modification), IHC (S-100, GFAP, PGP 9.5, vimentin, desmin, Factor VIII)

Case Presentations

CASE 5: MAIKE HUISINGA AND SIBYLLE GROETERS

BASF SE Ludwigshafen, GV/TD, Z470, 67056 Ludwigshafen, Germany

• Species, breed	• Rat, Crl:WI(Han)
• Sex	• Female
• Age	• 2.5 to 4.5 month
• Study type	• Various (28 day oral toxicity study, 90 day oral toxicity study, 1-generation study) with different test compounds
• Treatment	• 1 control animal, 1 animal of high dose test group, • 1 animal of mid dose test group
• Clinical findings	• One animal showed a decreased motor activity
• Organ(s)	• Pancreas
• Gross finding(s)	• Multiple, 1 to 2 mm in diameter sized, dark red foci
• Staining	• Hematoxylin/Eosin; Immunohistochemistry (insulin, glucagon, somatostatin)

Case Presentations

CASE 6: MAJA RUETTEN

Vetsuisse-Faculty Zürich, Winterthurerstrasse 268, 8057 Zürich, Switzerland

<ul style="list-style-type: none">• Species, breed	<ul style="list-style-type: none">• “Lisa”, a domestic german large white pig
<ul style="list-style-type: none">• Sex	<ul style="list-style-type: none">• Female
<ul style="list-style-type: none">• Age	<ul style="list-style-type: none">• 2 years
<ul style="list-style-type: none">• Clinical findings	<ul style="list-style-type: none">• The animal developed multifocal reddened and swollen papules and pustules on the udder, abdomen, flanks and hind legs during the final 3 to 4 weeks of pregnancy. Shortly thereafter, multiple fluid filled vesicles appeared on the same localisations, which then ruptured leaving deep, crusted ulcerations behind. Lisa developed severe decubitus of the right shoulder from laying on the less affected side during weaning of the piglets, and was euthanized because of progressive lameness.
<ul style="list-style-type: none">• Organ(s)	<ul style="list-style-type: none">• Haired skin
<ul style="list-style-type: none">• Gross finding(s)	<ul style="list-style-type: none">• Multifocal, reddened and swollen papules and pustules on the hind legs, abdomen and udder. Some of the foci were severely ulcerated and covered with thick serocellular crusts. On the right shoulder was a large, round, protruding, ulcerated mass that consisted of mineralized granulation tissue (decubitus). The articular cartilage of multiple joints were ulcerated or thickened (osteocondrosis dissecans).
<ul style="list-style-type: none">• Staining	<ul style="list-style-type: none">• H & E

Case Presentations

CASE 7: MATT JACOBSEN

Astrazeneca, 19F58a, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG, UK

• Species, breed	• Dachshund (adult)
• Sex	• Male
• Age	• 3 years
• Clinical findings	• Pustular dermatosis
• Organ(s)	• Skin
• Gross finding(s)	• Pustular skin eruption
• Staining	• Haematoxylin and Eosin

Case Presentations

CASE 8: KEN SCHAFER

Vet Path Services, Inc., 6450 Castle Drive, Mason, OH 45040 USA

• Species, breed	• Chinchilla, Laniger
• Sex	• Male
• Age	• 7 months
• Study type	• Single dose middle ear administration and followed for 4 days
• Treatment	• Administration of vehicle control or proprietary test article to the tympanic bulla of the left ear. Right ear served as untreated control.
• Clinical findings	• None
• Organ(s)	• Inner ear
• Gross finding(s)	• None
• Staining	• Hematoxylin and eosin

Case Presentations

CASE 9: JERRY WARD

Global Vet Pathology and NIAID, NIH, USA

<i>Species, breed</i>	<i>Mouse</i>
<i>Sex</i>	<i>Male</i>
<i>Age</i>	<i>6 months</i>
<i>Study type</i>	<i>Aging</i>
<i>Treatment</i>	<i>None</i>
<i>Clinical findings</i>	<i>Enlarged lymph nodes and spleen</i>
<i>Organ(s)</i>	<i>Mandibular Lymph nodes</i>
<i>Gross finding(s)</i>	<i>Greatly enlarged</i>
<i>Staining</i>	<i>H&E</i>

Case Presentations

CASE 10: PEDRO PINCZOWSKI

Universidad de Zaragoza, Spain

<i>Species, breed</i>	<i>Ovine, Rasa Aragonesa breed</i>
<i>Sex</i>	<i>Female</i>
<i>Age</i>	<i>Adult</i>
<i>Study type</i>	<i>Aging</i>
<i>Clinical findings</i>	<i>Posterior ataxia</i>
<i>Organ(s)</i>	<i>Thoracic spinal medulla</i>
<i>Gross finding(s)</i>	<i>Light-brown areas at the right ventral horn of the thoracic spinal medulla</i>
<i>Staining</i>	<i>H&E</i>

Notes

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