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European Society of Veterinary Pathology

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Welcome Message

On behalf of the Scientific and Organising Committee and the Institute of Veterinary Pathology of the Ludwig-Maximilians-University, it is an honour and a pleasure to welcome the participants of the 25th Meeting of the European Society of Veterinary Pathology to Munich, Germany.

This is the first meeting to take place in Munich since the ESVP became a proper "European Society". If one includes the "Autumn Meetings", the society can celebrate its 25th anniversary this year. It is a particular pleasure for us to be able to celebrate this year's meeting together with some of those who have made a significant contribution towards the 'internationalisation' of our society. Those who deserve special recognition are, amongst many others, Franco Guarda, Torino, Johan Mouwen, Utrecht and A. Parodi, Alfort. The history of our society will be illustrated through a reprint of the titles of the poster and oral presentations from the last 25 meetings, which started in Bern in the year 1970.

This year the meeting contains 190 contributions, of which 90 will be held as oral presentations with the remaining 100 held as poster presentations. In Munich we would like to establish the poster presentation as an appreciated form, as it indeed has been in other societies for many years. The presenter of each poster is required, under the supervision of a moderator, to summarize the contents of his / her work and to discuss it with the participants present. In this way we hope to afford the poster the recognition it deserves. This method calls for the poster presentation to require just as much preparation as for an oral one.

The Scientific Committee has thoroughly reviewed all contributions and made recommendations which could be accommodated in most cases by the final programme. During this process some poster presentations became oral presentations and vice versa. In the future, the Scientific Committee will place even more importance on this task to ensure that the quality of the presentations and, subsequently, the conference as a whole continues to rise.

I would like to express my sincere gratitude to all my collaborators at the Institute, who have offered much support in the preparation of this congress.

Munich is a beautiful city and we hope that you will find some opportunities to visit our museums, take a walk around the historic sights or to enjoy yourself in one of the many other ways which Munich has to offer.

Finally, the organisation of this conference would not have been possible without the generous support of our sponsors and trade exhibitors. We would like to thank them all very much and ask you, the participants, to give the exhibition your full attention.

We wish you a pleasant, informative and stimulating stay in Munich.

Walter Hermanns

Abstracts of the

Keynote Lectures

chronologically

Cell differentiation markers in the diagnosis and classification of histiocytic proliferative disease of dogs.

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HISTIOCYTIC DIFFERENTIATION AND CANINE HISTIOCYTOSIS

The development of canine specific monoclonal antibodies for many of the functionally important molecules of macrophages and dendritic antigen presenting cells (DC) has enabled the identification of the cell lineages involved in canine histiocytic disorders [1-4]. Despite the large variation of clinical and pathological features of canine histiocytic diseases, most represent proliferations of cells of various DC lineages.

Histiocytes differentiate from $CD34^+$ committed stem cell precursors into macrophages and several DC lineages, which include epithelial DC or Langerhans cells (LC), interstitial DC in many organs (eg dermal DC in skin), and interdigitating DC of T cell domains in peripheral lymphoid organs. Cytokines influencing DC development include GM-CSF and TNF-alpha. Macrophage development from $CD34^+$ precursors is influenced by GM-CSF and M-CSF. Blood monocytes can differentiate into either macrophages under influence of M-CSF, or into DC under influence of GM-CSF and IL-4 [5, 6].

Dendritic cells are the most potent antigen presenting cells (APC) for induction of immune responses in naïve T cells. Canine DC have been best defined in canine skin. They occur in 2 major locations: within the epidermis (LC), and within the dermis especially adjacent to postcapillary venules (interstitial DC or dermal DC). Canine DC abundantly express CD1 molecules [3, 4], which together with MHC class I and MHC class II molecules, are responsible for presentation of peptides, lipids and glycolipids to T cells. Hence, DC are best defined by their abundant expression of molecules essential to their function as APC. Of these, the family of CD1 proteins is largely restricted in expression to dendritic APC in skin; while MHC class I and II are more broadly expressed. Successful interaction of dendritic APC and T cells in response to antigenic challenge also involves the orderly appearance of co-stimulatory molecules (B7 family – CD80 and CD86) on dendritic APC, and their ligands (CD28 and CTLA-4) on T cells. DC also utilize adhesion molecules, such as the beta-2 integrins (CD11/CD18), in their function as APC.

CD11/CD18 expression is highly regulated in normal canine macrophages and DC. CD11c is frequently expressed by DC; while macrophages predominately express CD11b (or CD11d in the splenic red pulp and bone marrow) [7, 8].

Langerhans cells (epidermal DC) and interstitial DC are distinguishable by their differential expression of E-cadherin (LC^+) and Thy-1 (CD90) (DC^+). Lineage distinctions among histiocytes are best made via immuno-histochemistry (IHC) performed on frozen sections (CD1, CD11b, CD11c, CD11d, CD18, CD90, MHC II, and E-cadherin expression). Regardless, it is important to realize that dendritic APC arise in bone marrow and migrate through blood to a variety of epithelial sites (cutaneous and mucosal), where they reside either within epithelia or in dermis and lamina propria. In these sites they function as antigen processing and ultimately antigen presenting cells, which interact with T cells. Migration of DC beyond the skin to the paracortex of

lymph nodes occurs continuously. The interdigitating dendritic APC of lymph node paracortex are partially derived from such migration. Aspects of this developmental and migratory program of cutaneous DC are recapitulated in the DC proliferative disorders of canine skin.

AN OVERVIEW OF CANINE HISTIOCYTIC DISEASES

There are at least 4 well-defined histiocytic proliferative diseases recognized in dogs. They can be difficult to differentiate from granulomatous, reactive inflammatory diseases or from lympho-proliferative diseases without IHC. Clinical and pathological images of canine histiocytic diseases are available on the web (http://www.histiocytosis.ucdavis.edu).

Canine Cutaneous Histiocytoma (CCH) Complex

CCH usually occurs as a single lesion in young dogs and spontaneously regresses [9]. Multiple histiocytomas and metastatic histiocytomas are rare examples of aggressive behavior of this tumor, which must be differentiated from cutaneous lymphomas [3, 10]. Histiocytoma is readily distinguished from other histiocytic disorders and cutaneous lymphoma with the aid of IHC. Our work has clearly shown that histiocytomas have the phenotype of epidermal Langerhans cells [3]. They express CD1a, CD1b, CD1c, MHC class II, CD11c, and E-cadherin. Hence cutaneous histiocytoma is a localized epidermal Langerhans cell tumor. The rare cases of multiple histiocytoma with metastasis to local lymph nodes, and the cases in which confluent histiocytic lesions occur in many cutaneous sites with rapid systemic spread represent a spectrum of diverse clinical behavior, and are perhaps best characterized as Langerhans cell histiocytosis (LCH) [11]. LCH is also recognized as a rare disease of humans, in which marked variation in clinical behavior is recognized [12, 13]

Canine Reactive Histiocytosis

Cutaneous histiocytosis (CH) presents with single or multiple lesions, which may wax and wane, and even spontaneously regress. Few cases respond to corticosteroids, the remainder persist and may require more aggressive immunosuppressive therapy. Systemic histiocytosis (SH) is a familial disease of Bernese Mountain Dogs, and also occurs sporadically in other breeds. SH presents with prominent skin manifestations identical to those seen in CH, but mucous membranes (ocular and nasal) and a variety of other organ systems, including lymphoid organs, lung, and bone marrow may also be involved [14, 15]. Histiocytes in SH and CH express markers expected of DC such as CD1, C11c, and MHC II. However, the lack of consistent epidermotropism in SH and CH lesions, and the expression of Thy-1 (expressed by dermal DC) and CD4 (a marker of DC activation) suggest that histiocytes in these diseases are activated interstitial type DC [1]. Although the lesions may wax and wane, SH is a progressive disease that often requires continuous immunosuppressive therapy. Successful therapeutic agents include Cyclosporine A or Leflunomide [16]. The clinical behavior and consistent clinical response to immuno-suppressive therapy reinforce the concept that SH and CH are disorders of immune regulation, and arise from defective interaction of DC and T cells leading to chronic proliferation of both lineages. The initiation of the process is probably antigen driven, although studies to identify the nature of the antigens involved have been unrewarding. Hence, it is important to perform tests to rule out infectious agents in the initial workup of a reactive histiocytosis case.

Histiocytic Sarcoma Complex

Histiocytic sarcoma (HS) and the related disorder, malignant histiocytosis (MH), occur with high incidence in Bernese Mountain Dogs, Rottweilers, Flat Coat Retrievers, Golden Retrievers and sporadically in many other breeds. HS occur as localized lesions in spleen, lymph nodes, lung, bone marrow, skin and subcutis, brain, and periarticular tissue of large appendicular joints. HS also occur as multiple lesions in single organs (especially spleen), and rapidly disseminate to involve multiple organs. Hence, disseminated HS is difficult to distinguish from MH, which is a multi-sys-

tem, rapidly progressive disease in which there is simultaneous involvement of multiple organs such as spleen, lymph nodes, lung, bone marrow, skin and subcutis [2].

Clinical signs of (HS/MH) include anorexia, weight loss, and lethargy. Other signs depend on the organs involved. Pulmonary lesions are associated with cough and dyspnea. CNS involvement (primary or secondary) can lead to seizures, incoordination and paralysis. Regenerative and non-regenerative anaemias have been consistently documented in haemophagocytic HS. Lameness is often observed in periarticular HS. Response of HS and MH to chemotherapy is variable, but often brief.

HS (MH) lesions express leukocyte surface molecules characteristic of DC (CD1, CD11c and MHC II). Diffuse expression of E-cadherin, Thy-1 and CD4 has not been observed in HS or MH in skin or other sites; this together with cytomorphology assists in the distinction of HS (MH) from histiocytoma and reactive histiocytosis (SH and CH) [2]. In haemophagocytic HS, histiocytes express CD11d and MHC II. Expression of CD1 molecules is inconsistent. This phenotype is identical to that of macrophages in splenic red pulp and bone marrow, rather than that of DC, in which abundant expression of CD1 and CD11c is expected. Haemophagocytic HS is a rapidly progressive disease with a characteristic clinico-pathologic profile [17].

