



CUTTING EDGE

PATHOLOGY

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## Keynote lectures

### CUTTING THROUGH TO THE TRUTH: LASER CAPTURE MICROSCOPY AND ACUTE TOXIC BILIARY INJURY

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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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## 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 1<sup>st</sup> impression in pattern recognition with deliberate analysis.***

### COGNITIVE ERRORS IN DIAGNOSTIC VETERINARY 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 ECVP 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.

## **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”*

# 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.

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*Journal of Comparative Pathology Plenary Lecture 2011*  
**OVINE RETROVIRUSES...IN-BETWEEN PATHOLOGY AND PHYSIOLOGY**

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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.

## **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.

## 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.

## 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.

## 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.

## 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.

## 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.

## 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.

### 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<sup>-m</sup> 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.

## 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*<sup>-/-</sup>) 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*<sup>-/-</sup> mice. The affected colon of *uPA*<sup>-/-</sup> mice, however, had significantly lower levels of active Tgf-β1 compared to that of *wt* mice. Importantly, at 7 months, with no colitis evident, half of the *uPA*<sup>-/-</sup> 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-β1. Tumor suppressor roles of Tgf-β1 are well-established both in humans and mice. The low levels of active Tgf-β1 due to uPA deficiency may explain inflammatory-induced carcinogenesis in *uPA*<sup>-/-</sup> mice.

## 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.

## BIODISTRIBUTION OF RADIOACTIVELY LABELED NANOPARTICLES IN THE MOUSE

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**Introduction:** Fluorochromatic labeling of organic nanoparticles is often used to investigate their bio-distribution 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,  $7 \pm 1,5$  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.

## 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 (*ie* 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.

## 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 immunocytochemistry 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.

### 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<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>,  $\gamma\delta$ 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<sup>+</sup>, CD4<sup>+</sup> and  $\gamma\delta$ TCR labelled cells; whereas CD8<sup>+</sup> 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.

## 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.

### 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- $\alpha$  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.

## 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 significantly 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.

## 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.

## 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 cm<sup>2</sup>) 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.

### 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.

## 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.

## 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.

## 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.

## ROLE OF $\beta$ -CATENIN IN CANINE OSTEOSARCOMA

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**Introduction:** The role of Wnt/ $\beta$ -catenin signaling pathway in the pathogenesis of osteosarcoma (OS) is not yet completely known, as well as the prognostic significance of  $\beta$ -catenin expression. Recently, the Wnt/ $\beta$ -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  $\beta$ -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.  $\beta$ -catenin was also evaluated by immunofluorescence in canine OS cell cultures.

**Results:** Nuclear  $\beta$ -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  $\beta$ -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  $\beta$ -catenin immunolabelling and a longer survival time.

## P53 GENE ALTERATIONS AND OUTCOME IN CATS WITH INJECTION-SITE ASSOCIATED SARCOMAS (ISAS)

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**Introduction:** We retrospectively studied p53 gene alterations by mutational analysis and immunohistochemistry in ISAS from cats treated with surgery and adjuvant curative radiotherapy, and their correlation with outcome.

**Material & Methods:** Formalin-fixed, paraffin-embedded tumour tissues from 52 animals were included. Sequences of p53 exons 5 to 8 were determined by PCR and direct sequencing. Immunoreactivity for p53 was assessed using tissue arrays reacted with a novel monoclonal (clone P6/5) and a commercially available polyclonal (CM-1) antibody. The cut-off for positivity was determined based on the results of the mutational analysis. Correlations of p53 gene alterations with progression free interval (PFI) or survival were investigated using Kaplan-Meier statistics.

**Results:** Among cases with amplifiable DNA, 6 tumours yielded a coding mutation, 34 tumours yielded wild type p53. There was a very good agreement between immunohistochemical results with the two antibodies ( $k = 0.865$ ) and substantial agreement ( $k > 0.61$ ) between immunohistochemistry and mutational status. Survival analysis revealed no significant association between presence of a mutation in the p53 gene, or immunoreactivity for p53, and survival or PFI.