REFERENCES

1. Affolter VK, Moore PF: Canine cutaneous and systemic histiocytosis: reactive histiocytosis of dermal dendritic cells, Am J Dermatopathol 2000, 22: 40-48
2. Affolter VK, Moore PF: Localized and disseminated histiocytic sarcoma of dendritic cell origin in dogs, Vet Pathol 2002, 39: 74-83
3. Moore PF, Schrenzel MD, Affolter VK, Olivry T, Naydan D: Canine cutaneous histiocytoma is an epidermotropic Langerhans cell histiocytosis that expresses CD1 and specific beta 2-integrin molecules, Am J Pathol 1996, 148: 1699-1708
4. Moore P, Affolter V, Olivry T, Schrenzel M: The use of immunological reagents in defining the pathogenesis of canine skin diseases involving proliferation of leukocytes. Edited by Kwotchka K, Willemse T, von Tscharner C. Oxford, Butterworth Heinmann, 1998, p. pp. 77-94
5. Shortman K, Liu YJ: Mouse and human dendritic cell subtypes, Nat Rev Immunol 2002, 2: 151-161
6. Shortman K, Caux C: Dendritic cell development: multiple pathways to nature's adjuvants, Stem Cells 1997, 15: 409-419
7. Danilenko DM, Moore PF, Rossitto PV: Canine leukocyte cell adhesion molecules (LeuCAMS): characterization of the CD11/CD18 family, Tissue Antigens 1992, 40: 13-21
8. Danilenko DM, Rossitto PV, Van der Vieren M, Le Trong H, McDonough SP, Affolter VK, Moore PF: A novel canine leukointegrin, alpha d beta 2, is expressed by specific macrophage subpopulations in tissue and a minor $CD8^+$ lymphocyte subpopulation in peripheral blood, J Immunol 1995, 155: 35-44
9. Taylor DN, Dorn CR, Luis O: Morphologic and biologic characteristics of the canine cutaneous histiocytoma, Cancer Research 1969, 29: 83-92
10. Moore PF, Olivry T: Cutaneous lymphomas in companion animals, Clinics in Dermatology 1994, 12: 499-505
11. Nagata M, Hirata M, Ishida T, Hirata S, Nanko H: Progressive Langerhans cell histiocytosis in a puppy, Veterinary Dermatology 2000, 11: 241-246
12. Favara BE, Feller AC, Pauli M, Jaffe ES, Weiss LM, Arico M, Bucsky P, Egeler RM, Elinder G, Gadner H, Gresik M, Henter JI, Imashuku S, Janka-Schaub G, Jaffe R, Ladisch S, Nezelof C, Pritchard J: Contemporary classification of histiocytic disorders. The WHO Committee On Histiocytic/Reticulum Cell Proliferations. Reclassification Working Group of the Histiocyte Society, Med Pediatr Oncol 1997, 29: 157-166
13. Schmitz L, Favara BE: Nosology and pathology of Langerhans cell histiocytosis, Hematology and Oncology Clinics of North America 1998, 12: 221-246
14. Mays MB, Bergeron JA: Cutaneous histiocytosis in dogs, J Am Vet Med Assoc 1986, 188: 377-381
15. Moore PF: Systemic histiocytosis of Bernese mountain dogs., Vet Pathol 1984, 21: 554-563
16. Gregory C: Immunosuppressive agents. Edited by Bonagura J. Philadelphia, W.B. Saunders, 2000, p. pp. 509-513
17. Moore PF, Affolter VK, Vernau W: Canine hemophagocytic histiocytic sarcoma: a proliferative disorder of $CD11d^+$ macrophages, Veterinary Pathology 2006, 43: 632-645

Prognostic Evaluation of Canine Cutaneous Mast Cell Tumors

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Cutaneous mast cell tumors (MCTs) are one of the most common neoplasm in dogs, accounting for up to 21% of cutaneous neoplasms. They are found in any region of the skin, with the thigh, groin, and scrotum being frequently involved. There is no age or sex predilection, but they occur with greater frequency in breeds such as the Boxer, Boston terrier, Bull terrier, Staffordshire terrier, Fox terrier and young Shar-Peis.

MCTs may present as well circumscribed cutaneous nodules or as diffuse, poorly delineated skin swellings. Histologically, they most commonly represent as poorly or non circumscribed, nonencapsulated infiltrates of neoplastic mast cells accompanied by variable numbers of eosinophils. Neoplastic cells occur mostly in the dermis but may extend into the subcutis and underlying muscles. Collagen degeneration is frequently observed and appears as eosinophilic amorphous material surrounded by and infiltrated with eosinophils. Because mast cells contain several active compounds including histamine and heparin, systemic lesions, including gastric ulcers, focal glomerulonephritis, immune system and blood coagulation defects, may develop along with MCTs.

MCTs have an extremely variable biologic behavior. Clinically, they can range from a localized benign mass to potentially fatal metastatic disease. Currently, prognostic and therapeutic determinations of MCTs are based primarily on their histologic grade and clinical staging. Several grading systems based on cellular differentiation of the neoplastic mast cells have been proposed. However, none of these systems is based on multivariate survival statistics or censored data for non-MCT related death. The majority of MCTs are identified as grade 2, and the frequency and prevalence of recurrence and metastasis correspond poorly with histopathological grading. This grading problem is also reflected in the significant variation of percentages of each grade assigned to MCTs in different studies.

Therapeutically, for the vast majority of dogs with solitary cutaneous MCTs with no local spread to lymph nodes or distant metastasis, complete surgical excision of the tumor is considered curative. If no clean tumor margins can be achieved radiation therapy is commonly applied. For dogs with metastatic MCTs or MCTs with metastatic potential, chemotherapy has been used with varying success. A main problem in selection of optimal therapy is the small numbers of dogs in some clinical trials and the lack of a reproducible classification to allow comparisons of survival data among studies.

Any useful prognostic evaluation of a neoplastic entity has to be associated with a specific therapy. Survival times, disease free interval or rate of metastasis may be significantly altered when using different treatment protocols. Therefore today's pathologists are not only required to diagnose a neoplastic entity, but also to closely communicate with the oncologist and to follow recent clinical developments and therapeutic changes. Even more critical, any existing prognostic classification of a neoplastic entity has to be re-evaluated for each novel therapeutic approach. The utility and validity of any grading system has to be proven based on carefully controlled, double blinded

prospective clinical trials utilizing a statistically relevant number of animals treated by single protocol, analyzed using multivariate survival methods, and the results have to be published in a peer-reviewed journal.

Our laboratory has been working for the last 5 years to determine criteria that can be used to identify canine cutaneous MCTs with metastatic potential to more accurately select the appropriate therapeutic approach. We investigated 4 different aspects: 1. The morphology of canine cutaneous MCTs including a review of the current histologic grading, but also tumor depth within tissue sections, tumor location on the body and multiple simultaneous tumor occurrence. 2. The qualitative and quantitative expression of various proteins within neoplastic MCTs. 3. The proliferation activity of canine cutaneous MCTs. and 4. Genetic characteristics of canine cutaneous MCTs.

During the first phase of the project our study population consisted of 100 formalin-fixed, paraffin embedded cutaneous MCTs that had been surgically removed from 100 dogs. None of the dogs had received chemotherapy or radiation therapy. For each case complete follow-up data, including sex, breed, weight, age, tumor location, number of tumor masses, development of additional MCTs at the surgical site (local recurrence) and at distant sites outside the surgical margins, overall survival time (OS), disease free interval (DFI) and cause of death, were compiled.

In a blinded study 31 pathologists from 16 institutions worldwide graded this population in order to evaluate the consistency of the microscopic grading and its prognostic significance. There was an agreement of 74.6% for the grading of grade 3 MCTs, but only a 63.0% and 63.1% agreement for grade 2 and grade 1 MCTs, respectively. MCT depth within tissue section represents an important feature to differentiate between grade 1 and grade 2 MCTs in one grading system, but not the other. This particular criterion was one of the main reasons for the assignment of different grades (1 or 2) by various pathologists, even though all pathologists provided grades according to one grading system for consistency analysis. Assignment of a grade 3 to a MCT by at least 50% of the participating pathologists was associated with a poor prognosis. Approximately 70% of dogs with such grade 3 MCTs died or were euthanized due to MCT related disease within 6 months after the original diagnosis. Differentiation between grade 1 and grade 2 MCT was of no prognostic significance. Marked anisocytosis and anisokaryosis were consistently observed in MCTs assigned a grade 3 by at least 50% of participating pathologists. Based on these data and their prognostic association, a simple classification of canine cutaneous MCTs into high and low grade may be more consistent and prognostically significant than the currently used grading systems. In support of these data, we also investigated the significance of tumor depth, tumor location and multiple synchronous tumor masses for the prognostic evaluation of canine cutaneous MCTs. Based on multivariate survival analysis, tumor depth or location was of no prognostic significance for dogs with cutaneous MCTs. Dogs with multiple synchronous cutaneous MCTs at the time of diagnosis had a worse prognosis compared to dogs with single tumors. Additional treatment beyond surgical excision alone should be considered for these animals. Older dogs and Boxers with cutaneous MCTs were at higher risk to develop additional MCTs at distant sites (outside the surgical margins), and older and male dogs with cutaneous MCTs had significantly shorter OS.

Recent research on MCTs has focused on c-kit (stem cell factor receptor or steel factor receptor), a type 3-receptor tyrosine kinase, belonging to the platelet-derived growth factor receptor subfamily. The c-kit proto-oncogene encodes a receptor tyrosine kinase, KIT, that is known to play a crucial role in mast cell growth and differentiation. In the past decade, several gain-of-function c-kit mutations have been implicated in human disease. Mutations, including activating internal tandem duplications (ITD) in exons 11 and 12 of the juxtamembane domain of c-kit have been identified in canine cutaneous MCTs. Duplication mutations in the juxtamembrane region likely activate KIT kinase by inducing dimerization.

When analyzing the prognostic significance of KIT and tryptase expression patterns in canine cutaneous MCTs using immunohistochemistry (IHC), the KIT staining pattern was found to be a significant prognostic indicator for MCT biological behavior. The following 3 KIT staining patterns were identified: 1. Membrane-associated staining 2. Focal to stippled cytoplasmic staining with decreased membrane-associated staining 3. Diffuse cytoplasmic staining. Based on multivariate survival analysis, increased cytoplasmic KIT staining was significantly associated with an increased rate of local recurrence, and a decreased OS. The tryptase staining patterns were not significantly associated with any survival parameter. We also wanted to determine the prognostic significance of KIT protein levels (quantitative) in MCTs. Punches from 50 MCTs were incorporated into a tissue microarray and microarray sections were stained with an anti-KIT antibody followed by fluorescent-labeled secondary antibodies. Fluorescence was quantitated using a microarray scanner. The KIT protein levels were not significantly associated with any survival parameter.

Analysis of proliferation activity has received special attention in the search for prognostic parameters of neoplasms. Proliferation activity results from cell cycling and contributes to tumor growth. To evaluate the proliferation activity of a neoplasm, it is necessary to assess the speed of the cell cycle (proliferation rate) and the proportion of cells committed to cycle (growth fraction). Ki-67 nuclear antigen is a non-histone protein that is expressed in all phases of the cell cycle except G0, making Ki-67 the ideal marker for the growth fraction. Interphase argyrophilic nucleolar organizer regions (AgNORs) are structural-functional units of the nucleolus that contain all necessary components for ribosomal RNA synthesis. Two non-histone argyrophilic proteins, C23 (nucleolin) and B23 (nucleophosmin), are associated with interphase AgNORs and are responsible for their stainability with silver methods. The number of AgNORs has been demonstrated to be strictly related to rRNA transcriptional activity and in vitro studies have confirmed AgNORs as one measure of proliferation rate. An additional parameter is the phase index. A longer phase index results most likely from karyologic abnormalities and can therefore be used to analyze the quality of the proliferating cells, but not the actual proliferation activity. The S-phase index can be recognized by the Proliferation Cell Nuclear Antigen (PCNA). PCNA is a 36-kd acidic non-histone protein required for DNA synthesis through its relationship with polymerase delta. By combining proliferation rate and growth fraction in a survival analysis, it is possible to assess whether proliferation activity is important in determining the biological behavior of canine cutaneous MCTs. In a study of 56 MCTs treated with surgical excision alone, we demonstrated that increased Ki67 and AgNOR counts were both associated with a significantly decreased survival. Ki67 was better for identifying MCTs that were associated with a decreased OS, whereas Ag67 was a better marker for identifying MCTs with a decreased DFI. Therefore, based on these results MCTs should be evaluated for both their Ki67 and Ag67 indices in order to best identify patients that are likely to have subsequent local and distant MCT occurrences, and in order to identify patients that are likely to succumb to their mast cell disease.

To analyze the prognostic significance of ITD mutations of the c-kit proto-oncogene, 50 MCTs from 50 dogs that had been treated with surgical excision only were studied. Neoplastic mast cells from each tumor were isolated using laser capture microdissection and DNA was extracted for mutation analysis using PCR and sequence analysis. The occurrence of ITD mutations was significantly associated with a decreased OS. When investigating the phosphotransferase region of the kinase domain of the c-kit proto-oncogene for potential activating mutations no mutations or polymorphisms were identified in 35 canine cutaneous MCTs.

In a retrospective study we evaluated the prognostic utility of above described markers MCTs from 28 dogs treated with vinblastine and prednisone, and compared the outcome of dogs treated with chemotherapy to that of a prognostically matched group treated with surgery alone. Histologic grade 3 MCTs and MCTs with c-kit mutations had decreased DFI and OS times. MCTs

with increased cytoplasmic KIT localization were associated with decreased OS time. MCTs with increased Ki67 and AgNOR values were associated with decreased DFI and OS times. We than compared DFI and OS times between dogs treated with the combined chemotherapy and dogs treated with surgery alone using the same pretreatment prognostic indices. Dogs with histologic grade 3 MCTs or with MCTs with c-kit mutations had significantly longer DFI and OS times when treated with chemotherapy rather than surgery alone. This study demonstrates the utility of vinblastine and prednisone for grade 3 MCTs and MCTs with c-kit mutations.

One aspect of our current research projects focuses on the identification of genes that are involved in the malignant transformation of canine cutaneous MCTs. The goal of this study is to compare whole genome expression patterns in malignant and benign canine cutaneous MCTs using an oligoarray of approximately 850 canine genes that has been developed in our laboratory. We wanted to identify genes that are consistently up-regulated or down-regulated in malignant MCTs as compared to benign MCTs, and therefore potentially play a key role in the malignant transformation of canine cutaneous MCTs. This information will allow us to direct our future research towards identifying and understanding genes that are important for both prognosticating canine cutaneous MCTs, and whose products may serve as therapeutic targets for canine MCTs. In this study we have identified 17 genes that are significantly differentially expressed in high grade MCTs compared to intermediate grade MCTs. Sixteen of these genes were significantly down-regulated while only one gene was significantly up-regulated. The genes identified in this study fall into a broad range of functional classes. Among these genes, two groups are of particular interest, namely death receptor associated genes and cytokine/chemokine signaling associated genes. Further studies are ongoing to analysis the role of these genes in MCT tumorigenesis.

Considering the high prevalence of canine MCTs, the high costs of aggressive therapies such as radiation and chemotherapy and the emotional stress for the owner, a more accurate prognostic classification is urgently needed. Based on our research, we recommend histologic evaluation and grading of canine cutaneous MCTs following a revised grading scheme as well as special staining for KIT, Ki-67, PCNA, AgNORs and ITD mutation analysis on routinely formalin fixed specimen to more accurately prognosticate canine cutaneous MCTs.

Modern Methods in Pathology - Genomics: Generating animal models by forward and reverse genetics.

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Animal models are required for studying the pathobiology of diseases as well as for the development and evaluation of therapeutic strategies. Novel animal models for the functional genome analysis are generated by two complementary strategies either starting with the specific modification of selected genes in the host genome and subsequently analyzing the resulting phenotype (gene-driven approach, reverse genetics) or starting with interesting phenotypes and subsequently analyzing the causative genotype (phenotype-driven approach, forward genetics).

Reverse genetics is carried out by using genetic engineering techniques. Transgenes are transferred into the host genome with methods leading to non-homologous DNA recombination (DNA microinjection, viral vectors, sperm mediated gene transfer) which results in the additive gene transfer and the random integration of transgene copies, as well as with methods for the homologous DNA recombination (embryonic stem cells, somatic nucleus transfer) leading to gene targeting with the exchange of known genome sequences by the transgene.

The transgenic animals show a gain of function of the transgene and/or a loss of function of host genes. Loss of function includes inactivation of specific genes (gene knock-out), defined genome modifications (gene knock-in) and specific suppression of gene expression (gene knock-down, gene silencing by RNA interference (RNAi)). Expression of toxic transgene products leads to the inactivation or elimination of defined cells or cell compartments (genetic cell ablation). Transgenes may show spatio-temporal expression. Transgene expression may be controlled on the transcriptional, translational or post-translational level by regulatory elements. Conditional mutagenesis can be carried out leading to a defined alteration of the transgene locus by using recombination systems like the Cre/loxP system.

Genetic engineering techniques are currently improved especially for the homologous DNA recombination. In addition, mutations can be genetically analyzed in randomly mutagenized cells, and selected cells can be used for the generation of genetically modified animals for the further phenotypic analysis. The systematic phenotype analysis of the transgenic and genetically modified animals reveals the consequences of the genetic modifications and provides conclusions on the function of the respective genes. Thus, specific models for known genetic diseases can be generated by genetic engineering techniques which results in defined alterations of the animal genome. However, the resulting phenotypes of the mutant animals cannot be predicted and do not always mirror the respective human disease which leads to a "phenotype gap" in the animal models generated by reverse genetics techniques. Therefore, forward genetics is carried out by generating new alleles by random mutagenesis and screening for clinically relevant phenotypes.

Random mutagenesis of the host genome appears spontaneously and is induced by several means (chemicals like ethylnitrosourea (ENU) which has been used in various mouse mutagenesis projects; radiation; insertional mutagenesis by using gene trap constructs or transposons). The mutants may carry loss-of-function, hypomorphic or gain-of-function alleles of the affected genes. Specific pathologic states are identified by appropriate routine procedures allowing the screening of

large numbers of mice for a broad spectrum of phenotypical parameters. Breeding of the affected mice and screening of the offspring confirms the transmission of the altered phenotype to the subsequent generations, thereby revealing a mutation as cause for the aberrant phenotype. Both the in-depth genotypic and phenotypic analyses of the mutant lines produce novel tools for the biomedical research of human diseases.

Modern Methods in Pathology - Transcriptomics

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The development of molecular biological tools has opened new windows to evaluate the pathophysiology of disease processes on the molecular level. Gene expression analysis has been at the forefront of these studies. The study of transcriptomics or genome-wide expression profiling examines the expression levels of mRNAs in a given tissue or cell population. As a global way of looking at gene expression patterns, it improves the understanding of genes and pathways involved in biological processes. Today the genomes of human, as well as of the species most commonly used in animal experimental studies are sequenced. Together with recent advances in genome-wide profiling techniques, this availability of genomic sequence data allows for a comprehensive analysis of disease-associated transcriptional programs.

Evaluation of gene expression patterns in tissue specimens using oligonucleotide DNA array and quantitative real-time polymerase chain reaction techniques provides novel insights into both physiological and pathogenetic mechanisms of gene expression regulation and serves as a starting point for novel molecular diagnostic tools. Oligonucleotide DNA microarray technology represents a practical and economical tool for detection of genome-wide differences in the expression level of genes, meaning both identification and detection of differences in the abundance of nearly all mRNA transcripts present in the cells of a respective sample at a given point of time. Real-time PCR provides an accurate and rapid determination of mRNA expression levels and therefore has become the standard among all PCR-based gene expression analysis techniques.

The keynote lecture will adress the planning of the experimental design of transcript profiling analyses and strategies for generation of suitable sample materials. An overview of methodologies and underlying principles of DNA microarray technology and real-time PCR will be provided. Emphasis will be laid on different approaches and principles of statistical analysis of microarray and real-time PCR data, as well as the application of available software tools for bioinformatical analysis, pathway mapping and identification of regulatory networks underlying gene expression.

Modern Methods in Pathology: Proteomics

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Four major „omics" techniques, comprising genomics, transcriptomics, proteomics and metabolomics have recently achieved enormous methodological progress. All of these represent hypothesis generating rather than hypothesis driven approaches and are currently used extensively as tools in functional genome analysis. The term „Proteome" was introduced by Marc Wilkins in 1994 as the „proteins corresponding to a genome" and is nowadays used more precisely to describe the quantitative proteome pattern of a biological unit (e.g., cell, organell, virus or body fluid) at a stage defined as precisely and comprehensively as possible.

Proteomic approaches are indispensable in the field of functional genome analysis, since in all biological systems a broad spectrum of regulation phenomena occur on the protein level (e.g., secretion, activation of protein precursors by protein cleavage or phosphorylation, feedback inhibition, translational regulation, miRNA dependant regulation, etc). These crucial events are not mirrored by mRNA abundancies, and their analysis is therefore addressable exclusively on the protein level.

As a consequence of substantial efforts at the level of instrument technology as well as bioinformatics and genome sequencing, the quantitative expression profiling and the reliable identification of thousands of proteins has recently become possible. The most prominent technical progress applied to protein identification by facilitating tandem mass spectrometry of peptides in combination with electrospray or laser desorption ionisation. The development of nano-chromatography based peptide separation technology and improved fluorophor labeling methods for protein quantification in gel based approaches are further technical highlights. Due to the bioinformatics algorithms used for MS-based protein identification, a comprehensive genomic information about the organism analysed (or at least a closely related species) is an undispensable prerequisite. However, the rapid increase of genomic data from mammals, including domestic animals, facilitates straightforward protein identifications in the corresponding species from as little as femtomol amounts of each protein.