**Conclusion:** The data suggest that alterations of the P53 gene as determined by mutational analysis or by immunohistochemistry are not predictive of outcome in cats with ISAS treated in this setting.

## Session F – Infectious diseases and Neuropathology

### INVESTIGATIONS OF ENCEPHALITIZOONOSIS 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.

## 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- $\gamma$ , TGF- $\beta$ 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<sup>+</sup> regulatory T cells and CD45R/B220<sup>+</sup> 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- $\alpha$  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.

## 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.

## 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.

## 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.

## 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 cachectic 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.

## 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 ( $\beta$ 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  $\beta$ 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  $\beta$ A deposits were observed intracellularly but also sporadic amyloid aggregates were found. Immunoblot analysis showed that  $\beta$ A was represented by both fragments  $\beta$ A 1-40 and 1-42 in selected brain areas.

**Discussion and conclusion:** This study demonstrates the presence of  $\beta$ A peptides in brain cattle at different ages and characterizes the phenotype of  $\beta$ 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.

## 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 $\alpha$  and PDGFR $\beta$  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 $\alpha$  and PDGFR $\beta$  expression in canine OSA tissues and primaries canine OSA cell lines.

**Materials and Methods:** PDGFR $\alpha$  and PDGFR $\beta$  transcripts were evaluated in 7 OSA cells line by q-PCR. PDGFR $\alpha$  and PDGFR $\beta$  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 $\alpha$  and PDGFR $\beta$  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 $\alpha$  and PDGFR $\beta$  are expressed in 71.4% and 82.1% respectively

**Conclusion:** These data showed that expression and distribution of the PDGFR $\alpha$  and PDGFR $\beta$  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.

### 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.

## 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  $\gamma$  (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.

## 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.

### 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.

## 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.

## 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.

### 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.

## 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 immunoexpression 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.

## 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 >15cm 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.

## 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.

## 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 PDGFβ-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.

## 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.

## 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.

### 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 (274-1543µmol/kg DM, n=7) compared with controls (28-98µmol/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.

## 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.

## 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.

**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.

# 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.

## 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.

## 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.

## Posters

### 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.

### EVALUATION OF <sup>213</sup>BI TOXICITY IN MICE AS PRECLINICAL APPROACH OF RADIO-IMMUNOTHERAPY

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**Introduction:** <sup>213</sup>Bi 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. <sup>213</sup>Bi coupled with specific antibodies is considered as a promising anticancer drug. In this preclinical study, <sup>213</sup>Bi coupled with bovine serum albumin (BSA) was injected to mice to determine its toxicity.

**Materials and methods:** Systemic injections of increasing doses of <sup>213</sup>Bi-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:** <sup>213</sup>Bi coupled with BSA shows mainly and hepatotoxicity (necrotizing hepatitis) and nephrotoxicity (glomerular, interstitial and tubular injuries).

## 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 clematidis* 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.

## 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.

## 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.

## 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

## 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.

## BARIUM SULFATE ASPIRATION IN AN ECLECTUS PARROT (*ECLECTUS RORATUS*)

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**Introduction:** Barium sulfate aspiration resulting in granulomatous pneumoconiosis is very rarely reported in animals. The presented psittacine biopsy case included 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 female eclectus parrot (*Eclectus roratus*) at a health screen. 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 a previous examination where barium sulfate (BaSO<sub>4</sub>) was given *per os*. Radiographs of the biopsy paraffin block showed obvious radio-opacity of the granulomas, confirming the suspicion that aspiration of a significant amount of barium sulfate had caused the lesions.

**Conclusion:** Diagnosing suspected barium granulomas can be helped by radiographing tissue biopsies, also in paraffin blocks.

## 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<sup>+</sup> 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 .

## ASSOCIATION OF AROMATASE AND 3 $\beta$ 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 $\beta$ 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 $\beta$ 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 $\beta$ 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 $\beta$ 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 $\beta$ HSD levels may be compensatory mechanism for new myelin formation in demyelination.

## 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/cm<sup>2</sup>), 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/cm<sup>2</sup>). 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.

## 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 lesion 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 mean values of systemic lesion 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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## EFFECT OF HYPERTENSION ON EXPERIMENTAL PERIPHERAL 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.