Nevertheless, proteomics currently comes to substantial limitations in so called holistic approaches, i.e. experimental setups where as many proteins as possible should be quantified and identified in a complex protein mixture. Samples of mammalian tissues or body fluids show an estimated complexity of 100.000 protein species and a huge dynamic range with respect to the abundance of individual proteins, comprising 8 – 12 orders of magnitude. Therefore, an extensive separation of these very complex protein mixtures prior to MS analysis is a prerequisite and presently one of the major challenges. A very common and effective strategy to separate proteins is the two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) already established in the middle of the 1970s, showing a separation power of several thousand proteins. By combination with covalently linked fluorescent dyes, a reliable quantification of proteins in 2D gels has recently become possible. A powerful alternative, avoiding 2D-PAGE separation, is coupling HPLC (high performance liquid chromatography) systems with tandem mass spectrometers. This so called LC-MS/MS technology, especially when downscaled to micro- or nano-liter flow-rates per minute, has proven to be

a very fast and sensitive method which in addition can be automated to a great extent.

In quantitative proteomics, the majority of experimental setups are "differential" approaches, where samples are analysed with respect to quantitative differences in the protein profile of two or more biological stages. These experiments are especially useful to detect proteins which have so far been not been recognised to be directly or indirectly affected by the biological phenomenon investigated, or even to be a trigger of this phenomenon. Another widespread application of quantitative proteomics is the identification of biomarker candidates for basically any relevant biological stage or disorder. The challenges in biomarker discovery are the extreme dynamic range of proteins in sera (up to 10 orders of magnitude) or, if tissues instead of sera are analysed, the reproducible preparation of a subpopulation of relevant cells by, e.g., laser microdissection, a step often necessary in order to gain enough selectivity.

Despite the impressive technical progresses and scientific results obtained during the last years, it should not escape our notice that substantial improvements still have to be made in order to enable a close-to-complete analysis of complex samples. Hence, proteomics seems to be just at its beginning.

Modern Methods in Pathology: Metabolomics

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Dramatic advances in the biological sciences over the past years have forged an exciting era of research including the emerging field of systems biology. Although the understanding of biological systems at the system level is still in its infancy, it is evident that investigations in genomics, proteomics and metabolomics will be important building blocks of this new science. Whereas genomics and proteomics have made significant strides in technology development, metabolomics is still a new field in the biosciences. It is often described as the comprehensive analysis of a biological system in which all metabolites are identified and quantified.

Examining metabolism, as a key aspect to phenotype, means describing the distribution of metabolites as a function of disease, genetic modifications or toxicity, but will also help to understand the mechanisms by which phenotypic changes develop. Individual metabolites have long been used as markers of disease or toxicity and now that pursuit has become dramatically enabled by advances in analytical chemistry and informatics. Nevertheless, the comprehensive evaluation of the metablome, the complete set of metabolites in a biological system, is still a daunting task. On the one hand the number of potential metabolites in any given system is quite large. According to conservative estimates, one can expect up to 3000 major metabolites in human metabolism. One the other hand, the physical and chemical properties of the compounds are very divergent and they vary dramatically in concentration. Moreover, the metabolome is a dynamic system subjected to significant environmental influences, e.g. temporal or dietary.

The successful implementation of metabolomics requires quantitative analyses with high throughput, resolution, reproducibility, and sensitivity. Due to the complexity of metabolites in biological samples, the assembly of different analytical platforms is necessary to obtain maximum coverage of the metabolome. Mass spectrometry coupled to chromatography is a powerful analytical platform offering high sensitivity and resolution, as well as the ability for structure elucidation of potential biomarkers. However, metabolomics in a true sense, namely the quantitative analysis of all metabolites in a biological system, represents a great challenge requiring different tools to probe the metabolome for dynamic changes.

Two complementary approaches are therefore generally used for metabolomic investigations: metabolic fingerprinting and metabolic profiling. The metabolic fingerprinting approach is not intended to identify each observed compound, but to identify patterns or “fingerprints” of metabolites, which change in response to perturbations. Being semi-quantitative and simultaneously applicable to a wide range of metabolites, this is a true “omics” approach. Such methods are attractive since they allow investigators to cast a wide net and both generate and test hypotheses. However, the nature of the data makes the observations instrument/platform dependent.

The real power of metabolomics is realized when analyses are quantitative and qualitative. Knowing the identity of metabolites and their quantitative perturbation as the basis of differences in specific phenotypes within a comprehensive metabolomic profile is the goal. The power of such approaches to identify not just existing diseases and toxicities but also to identify trajectories

towards diseases prior to their emergence is just one of the applications of metabolomics. Understanding who is at risk, why they are at risk and how to intervene are all benefits emerging from knowing precisely the metabolic basis of disease or toxicity.

If the metabolites have been identified, one can use metabolic profiling as the second approach towards metabolomics. In metabolic profiling, quantitative analytical methods are developed for metabolites in a pathway or for a class of compounds. This approach produces independent information that can be interpreted in terms of known biochemical pathway and physiological interactions. Due to the fact that absolute and validated quantitative data are generated, a platform independent legacy database can be built. The disadvantage is that the system is not a universal or 'omic' approach.

A central focus of the work in the field of metabolomics at the Institute of Functional Genomics at the University of Regensburg is the development of an array of robust methods to comprehensively probe the metabolome. A potpourri of those methods and their applications will be presented. This includes both metabolic fingerprinting and metabolic profiling methods. Using several chromatographic separation techniques, such as gas chromatography, liquid chromatography and capillary electrophoresis coupled to mass spectrometry a broad range of metabolites can be covered. Metabolic fingerprinting is primarily done using gas chromatography coupled to mass spectrometry after derivatization. The availability of comprehensive mass spectral libraries greatly facilitates the identification of unknown metabolites. Metabolic profiling focuses on the automated high throughput analysis of amino acids, amino acid metabolites, and metabolites of the central carbon metabolism as key components of metabolism.

A journey to the past:
Electron microscopy of the nervous system in health and disease.

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The electron microscope and the discipline of electron microscopy (EM) have played an important role in advancing human and veterinary medicine from the 1960's. This technology was crucial for furthering knowledge of normal cell structure, for investigating in fine detail the effects of diseases on various tissues, for identifying infectious agents especially viruses, and for a great variety of experimental investigations. While the role of EM has greatly declined over the last 20 years, it remains an important tool in medical centers for the diagnosis of certain spontaneous disorders of the kidney, skeletal muscle and peripheral nerve, and for the investigation of new clinical syndromes in animals and man.

EM in neuropathology has played a role in the investigation of disorders of many types including lysosomal storage diseases, viral infections, plant poisonings, demyelinating disorders, neoplasms, motor neuron diseases, the neuropathy of diabetes mellitus and so on. A quaint jargon has evolved to describe the novel findings identified in cells at the ultrastructural level – curvilinear profiles, zebra bodies, tubuloreticular inclusions, and so on. EM study of the parasite which causes equine protozoal myeloencephalitis, originally assumed to be toxoplasma, resulted in its' reclassification as a sarcocystis based on the method of reproduction (endopolygeny) as identified by EM. A study of the cytoplasmic organelles of another important apicomplexan parasite helped Dr.'s Bjerkas and Dubey to propose a new genus, neospora, distinct from toxoplasma.

This presentation will begin with a short review of the normal elements of the central and peripheral nervous systems showing the ultrastructural features of neurons, glial cells, myelin sheaths, synapses and peripheral nerves. This will be followed by a selection of spontaneous disorders affecting the CNS or PNS in several species, beginning with general processes such as cellular degeneration, inflammation, gliosis and repair. Specific disease entities to be illustrated include Solanum plant intoxications in ruminants which target Purkinje neurons and induce changes reminiscent of a gangliosidosis. We will look at demyelination illustrated by canine distemper infection and see how the choroid plexus has a bearing on the distribution of demyelinating lesions in this disease. Infectious agents illustrated will include protozoa and viruses and some cellular inclusions which can be confused with viruses at the ultrastructural or light microscopic level. We will compare a few lysosomal storage diseases and finally look at the ultrastructural features of a selection of brain tumors.

Prion diseases

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Since BSE has been receding in most countries of the world, interest in prion research of the scientific community is waning. It may be premature, however, to lower the guards on transmissible spongiform encephalopathies (TSE) or prion diseases, since on the one hand novel variants (prion strains) have been identified only recently and may continue to emerge in the future. In particular atypical BSE (H- and L-type BSE), atypical scrapie, and the rapid spread of chronic wasting disease are of major concern and warrant close attention. On the other hand, the basic mechanism of prion replication is only incompletely understood, there is no effective in vivo diagnosis and no therapy.

Recently developed methology such as the protein misfolding cyclic amplification (PMCA) (Saborio et al., 2001) which enables us to replicate prions in the test tube may help to develop highly-sensitive tests, it may also help understand the origin and development of spontaneous prion disease.

Emerging diseases:
Bluetongue - vectors, epidemiology and climate-change.

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The presentation will begin with a brief discussion of those climatic variables that are likely to influence the distribution and incidence of vector-borne diseases such as bluetongue. An explanation of how these variables may induce their own particular effects will be included.

The talk will then move on to describe recent changes in the world distribution of bluetongue virus and its vectors focussing on Europe from 1998 until 2007. It will be argued that the recent changes, both in terms of virus distribution and the species of vectors transmitting the virus can be linked to climate-change.

Suggestions of what this might mean for the future, in a time of on-going climate-change will be set out.

Comparative pathology of avian influenza: recent advances

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The currently ongoing outbreaks caused by highly pathogenic avian influenza A virus of the subtype H5N1 are not only of great concern to the poultry industry and the health of other domestic and wild animals, but also to public health. It is feared that this virus, which causes a high fatality rate in infected patients, will adapt to efficient human-to-human transmission and thus initiate a new human influenza pandemic. Since 1996, when the ancestor virus was identified in domestic geese from China, the H5N1 virus outbreaks have spread and now encompass countries in Asia, the Middle East, Europe, and Africa.

In recent years we have studied several unusual aspects of H5N1 virus. First, it is the first-known influenza virus to cause lower respiratory tract disease in humans. This exposed a large gap in our knowledge of the tissue tropism of H5N1 virus in the lower respiratory tract. Although attachment is not the only factor required for virus replication, this information is important to better understand the pathogenesis of H5N1 influenza. By use of virus histochemistry, which directly displays the attachment of influenza virus to tissues, we determined that H5N1 virus attachment in the human respiratory tract is progressively more abundant towards the alveoli, where the virus attaches predominantly to type II pneumocytes and alveolar macrophages. This attachment pattern fits with the limited pathology data on H5N1 virus infection in humans, which show diffuse alveolar damage as the primary lesion. More recently, we also determined the pattern of attachment of two currently circulating subtypes of human influenza A virus (H3N2 and H1N1) in human respiratory tract, and showed that virus attachment in the trachea and bronchi was more abundant than in the bronchioles. This corresponds with the common presentation of human influenza A virus infection, which is tracheobronchitis. This difference in disease outcome between human influenza A viruses and H5N1 virus infection, where the primary lesion is severe pneumonia, fits with differences in virus attachment that we observed.

Second, H5N1 virus infection results in disease and mortality in multiple other species that were not previously known to become ill from influenza A virus infection. For example, during the 2003 to 2004 outbreak of H5N1 influenza in Asia there were reports of fatal H5N1 virus infection of domestic cats and zoo felids after feeding on virus-infected chickens. This is most unusual, because domestic cats are generally considered to be resistant to disease from experimental influenza A virus infection, and reports of natural disease are rare. To determine the pathogenicity of this virus for domestic cats, we experimentally infected cats with H5N1 virus by different routes and examined them by virologic and pathologic techniques. The results demonstrated that H5N1 virus can productively infect domestic cats, cause diffuse alveolar damage, and result in clinical disease or death.

Third, H5N1 virus in humans and other mammals is not limited to the respiratory tract, as is usually the case with influenza A virus infection, but may also extend to other organs. The question of extra-respiratory tissue tropism of H5N1 virus in humans was once again raised by the recent isolation of H5N1 virus from a human patient with severe neurological symptoms. To study this question, we performed detailed virological and pathological studies of domestic cats infected

experimentally with H5N1 virus by different routes of inoculation. Our results showed that virus replicated not only in the respiratory tract but also in multiple extra-respiratory tissues, and was associated with severe necrosis and inflammation. An interesting observation in some cats was virus-associated ganglioneuritis in the submucosal and myenteric plexi of the small intestine, suggesting direct infection from the intestinal lumen.

Fourth, free-living wild birds appear to act as a vector for H5N1 virus. Traditionally, spread of highly pathogenic avian influenza virus among poultry flocks is thought to occur by transport of infected poultry, contaminated equipment, and people associated with the poultry industry. In close proximity to affected poultry flocks, highly pathogenic avian influenza virus occasionally has been detected in wild birds, but they have had a very limited or no role in its dissemination. However, in the current outbreaks wild birds are suspected to play a significant role as long-distance virus vectors. Evidence in favour of spread by wild birds includes the detection of H5N1 virus in many wild waterbirds in western Europe at the beginning of 2006, often in areas where no outbreaks had previously been detected in intensively surveyed poultry. This event overlapped with unusual waterbird movements associated with cold weather in the Black Sea area. The main argument against wild waterbirds as long-distance virus vectors of H5N1 virus is that most wild waterbirds in which this virus was identified were either sick or dead, suggesting that they were too severely affected to spread the virus over any substantial distance. To test the hypothesis that wild waterbirds can excrete H5N1 virus in absence of debilitating disease and so act as potential long-distance virus vectors, we experimentally infected six species of wild ducks with a recent avian isolate of H5N1 virus from Europe. All six species are listed as presenting higher risk to avian influenza by the European Union. We found that some species of ducks excreted significantly more virus than others, while only two of six species became ill or died. These findings suggest that some wild duck species are potential long-distance vectors of H5N1 virus, while others are more likely to act as sentinels. This information has several implications for both active and passive surveillance of influenza in wild ducks.

Abstracts of the

Oral and Poster Presentations

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Pathological characterization and gene mapping of malignant histiocytosis in the Bernese Mountain Dog.

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Introduction: Canine histiocytic proliferative disorders are heterogeneous diseases which include reactive disorders such as cutaneous and systemic histiocytosis, and neoplasia such as cutaneous histiocytoma and localized and disseminated histiocytic sarcoma (malignant histiocytosis, MH). MH is highly breed related: its incidence in Bernese Mountain Dogs (BMD) is high, and MH may represent 20 to 25 % of the cause of death in the French BMD population. The aim of our study was to better characterize the nosology and pathological features of malignant histiocytosis in BMD and to search for the potential genetic causes of this disease.

Material and methods: We undertook the collection of cases and DNA samples from BMDs affected or not by MH. All suspected cases of MH were submitted to histological and immunohistochemical confirmation. The epidemiologic and pathological features were investigated by sending a questionnaire to each referring veterinarian. Regarding genetic studies, a total of 191 dogs including 73 cases of MH were analyzed by genetic linkage analysis, with 260 microsatellite markers. Results were compared with those obtained from a distinct US collection of 52 validated MH cases and 103 aged control dogs which were analyzed by an association method with a set of 500 microsatellite markers.

Results: We constructed a BMD pedigree of 300 dogs, of which 100 are affected by MH. We showed that the transmission mode of the disease is most probably oligogenic, involving a small number of genes.
In a selected population of 70 affected BMD from the pedigree, MH is characterized by a mean age of 6 years and 3 months at the time of diagnosis (range 2 to 12 years) without sex predisposition. Most common clinical signs reported were asthenia and weakness (88% of cases), anorexia (88%), weight loss (79%) and hyperthermia (43%). Splenomegaly was present in 54% of the dogs. In 75% of the cases, internal organs, primarily the spleen, the lungs and mediastinum, internal lymph nodes and the liver were involved. Involvement of several organ systems at the time of presentation was present in more than 50% of the dogs. Skin and subcutis were less frequently affected (only in 17% of the cases). Haematological investigations revealed anaemia, frequently severe, and thrombocytopenia in 50% of the cases at the time of diagnosis. Clinical outcome reflected the poor prognosis of this disease with a mean survival time of 92 days after the diagnosis and a survival median of 1 month.
Genetic analyses based on comparison of DNA samples from MH cases and control dogs point out at least four loci on two chromosomes as containing genes involved in the disease. Candidate genes present in these loci are under active investigation.

Discussion: Altogether, these approaches lead to a better understanding of MH in BMD and may enable us to identify genes implicated in histiocytic transformation in dogs.

Expression profile of Borna Disease Virus specific proteins and RNAs in naturally infected horses.

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Introduction: Borna Disease (BD) is a naturally occurring viral infection of the central nervous system (CNS) in horses and sheep, mostly leading to an acute neurological disease and a severe non-purulent meningoencephalitis. However, inapparent or chronic, sometimes recurrent courses of the disease are also observed. Whether different clinical signs and severity of inflammatory reactions correlate with a specific expression profile of viral proteins and their viral RNA has yet not been investigated.

Material and methods: For this retrospective study, brain samples of 14 naturally with the Borna Disease Virus (BDV) infected horses were used. Formalin fixed, paraffin embedded and H&E stained tissue samples of the hippocampal region were examined histopathologically and histopathological changes were evaluated semiquantitatively. In addition, glial cell activation was examined using GFAP specific immunoreactivity. Detection of the viral nucleoprotein (BDV-N), glycoprotein (BDV-GP) and matrixprotein (BDV-M) was performed by immunohistochemistry using a monoclonal and polyclonal monospecific antibodies, respectively. Viral mRNA specific for BDV-N, BDV-GP, BDV-M, BDV-phosphoprotein (BDV-P) and the viral RNA dependent polymerase (BDV-L) as well as the corresponding sequences of the viral genome were demonstrated by in situ hybridization (ISH).

Results: Histopathologically, 3 groups of inflammatory lesions were noted. One group of horses (9/14) showed predominantly perivascular mononuclear infiltrates. The second group (3/14) displayed parenchymal and perivascular mononuclear inflammatory infiltrates, whereas in the third group (2/14) only discrete perivascular inflammatory cell infiltrates were noted. All groups showed astroglial activation varying from mild to severe. Expression of the BDV-N and BDV-M protein was found in 100% of the horses investigated, whereas the BDV-GP was only detectable in 50% of the animals. Unlike the nuclear and cytoplasmatic reaction of BDV-N and BDV-M, BDV-GP was only found in the cytoplasm of a few neurons. By ISH, BDV-N and BDV-P specific mRNA was mainly found in the cytoplasm, whereas BDV-GP, BDV-M and BDV-L mRNA were predominantly detected in the nucleus. The corresponding sequences of the viral genome were consistantly found in the nucleus.

Discussion: In conclusion, viral protein and RNA expression seem to be tightly regulated in horses naturally infected with BDV, comparably to experimentally infected animals.

Pathological evaluation of the effect of Bone Morphogenic Protein on the bone healing process in metatarsal bones of horses.

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Introduction: Bone morphogenic protein (BMP) can cause molecular and cellular impulses and induce rapid bone healing process. Therefore it is good for cases of delayed or non-union fractures as well as filling up of intervertebral gaps by osteogenic activity.

Material and methods: The long bones of horses were collected and processed by defating, grinding, decalcification, degreasing, protein extraction, dialyzing, purification, and lyophilization. Then the extracted BMP was collected as powder and kept in the refrigerator.

Ten adult horses of both sex were included in the study and divided into two equal random groups. All horses were handled according to the Shiraz University regulation for animal rights.

Under general anaesthesia with xylazin, diazepam and ketamin-HCl a window defect was made in the midmetatarsal region using an electric osteotom in all horses. The horses of the first group received BMP which was injected at the window site of the metatarsal bone. The wound was closed, bandaged and splinted in both groups. Patients were monitored clinically for 12 weeks and histomorphometric evaluation was performed. Clinically the horses in BMP group were using their legs slightly from the 4th week onwards and completely after 6 weeks. The horses in control group did not used their legs even 6 weeks after the operation.

Results: Histomorphometric evaluation showed that a good thickness of cortical and trabecular bone and periosteum was present and intact at the window site in patients of treatment group as compared to control group.

Discussion: It was concluded that the extracted protein was BMP and that it has accelerated the bone healing process by osteogenic activities.

Distribution of nitric oxide synthase (NOS) in rat normal liver and in acute hepatitis induced with galactosamine.

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Introduction: Hepatotoxicity by galactosamine has been used as a model of acute hepatitis in multiple pharmaco-toxicological studies. Nitric oxide (NO) is a short-living biological mediator generated from L-arginine by NO synthase (NOS). The NOS family of enzymes identified up to now includes constitutively expressed endothelial NOS (eNOS or type 3 NOS) and neuronal NOS (nNOS or type 1 NOS), as well as inducible NOS (iNOS or type 2 NOS). In the liver, NO is generated by eNOS and iNOS. This generation can mediate a number of physiological and disease reactions. Excessive NO production can be detrimental since it might downregulate cytochrome P450, suppress liver protein and DNA synthesis, induce apoptosis and necrosis. In pathological conditions, such as hepatitis, iNOS is activated and produces sustained amounts of NO. In this study we try to evaluate iNOS and eNOS expression in rat livers with acute hepatitis induced by galactosamine and the correlation to cellular death caused by apoptosis.