## 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.

**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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## 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.

## 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 Giroviridae.

**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 inoculated 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.

## 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.

## 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.

## 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.

## 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.

## 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.

## 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.

## A CASE REPORT OF TUBERCULOSIS IN PEA FOWL (*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.

## 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.

## **BALANTIDIUM COLI INFECTION IN A BELGIAN WARBLOOD 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.

## **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 ( $4.16 \times 10^{-5}$ ) when compared with the control group ( $5.32 \times 10^{-4}$ ). TGF-β was also lower ( $P=0.0047$ ) in the infected group ( $2.58 \times 10^{-3}$ ), in comparison with the control group ( $1.36 \times 10^{-2}$ ).

**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.

## 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.

## 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.

## 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.

## 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.

## 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.

## 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.

## 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- $\gamma$  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- $\gamma$  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- $\gamma$  assay using ESAT-6/CFP-10 performed better (72% of sensitivity and 90% of specificity) than the other skin test and IFN- $\gamma$  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.

## MYCOBACTERIOSIS IN PSITTACINES: A POTENTIAL FOR ZONOTIC 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.

## 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 i 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.

## 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.

## 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.

## 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.

## PATHOLOGIC FINDINGS IN RED-LEGGED PARTRIDGES (*ALECTORIS RUFUS*) 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.

## **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.

## **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.

## 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.

## 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 fetuses 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 fetuses 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.

## 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 MHCI was also established.

The only significative 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.

## 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* subs. *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.

## 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.

## 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, unbiten 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.

## 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 packaging 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.

## 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.

## 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.

## **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*.

## **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 diarrhoea 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.

## 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.

## 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.

## 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.

## 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 (4µm) 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<sup>Sc</sup>) and amyloidβ precursor protein (AβPP) was performed. Molecular techniques including PCR 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<sup>Sc</sup> within the vessel walls and perivascularly. Plaque-like areas positive for PrP<sup>Sc</sup> 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.

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

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**Introduction:** GM2-gangliosidoses are 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.

## 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 2<sup>nd</sup>, 5<sup>th</sup> and 6<sup>th</sup> 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.

## 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.

## 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.

## 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.

## 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.

## 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  $\alpha$ -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.

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## 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.

## 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.

## 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.

## 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.

## 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.

## 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.

## 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.

## 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.

## 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.

## 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).

## 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  $\alpha$ -Smooth Muscle Actin ( $\alpha$ -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  $\alpha$ -SMA, stained basal myoepithelial cells. Stellate myoepithelium lost the labelling with p63, but maintained its positivity for VIM and  $\alpha$ -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.

## 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.

## 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.

## 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 chromogranin 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.

## 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.

## 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.

## 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.

## 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.

## 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  $\geq$  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.

## 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<sup>®</sup>. 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.

## 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.

## 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.

## 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 $\alpha$ , ER $\beta$ , 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 $\alpha$ +, ER $\beta$ +, 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.

## NUCLEAR FACTOR- $\kappa$ B SIGNATURE OF FELINE INJECTION SITE SARCOMA BY IMMUNOHISTOCHEMISTRY

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**Introduction:** The nuclear factor- $\kappa$ B (NF- $\kappa$ B) transcription factors consist of dimeric proteins of the Rel homology family known to be involved in inflammatory and stress responses. Inappropriate NF- $\kappa$ B activation can stimulate cell transformation and growth. In addition NF- $\kappa$ B seems to have a role in tumours with a high inflammatory component. The NF- $\kappa$ B protein family is composed by different members: p50, p52, p65, cRel, RelB, that form various homodimers and heterodimers, Only specific combinations of NF- $\kappa$ B family members are transcriptionally active. The aim of this study was to evaluate immunohistochemically the expression of the different NF- $\kappa$ 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- $\kappa$ 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  $\geq 7$  as high expression. Then for each case we looked for transcriptionally active combinations of NF- $\kappa$ 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- $\kappa$ B dimers were identified the tumour was considered positive for NF- $\kappa$ B.