Material and methods: The test group of the animals (adult male Wistar rats) were exposed (ip) to galactosamine (400mg kg^{-1} b.w.); the control group received only saline (0.9%). At intervals of 24, 48, 72 and 96 hours, groups of five animals were sacrificed, livers removed, fixed in 10% of neutral buffered formaldehyde, embedded in paraffin, and sectioned at 4 μm. The immunohistochemical study was performed according to the ABC technique using polyclonal antibodies against eNOS and iNOS. TUNEL technique was used to study apoptosis.

Results: As expected cellular death is almost absent in the hepatic tissue of the control group. After 24 and 48 hours apoptosis achieved its maximum expression, after 96 hours the result was identical to control group. iNOS was found in cytoplasmic and membranous regions of the hepatocytes in normal livers. Surprisingly, Kupffer cells did not stain. At 48 hours the hepatocytes and Kupffer cells exhibited a strong immunoreactivity to iNOS and at 96 hours the result was identical to the control group. In the control group eNOS was localized typically in endothelial cells as well as in the cytoplasm of hepatocytes, but with a less intense immunoreactivity. The hepatocytes of the animals exposed to galactosamine after 48 hours showed a maximum immunoreactivity. At the same time enhanced staining was noted in endothelial cells. After 96 hours immunoreactivity of the hepatocytes was similar to the control group, but the endothelial cells had a higher intensity when compared with the control group.

Discussion: This study shows that the intensity of iNOS, eNOS and the TUNEL score was highest or achieved the maximum expression at the same group (48 hours).
The differences of NOS expression might be due to its differing role in maintaining liver homoeostasis and/or involvement in the pathology of hepatitis.

Diagnostic value of immunofluorescence exam in canine kidney disease.

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Introduction: Immune complex glomerulonephritis represents one of the most common forms of primary glomerular disease. Renal lesions can also be secondary to vascular alterations or other systemic diseases.

Material and methods: Seventy-four renal cortical biopsies from dogs with different levels of renal failure were studied by immunofluorescence in order to look for the frequency and potential predominance of IgG, IgM, IgA and complement C3. Due to the clinical analysis and histological exam, the dogs were divided into two groups according the origin of the disease: immune-mediated nephropathy and non-immune-mediated nephropathy.

Results: A strong association between immune-mediated nephropathy and the positivity of immunofluorescence was detected for IgG in the mesangium and glomerular basement membran, for IgM only in the mesangium. Immunofluorescence evaluation resulted to be essential for the diagnosis of inflammatory lesions caused by immunopathological processes.

Discussion: The mechanism of trapping into the glomerulus caused positive reactions to IgM in non-immune-mediated nephropathy. In conclusion, the immunofluorescence exam was highly characteristic and provided diagnostic clues in association with histological and electron microscopy diagnosis.

Severe chronic endometritis in an Asian elephant (Elephas maximus) - Clinical, microbiological and pathological findings.

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Introduction: Reproductive pathology described in elephants includes leiomyomas, endometrial glandular cysts and hyperplasia, polyps, and periglandular fibrosis. In contrast, inflammatory diseases of the female genital system of probiscidea are extremely rare.
We present the case of a 48-years-old female Asian elephant with a severe chronic endometritis, which appeared clinically as recurrently expulsed bloody-clotty material during urination.

Material and methods: The animal was investigated microbiologically, biochemically, and clinically by transrectal ultrasonography and endoscopic examination. When the elephant died suddenly, detailed necropsy, histopathology, and microbiology were performed.

Results: Ultrasonography and endoscopic examination identified the uterine as the origin of the bloody material and excluded a haemorrhagic cystitis. Furthermore the animal suffered from recurrent pododermatitis. The elephant was intensively treated with Desloreline implants, antibiotics, and homoeopathic drugs.
During necropsy the uterus was severely enlarged and contained about 250 l of a yellow-reddish creamy fluid with pieces of necrotic tissue. Microscopy showed a severe ulcerative purulent endometritis with total destruction of the endometrium. Furthermore, severe purulent chronic pododermatitis, leiomyoma in the mesometrium, and numerous large calcified nodular hyperplasia of abdominal fat tissue were found.
Microbiological investigations identified a high number of two different strains of Streptococcus equi ssp. zooepidemicus and E. coli from the uterine content. From the lesions of the toes high numbers of Streptococcus agalactiae, Streptococcus equi ssp. zooepidemicus, Staphylococcus sp., Corynebacterium sp., and Entercoccus sp. were isolated.

Discussion: This is the first clinical and pathological report of a severe purulent endometritis in an Asian elephant described in literature. The clinical diagnosis of the endometritis was difficult, because the material expulsed from the vagina with the urine suggested a haemorrhagic cystitis until ultrasonographic investigations identified its uterine origin.
Several other findings, such as pododermatitis, leiomyoma and fat tissue hyperplasia were not the main problems. Pododermatitis is a well known disease in captive elephants, and several cases have been reported. In the present case, it may be speculated whether the streptococci in the uterus were related to the bacteria of the pododermatitis.

The role of MMPs and TIMPs in chronic valve disease (syn. endocardiosis) in dogs.

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Introduction: Chronic valve disease (CVD) is a common disease in old, small and medium-sized canine breeds (syn. endocardiosis). The pathogenesis includes early endothelial damage and an atypical proliferative, metabolic and enzymatic activity of endothelial and stromal cells. Valvular stromal cells transdifferentiate into myofibroblasts and acidic mucopolysaccharids, collagen and elastic fibers accumulate in the valvular spongiosa, finally leading to a progressive disintegration of collagen fibers in the valvular fibrosa. Metabolism of extracellular matrix (ECM) mainly depends on matrix metalloproteinases (MMPs) and their specific tissue inhibitors (TIMPs). The aim of this study is the characterization of the expression patterns of MMPs and TIMPs in normal mitral valves and in canine CVD by immunohistochemical methods.

Material and methods: 35 dogs of different breeds and age (1-16 years) were included in the study. Heart valves were fixed in 4% formaldehyde. Formalin fixed specimens were embedded routinely, stain-ed with haemalaun-eosin and picrosirius red.
The expression of MMP-1, -2, -9, -14 and TIMP -2, -3, -4 in normal mitral valves (MV) (n=6) and in mild (n=6), moderate (n=14), or severe (n=9) CVD was investigated immunohistochemically.

Results: Immunohistochemistry: In normal mitral valves MMP-2 was mildly expressed in numerous stromal cells. MMP-9, MMP-14, TIMP-2 were mildly expressed in only single stromal cells. TIMP-3 was intensively expressed in numerous stromal cells and appeared in the extracellular space of the atrial layer. Endothelia covering the mitral valve expressed minimal amounts of MMP-2, MMP-9, TIMP-2. MMP-14 and TIMP-3 expression was mild to moderate. MMP-1 and TIMP-4 were negative with the antibodies, used in our experiments.
In mild CVD, the number of MMP-2 and MMP-9 positive cells decreased. MMP-14 and TIMP-2 were detectable in numerous valvular stromal cells. Furthermore, mild staining for TIMP-2 appeared in the extracellular space. In myxomatous nodules, the extracellular TIMP-3 staining was less than in the normal atrial layer, but numerous stromal cells were moderately positive.
In moderate/severe CVD, MMP-2 and MMP-9 were absent in most cases (21/23). MMP-14 and TIMP-2 were intensively increased in numerous stromal cells. Additionally, in 11/23 cases TIMP-2 showed moderate staining reaction in extracellular space. TIMP-3 was intensively expressed in stromal cells and multifocally in the extracellular space of the myxomatous nodules.

Discussion: This is the first study describing the expression of MMPs and TIMPs in the normal canine mitral valve. The expression pattern of MMPs and TIMPs was markedly changed in CVD. The increased expression of TIMPs in CVD may correspond to a depressed degradation of extracellular matrix in the mitral valve, resulting in an atypical accumulation of mucopolysaccharids, elastic and collagen fibers.

Clinical, morphological and immunohistochemical characterization of canine perivascular tumours.

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Introduction: Perivascular Wall Tumours (PWT) are neoplasms deriving from the different cellular components of the vascular wall excluding the endothelial lining. The spectrum of human cutaneous PWT comprises several entities including glomus tumour, haemagiopericytoma (HEP), myopericytoma, angioleiomyoma/-sarcoma, angiomyofibroblastoma and angiofibroma. Glomus tumours, HEPs and angioleiomyomas/-sarcomas have been described in dogs. HEP is the most frequent PWT described in dogs. Nonetheless, its histogenesis remains uncertain. The purpose of this study is to revise clinical presentation, histopathology, and immunohistology of canine cutaneous PWT.

Material and methods: Cases were selected on the basis of cytological features suggestive of HEP. One half of bioptic sample was fixed in formalin and the other half was snap frozen. H&E stained sections were evaluated for the presence of vascular patterns (staghorn, placentoid, perivascular whorling, bundles from media). Immunohistochemistry was performed on frozen sections applying a large panel of antibodies including 7 smooth muscle markers. Immunofluorescence for the membrane ganglioside 3G5, a marker of human dermal pericytes, was also performed. Diagnosis was established based on the association of prevalent vascular growth pattern and degree of muscular differentiation evaluated by immunohistology.

Results: Twenty cases were collected. Age ranged from 6 to 13 years, 12 dogs were males and 8 females with a prevalence of crossbreeds. Tumours arose from a single site with preferential acral location (11/20). Five angioleiomyomas, 2 angioleiomyosarcomas, 6 myopericytomas, 2 HEP, 1 perivascular adventitial tumour, and 1 angiofibroma were diagnosed. A definitive diagnosis was not possible in 3 cases. Angioleiomyosarcomas and HEPs beared a poor prognosis. Only one myopericytoma recurred once.

Discussion: Smoothelin, heavy caldesmon, desmin, myosin, calponin and 3G5 were the most valuable markers to differentially diagnose canine PWT. The widely applied term of canine HEP embraces a spectrum of neoplastic entities arising from different cellular components of the vessel wall, where true HEP seems to be a rare and aggressive tumour while myopericytoma seems more common and is generally benign. Prior to the assimilation of canine PWT into the prognostic category of "spindle cell tumours of canine soft parts" a consistent classification and characterization of their biology is necessary.

Macroscopic evaluation of handsewn laparoscopic gastrojejunostomy in dogs.

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Introduction: Gastrointestinal anastomosis is one of the most common procedures, performed in the intestinal tract procedures for management of gastric outflow obstruction.The feasibility and safety of the laparoscopic gastrojejunostomy have been evaluated by many surgeons. The objective of this experimental study was to determine the suitability and feasibility of a single layer continuous technique for handsewn laparoscopic gastrojejunostomy in dogs.

Material and methods: Sixteen healthy male and female dogs were randomly divided in two groups. In the control group the two layer side to side handsewn laparoscopic gastrojejunostomy was performed in contrast to the one layer continuous anastomosis in the case group. Four weeks after the operation the cases were sacrificed and necropsy findings were evaluated precisely.

Results: There was no gross inflammation, haemorrhage, infection, ischaemia or apparent granulation tissue, abcess or fistule formation. There was no evidence of anastomotic leakage or stricture and all anastomotic sites were patent. The mean diameter of the anastomotic sites was higher in the case group. Several adhesion formations were found in the abdomen, with higher incidence in the control group. Mesenteric lymphadenopathies were found in two cases of the control group.

Discussion: Single layer continuous handsewn laparoscopic gastrojejunostomy is a feasible and safe technique in dogs and has advantages over the two layer technique. There was no evidence of anastomotic leakage or stricture and all anastomotic sites were patent in both groups in necropsy. There were several adhesions in the abdomen of the cases with higher incidence in the control group.

Early granulosa cell tumours in the bitch.

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Introduction: Granulosa cell tumour (GCT) is frequently seen in the bitch representing 23-52% of all ovarian tumours. It is assumed that the tumour arises from follicular granulosa cells and might appear with different histological patterns, but the real histogenetic mechanisms are not completely understood.

Material and methods: In a survey of 762 canine ovaries, 52 primary ovarian tumours were diagnosed including: 31 epithelial tumours, 5 germ cell tumours, and 16 sex-cord stromal tumours. In the last group, histology and immunohistochemical alpha-inhibin expression allowed to identify 13 GCTs, of which 6 early tumours, herein described, were not grossly identifiable.

Results: In two cases (I, II), the tumour showed a pseudotubular pattern, with Sertoli-like elements, originating probably from granulosa cell islands (GCIs).
In three others (III, IV, V), early GCTs arose in the wall of follicular cysts as focal papillary proliferations of granulosa cells. In these tumours, round or elongated cells showed crowding and mild nuclear atypia. Call-Exner bodies were also seen. In the last case (VI), the tumour, in a more advanced growth phase, was found all around a large epithelial cyst and included follicle-like and tubular structures. Furthermore, it was underlined that two of these tumours (III, IV) arose in ovarian remnants of ovariohysterectomized bitches.

Discussion: GCIs are atretic follicular structures, exclusive of the canine ovary, in which the granulosa cells become elongated and often assumed a palisading arrangement. It is likely that GCTs with tubular pattern and the so-called ovarian Sertoli cell tumour, reported with high prevalence in the bitch, originate from the proliferation of GCIs. On the other hand, the pathogenesis of follicular cysts is unclear in the bitch. According to the previous WHO classification (Nielsen et al., 1976), we agree to consider them as tumour-like lesions, able to originate follicular GCTs. The study of 6 early GCTs allowed to hypothesize a probable origin of canine GCTs from GCIs and follicular cysts, explaining the high prevalence of tubular and follicular patterns in canine GCTs. According to previous reports, the failure to remove all of a normal ovary at surgery could be a predisposing condition for development of GCT.

Treatment of neuronal cells with PrP GPI anchor analogues cures prion infection.

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Introduction: Prion diseases such as BSE in cattle, scrapie in sheep and goats, and CJD in man are a family of chronic progressive neurodegenerative diseases for which there is currently no proven cure. The pathogenesis of these diseases relates to abnormal folding of a host-encoded protein, PrPc, into pathogenic forms (PrPsc) that constitutes the major, if not only, component of the infectious agent. PrP is tethered to membranes via a glycosylphosphatidylinositol (GPI) anchor. Previous studies have demonstrated important roles for this GPI anchor in terms of cell signalling and neuronal toxicity. Thus, for example, [1] treatment of ScGT1 cells with glucosamine-phosphatidylinositol disrupted cell membranes, increased cholesterol concentrations and reduced the amounts of PrPSc and [2] treatment with glucosamine-phosphatidylinositol reduced activation of phospholipase A2, an enzyme that is required for PrPsc formation. The aim of this study was to use partial structures of GPI anchors (GPI analogues) to determine if such molecules could disrupt prion replication.

Material and methods: A scrapie-infected neuronal cell line (ScGT-1 cells) was incubated with 1 µM of either glucosamine-phosphatidylinositolglucosamine 2-O-methyl inositol octadecyl phosphate or inositol-1-monophosphate for 7 days. To challenge mice directly, 30 µl homogenate of treated ScGT1 cells containing 7.5 x 10^4 cell equivalents in 0.9% sterile saline, was injected intracerebrally under halothane anaesthesia. Mice were monitored for clinical signs of scrapie until reaching a pre-defined clinical end point.

Results: The mean incubation period for mice inoculated with untreated ScGT-1 cells was 164 days ± 4. Mice inoculated with treated cells survived beyond 550 days and showed no behavioural abnormalities suggestive of low-grade infection. Detailed histopathology and PrP immunocytochemistry of the surviving mice did not show any evidence of prion-related pathology.

Discussion: This study clearly demonstrates a role of the GPI anchor in prion biology and the neuronal propagation of infectious prions. The detailed cell biology of this role, and the role of the GPI anchor in determining intracellular processing pathways and resulting pathology will be discussed.

Congenital cataract in French 1-day-old Mulard ducklings.

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Introduction: Ocular opacity, associated with abnormal behaviour (reluctance to move and inability to feed properly), was observed in about 1% of all newborn females from several related flocks of Mulard ducks, produced by a French Brittany commercial facility. The vaccinal status, the commercial feed and the clinical exams were unremarkable

Material and methods: A 5 weeks follow up of ten 1-day-old affected females was performed by comparison with 10 control animals. Clinical, ocular and ultrasonographic examinations and a complete necropsy of 2 animals per group were performed weekly. The right and the left eyes were respectively processed for histological and electron microscopical examination.

Results: An immature (cortical) bilateral cataract was confirmed by ultrasonography in affected ducks, occasionally associated with a pupillary deformation. Severe cataract, with Morgagnian globules, severe anterior fibers liquefaction, disorganization, and lenticonus was shown by photonic and electron microscopy. No retinal and choroidal lesions were observed. Ultrasonographic and microscopic lesions did not progress during the five weeks of examination.

Discussion: The female predilection for the ocular lesions leads to the hypothesis of a congenital sex-linked recessive cataract.

Plasma cell granuloma of the brain in a cat.

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Introduction: Plasma cell granulomas are rare benign lesions that may affect all organ systems. In humans, they have the greatest frequency in children. Rare cases have been previously described in dogs and horses; to the author's knowledge, no description has yet been reported for brain and cat.

Material and methods: A 6 year-old male European cat was referred with a history of 2-month anorexia with progressive behavioural abnormalities and 4 limbs ataxia. Neurological examination showed a severely depressed cat, with tetraparesis more pronounced on the right side. There was no menace response and a vertical nystagmus could be elicited after neck hyperextension. The cat was negative for FeLV and FIV and blood biochemical analysis including ammoniac was normal. A left cortical lesion with increased intracranial pressure was suspected. A contrast-enhancing left temporo-parietal lesion with a severe mass effect was observed on CT scans. Differentials included neoplasia or inflammatory granuloma.

Results: At necropsy, a solitary 4 x 3 cm well-circumscribed, white, firm mass was found in the left temporo-parietal lobe. The other organs were normal. At histopathological examination, the mass appeared well-demarcated from the adjacent cerebral parenchyma, and constituted by an admixture of inflammatory cells enmeshed in a collagenous stroma containing a great number of spindle cells. The inflammatory component was constituted of predominantly plasma cells and lymphocytes, mixed with a smaller number of neutrophils and histiocytes. Staining for CD3 and CD79a revealed polyclonal B and T cell infiltrate. The spindle cells were arranged in a parallel fashion or in a storiform pattern showing regular and normochromic nucleus. They coexpressed vimentin and smooth muscle actin but were negative for desmine and cytokeratin. Stains for mycobacteries, bacterial and fungal diseases were negative.

Discussion: The clinical and pathological features of the present case are similar to those reported for plasma cell granulomas of children and of animals.

Histological, immunohistochemical and ultrastructural study of a toxoplasmosis in a Bennet´s wallaby.

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Introduction: Toxoplasmosis is a common infection in many species including Bennet's wallabies, a marsupial smaller and stocker built than a kangaroo. The present study summarizes findings of a fatal generalized toxoplasmosis in an adult, male wallaby (Macropus rufgriseus) submitted for diagnosis to our laboratory.

Material and methods: A dead wallaby was submitted for necropsy from a zoo near the School of Veterinary Medicine of Lugo (Spain). Before death, the animal showed non-specific symptoms: apathy, depression and emaciation. Tissue samples were fixed in 10% formaldehyde, processed routinely, sectioned and stained with H&E. Sections were subjected to immunohistochemical staining with a polyclonal antiserum against T. gondii. Small pieces from formalin-fixed tissues were processed for TEM study.

Results: A complete necropsy was performed and the main macroscopical changes were severe congestion of the lungs, petechia in several organs, stomach ulcers, atrophy of the main lipid reservoirs and occurrence of pleural, pericardial and peritoneal effusions. Histological examination revealed interstitial pneumonia, non-suppurative myocarditis and cholangiohepatitis. In the digestive tract, severe gastric ulcers with the presence of infiltrating neutrophils and a mild catarrhal enteritis were observed. We also detected intralesional parasitic forms, morphological consistent with T. gondii tachyzoites. These parasitic forms reacted positively to the immunolabelling technique. The TEM study revealed tachyzoites within parasitophorous vacuoles, which exhibited typical structures that allowed us to confirm toxoplasma infection.

Discussion: Toxoplasmosis has been diagnosed in captive wallabies and kangaroos in a number of countries. However, to our knowledge, this is the first report of this disease in a Bennet's wallaby in Spain. This parasitoses can produce considerable losses in captive macropods. On the other hand, subclinical infected animals represent a risk of infection for humans in countries where their raw or undercoocked meat is beeing consumed.

An abdominal cavity abscess associated with Salmonella enterica serovar typhimurium phage type DT2 in a dog.

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Introduction: This report describes and discusses the pathological and bacteriological findings of an abdominal cavity abscess associated with Salmonella enterica serovar typhimurium phage type DT2 in an Anatolian karabash dog. A male dog from humane society of Kayseri Municipal was presented to the Department of Pathology, Faculty of Veterinary Medicine, Erciyes University for necropsy. The referring veterinarian, indicated anorexia and lethargia in the history of the dog.

Material and methods: Following a gross necropsy examination, tissue samples were collected for histopathological and bacteriological evaluations. Content of mass was inoculated onto blood agar (with 7% defibrinated sheep blood) and the MacConkey agar. All media were incubated at 37° C, 24 - 48 h, in aerobic, microaerobic, and anaerobic conditions. After the incubation periods, grown colonies were picked and subjected to biochemical and serological tests to identify bacteria (phage typing and antibiotic suspectibility of bacteria were done at Danish Institute for Food and Veterinary Research, Department of Microbiological Food Safety, DK). The obtained isolate was resistant to Streptomycin and sensitive to Amoxicillin + Clavulanic, Ampicillin, Apramycin, Ceftiofur, Chloramphenicol, Ciprofloxacin, Colistin, Florfenicol, Gentamicin, Nalidixic acid, Neomycin, Spectinomycin, Sulfamethoxazole, Tetracycline and Trimethoprim. Obtained tissue samples were fixed in 10 % neutral-buffered formaldehyde, embedded in paraffin wax, sectioned at a thickness of 5 µm, mounted on glass slides, stained with H&E and Gram stain and examined with a light microscope.