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

**Conclusions:** This study show that NF- $\kappa$ B is more often activated in FISS compared with FSTF. Concluding, NF- $\kappa$ 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.

## 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<sup>-6</sup>M P, RU486, ZK299 or vehicle or with 10<sup>-6</sup>M RU486 or ZK299 before incubation with 10<sup>-6</sup>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:** Antiproggestins 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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## 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,  $\alpha$ -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.

## 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.

## 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.

## 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.

## 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<sup>-6</sup>M P, RU486, ZK299 or medium alone or with 10<sup>-6</sup>M RU486 or ZK299 before incubation with 10<sup>-6</sup>M 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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## 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  $\mu$ 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  $\mu$ M 17-AAG, while apoptosis was prevalent at 3  $\mu$ M (24 hrs) and necrosis at 5  $\mu$ 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.

## 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.

## 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.

## 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.

## 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  $\alpha$ -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.

## 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  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -catenin suggests that these cells undergo EMT.

## 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 trichoepiteliomas, 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 trichoepiteliomas, 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 pilomatixoma.

## 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*<sup>+/-</sup>*ptenb*<sup>-/-</sup> fish (10%) and 1 out of 42 *ptena*<sup>-/-</sup>*ptenb*<sup>+/-</sup> fish (2%) developed tumors.

**Results:** All except 4 of the tumors in *ptena*<sup>+/-</sup>*ptenb*<sup>-/-</sup> 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.

## 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.

## 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.

## 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 silymarine 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.

## 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 metastatize, 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.

## 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 condroid 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 jejuneal 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.

## EXPERIMENTAL HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS H5N1 INFECTION IN BLACK-HEADED GULLS (*CHROICOCEPHALUS RIDIBUNDUS*)

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**Introduction:** Little is known about highly pathogenic avian influenza virus (HPAIV) H5N1 infection in black-headed gulls, a common waterbird species in western Europe.

**Material and Methods:** We inoculated sixteen black-headed gulls with  $1 \times 10^4$  TCID<sub>50</sub> HPAIV H5N1 (A/turkey/Turkey 1/2005) intratracheally and intraesophageally to examine pathogenesis and virus dynamics. Birds were monitored daily for clinical signs or death until 15 days post inoculation (dpi). Oropharyngeal and cloacal swabs were collected daily to detect viral shedding. Four birds were euthanized on 2, 4, 6, and 15 dpi for necropsy. Histopathological and immunohistochemical evaluation for influenza A virus nucleoprotein was performed on all tissues.

**Results:** All birds became productively infected. Spontaneous deaths and neurologic signs were evident from 4 to 7 dpi. From 7 dpi onwards, there was a decrease of viral shedding and general signs. Viral antigen was detected as soon as 2 dpi in thymus and adrenal gland, and from 4 to 7 dpi in CNS, pancreas and adrenal gland, in association with inflammation and necrosis.

**Conclusion:** Black-headed gulls develop severe encephalitis from HPAIV H5N1 infection and are thus sentinel species for the presence of this virus in the environment.

## 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 (Hematoxylin & 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.

## 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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## 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. Peripherally, 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.

## 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.

## 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.

## 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.

## 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 microfilariae 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 microfilariae of *Onchocerca* spp. with bacterial infections and environmental factors potentially secondary causes.

The study was supported by the National Centre for Science, Poland, grant Nr: N N308 573340.

## 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.

## 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 its 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.

## 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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## 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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## 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.

## 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.

## 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.

## PATHOLOGICAL CHARACTERIZATION OF LARVA MIGRANS CAUSED BY *ALARIA ALATA* INFECTION IN 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.

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.

## Case Presentations

### A CASE OF FIBROSARCOMA OF THE PINNA IN 1-YEAR-OLD CAT

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**Introduction:** Fibrosarcoma is a malignant tumor of the connective tissue and has variable presentations depending on species, age and sex. Although big proportion of fibrosarcomas is seen in adults, it can be seen in six-month-old or younger cats and dogs with various etiologies.

**Material and Method:** Male, 1 year-old, mixed breed cat with a mass on pinna was examined. The mass was surgically removed and processed routinely. Haematoxylin & Eosin (HE), and immunohistochemical (IHC) stainings were applied.

**Results:** Microscopically, the mass was constituted by spindle to ovoid shaped pleomorphic fibrocytes and fibroblasts. Multinucleated giant cells, coagulation necroses and mitotic figures were noticed throughout the sections. The neoplastic cells and giant cells were positively stained with vimentin and the mass was diagnosed as primary fibrosarcoma of pinna.

**Discussion and Conclusion:** Fibrosarcomas are seen in various anatomic parts of body in domestic animals. The pinna is the preferential site for haemangiomas and haemangiosarcomas. According to the author's knowledge, this is the first description of fibrosarcoma located on pinna in cats.

### MALIGNANT PERIPHERAL NERVE SHEATH TUMOR ON THE TONGUE OF A DOG

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A 10-year-old male German Shepherd dog was presented for a lingual mass on the mid-dorsal surface of the tongue. The dog had inappatence and dysphagia. The protruding lingual mass having 2.5x5.5x5.5 cm size, had a rapid onset and was surgically removed. Microscopically, the mass was composed of spindle-ovoid shaped cells and collagen rich matrix which was demonstrated with triple staining. The invasion of the tumoral cells into the muscle fibers, pleomorfism of the tumoral cells indicated the malignancy. Verocay bodies were observed. Tumor cells expressed S-100, vimentin but were negative for desmin, factor VIII, c-KIT immunohistochemically. Depending on the findings the mass was diagnosed as malignant peripheral nerve sheath tumor. To the best of our knowledge, lingual location of this kind of tumor has not been reported previously.

## **PORCINE ULCERATIVE DERMATITIS SYNDROME IN SOWS: POSSIBLE HERPES ASSOCIATED ERYTHEMA MULTIFORME?**

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A 2 years old german large white sow developed during pregnancy, birth and weaning of her piglets, a severe multifocal ulcera of the abdomen, udder and hind legs. The histological lesions consisted of lymphocytic interface dermatitis<sup>2</sup> and folliculitis with vesicle formation at the epidermal-dermal junction and multifocal epidermal necroses and were interpreted as erythema multiforme. In addition, in the skin and in peripheral blood, leucocytes tested positive with a nested PCR for porcine lymphotropic herpesvirus 1<sup>3</sup>. No evidence for porcine circovirus 2 could be found with immunohistochemistry. This is our third case of ulcerative dermatitis in sows in the last year that was positive for porcine lymphotropic herpesvirus 1. All attempted treatments with dexamethasone were in vain in these animals. These findings suggest that herpesvirus 1 could play a roll in the development of erythema multiforme in pigs as it is described in the literature for humans and dogs.

## **LYMPHOPROLIFERATIVE DISEASE IN MICE**

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A 6 month old male C3H mouse had generalized enlarged lymph node and spleen enlargement. Histologically, there was loss of normal lymph node architecture and the node was composed of a monomorphic population of medium sized lymphocytes which were CD3+ and CD45R+. The lymph node lesions appeared morphologically as lymphomas. The case, however, represented non-neoplastic, non-clonal lymphoproliferative disease due to a heritable mutation, *Gld* (generalized lymphoproliferative disease) involving *Fasl*, of the TNF receptor superfamily member 6 (synonyms - Tnfsf6, APT1LG1, CD178, CD95L). The enlarged nodes show no clonal populations in Southern blotting. Later in life, however, the mice developed plasma cell neoplasms which were clonal. A comparable disease occurs in humans involving similar genes.

## **MEDULLAR VISNA IN A SHEEP**

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A sheep was presented to the veterinary hospital of the Universidad de Zaragoza showing posterior ataxia. The animal was euthanized and sent to necropsy. At necropsy the animal showed an enlarged and heavy lung with miliar grey spots on the surface. The thoracic spinal medulla showed light-brown discolored areas at the right ventral horn. The medulla exhibited microscopically an intense gitter cell infiltration predominantly at the right ventral horn, associated with severe necrosis of the white and grey matter, intense astrogliosis and mononuclear perivascular cuffing. The encephalus exhibited less severe similar lesions multifocal distributed.