Results: Gross pathology revealed a fluctuant, grayish-pink and well demarcated mass, measuring approximately 25 cm in size, in the abdominal cavity. Moreover, an adhesion to spleen, peritoneum and the distal portion of the gut were observed. The abscess was consistently surrounded by a thick fibrous capsule. The mass included a grayish-white content with a creamy consistence. Other organs were grossly normal. The histological examination revealed chronic inflammatory cells (bizarre and giant cells) in the fibro-vascular connective tissue of the abcess wall. In some areas, however, many eosinophils, neutrophils and macrophages were infiltrating the fibrous connective tissue, and there was a lymphoplasmacytic cellular infiltrate, too. These areas contained Gram-negative bacterial colonies. In some areas, there was advanced fibrosis, occasionally hyalinized. Similar findings were also observed in the spleen. Moderate inflammatory changes were observed in sections of other organs. In the bacteriological examination, Salmonella enterica serovar typhimurium phage type DT2 was isolated and identified from the content of the abscess in the abdominal cavity.

Discussion: The serotype typhimurium phage type DT2 is frequently isolated from pigeons. Therefore the findings are likely related to the consumption of a pigeon or contaminated materials by the dog.

Effects of anabolic and therapeutic dosages of dexamethasone on thymus morphology and apoptosis.

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Introduction: Glucocorticoids are currently one of the most efficacious agents used for the treatment of inflammatory diseases, autoimmunity and shock. They are also widely used in animal production as growth promoters, either alone or associated with sexual steroids and/or β-agonists. An experimental administration of dexamethasone (DEXA) was carried out in veal calves in order to assess the role of anabolic and therapeutic dosages on thymus morphology and apoptosis.

Material and methods: Thirty veal calves were included in this study: group A (n=10) was administered 5 mg of dexamethasone-21-isonicotinate intra-muscular at days 0 and 7; group B (n=10) was administered 0.4 mg/day of dexamethasone-21-phosphate per os for 20 days. Ten animals served as control group (Group K). Two animals from each group were slaughtered at day 3 (T3), 7 (T7), 14 (T14), 32 (T32) and 52 (T52). In situ apoptosis was evaluated by TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling) technique. Moreover, the presence of phosphatidyl-serine in apoptotic cells was detected by flow cytometric analysis.

Results: The weight and size of the thymus was progressively reduced in both treated groups. At T14 a 76% and 35% reduction of the relative thymus weight, compared to controls, was observed in group A and B, respectively. At T32 the reduction was 13% in group A and 50% in group B, whilst both groups showed a complete recovery of thymus weight at T52. Histological variations of thymus weight were associated with lymphoid depletion and fat replacement of the peripheric portion of the lobular cortex, particularly at T14 in group A. In group B at T14 and T32 lymphoid depletion was mostly associated to widening of the medulla and thinning of the lobular cortex. A clear correlation of these phenomena with apoptosis of thymocytes was detected. Apoptosis was evaluated by in situ and flow cytometric methods. The trend of apoptosis was similar in animals treated with anabolic and therapeutic dosages of DEXA, although a higher percentage of apoptosis was induced by anabolic treatment.

Discussion: The findings suggest that DEXA induces apoptosis and consequent thymus atrophy, which is soon reversible allowing the thymus gland to return to it´s original weight and structure in a few weeks after the last administration of the drug.

In conclusion, our investigation confirms, that thymus weight and histological lesion, as lymphoid depletion and fat replacement, can be good indicators of recent illegal corticosteroid administration in veal calves. This backs up the adoption of histopathology by the the National Health Service in prevention of the abuse of that drug in veal and beef production.

Differential proteomic analysis of renal glomeruli from two murine nephropathy models.

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Introduction: The variety of renal diseases implies various mechanisms leading to altered expression of proteins in the different renal microstructures. Therefore, analysis of the renal proteome during relevant disease states is crucial for a better understanding of the complexity of the pathogenesis and pathophysiology of different renal diseases. Our project focuses on progressive glomerulosclerosis, which represents a highly relevant problem in nephrology. We analysed the glomerular proteome of two different mouse models of nephropathy at defined disease stages in order to identify candidate proteins with potential diagnostic or pathogenetic significance: Transgenic mice expressing a dominant negative glucose-dependent insulinotropic polypeptide receptor (GIPRdn) represent a model for diabetes-associated glomerular lesions. Growth hormone (GH) transgenic mice are a well characterized model for progressive glomerulosclerosis. Because glomeruli represent only ~2% of the whole kidney volume, a subproteome approach targeting isolated renal glomeruli is the method of choice.

Material and methods: A modified version of a recently published method for the isolation of glomeruli from murine kidneys perfused with spherical superparamagnetic beads was used, enabling a very high yield of approximately 10,000 glomeruli per mouse kidney within 90 minutes. For differential quantitative proteomic analysis of glomerular proteomes from GIPRdn transgenic (tg) as well as GH transgenic mice vs. their corresponding wild-type controls (co), the two-dimensional differential in gel electrophoresis (2D DIGE) technique was applied in a pH gradient 4-7. Spots of interest were identified by matrix assisted laser desorption ionization - time of flight / time of flight (MALDI-TOF/TOF) mass spectrometry analysis.

Results: Several differentially abundant proteins could be found in the group of GH tg vs. co and in the group of GIPRdn tg vs. co, respectively. Most of the identified proteins have protein binding function or belong to the cytoskeleton. Molecular functions of other identified proteins include calcium ion binding, hydrolase activity or transporter activity. Some of the differentially abundant and identified proteins could be found in both groups.

Discussion: Differentially expressed proteins in the stage of beginning albuminuria could be detected in the two different murine glomerulopathy models as compared to wild-type controls. The abundance alterations of some of these proteins were identical in both models, suggesting a common pathogenetic mechanism. Further studies will reveal their pathogenetic relevance and their potential as diagnostic markers.

Common patterns of glomerular gene expression in early stages of progressive kidney disease.

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Introduction: Development of glomerulosclerotic alterations is a common feature of various progressive kidney diseases. The earliest stages of these different disease entities are characterized by common morphological and functional alterations of the glomeruli, such as glomerular hypertrophy and consecutive development of albuminuria. To address the question, if such common patterns of morphological and functional glomerular alterations would also find a reflection in glomerular gene expression profiles, we performed differential transcript profiling analyses.

Material and methods: Kidney glomeruli were isolated from two different murine models of nephropathy: a novel model of diabetes mellitus: transgenic mice expressing a dominant negative glucose-dependent insulinotropic polypeptide receptor (GIPRdn); and growth hormone-transgenic mice (bGH). Both models develop glomerular hypertrophy and microalbuminuria. Male mice were investigated in two early comparable stages of glomerular alteration. Stage I: glomerular hypertrophy: significant increase of the mean glomerular volume of the transgenic animals. Stage II: glomerular hypertrophy and onset of albuminuria in transgenic animals, detected by recurrent SDS-PAGE based urine analyses of urine samples taken on consecutive time points and verified by ELISA. Glomerulus isolation was performed according to a modified protocol of a method for magnetic isolation of glomeruli from kidneys being perfused with spherical superparamagnetic beads. Total RNA was extracted from glomerulus isolates and processed for Affymetrix® GeneChip microarray analysis. In each group (model) and stage, transcripts that were differentially expressed between transgenic animals and their corresponding controls were identified, using the Genomatix® ChipInspector 1.20 software.

Results: Commonly differentially regulated transcripts, representing the intersections of congeneric differentially regulated transcripts in both groups in comparable stages of glomerular alteration, were identified. The numbers of these commonly differentially regulated transcripts (86 in stage I and 469 in stage II) were found to be statistically significantly enriched. 21 transcripts were congeneric differentially regulated in all groups and stages. Differential expression of 5 of these transcripts was confirmed by real-time PCR.

Discussion: Thus, in two different mouse models of glomerulopathy, common patterns of glomerular gene expression profiles in early stages of glomerular alteration could be identified. Further studies will reveal the meaning of these shared expression signatures in terms of our understanding of the molecular pathogenesis of early stages of progressive glomerulopathies, as well as their potential use as diagnostic markers or therapeutic targets.

Expression of proliferation markers and evaluation of glycosaminoglycans in Achilles tendon healing in a rat model.

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Introduction: Rupture of the Achilles tendon is a particularly debilitating injury for which the current treatments are limited. The inflammatory phase and the subsequent reparatory and remodeling phases are characterized by fibroblast migration and proliferation, collagen synthesis and reorganization. Tenocyte growth and neovascularization are the most critical features during tendon healing. Tenocytes are able to convert biophysical stimulations into a biochemical response, releasing several growth factors, i.e. tumour growth factor-beta (TGF-beta) and insulin-like growth factor-I (IGF-I). Supplementing the diet with amino acids has proven useful in accelerating healing of ligament and tendon injuries. The aim of the investigation was the evaluation of the efficacy of a pharmaceutical amino acid composition in Achilles tendon healing in the rat.

Material and methods: Twelve male Wistar Sprague-Dawley rats aged 11 weeks underwent surgical transection of the Achilles tendon. A pharmaceutical amino acid composition was administered to the treated group (6 animals) per os for 4 weeks; 6 animals served as control. At the end of the treatment period, animals were euthanatized and the Achilles tendon was removed, formalin-fixed, and H&E stained. Further sections were immunohistochemically stained with anti-TGF-beta, anti-IGF-I and anti-PCNA antibodies. Furthermore glycosaminoglycans (GAGs) were extracted and determined from tissue fragments.

Results: Histological examination revealed a higher percentage of fibroblasts in treated animals, while controls showed a higher percentage of macrophages, granulation and fibrous tissue, necrosis and hemorrhages. Significant differences were revealed in the expression of proliferation markers: IGF-I was expressed in a higher percentage in treated than control animals (P=0.0080), while TGF-beta and PCNA scored higher in control than in treated animals (P= 0.0256 and P=0.0753 respectively). The total amounts of GAGs and hyaluronic acid were higher in the control group, while dermatan sulphate, heparan sulphate and chondroitin sulphate scored higher in treated animals.

Discussion: The treatment of Achilles tendon surgical lesions with a pharmaceutical aminoacid composition in a rat model apparently induces a positive evolution of the lesions by anticipating the proliferation of cells responsible for the healing.

Familial cutaneous vasculopathy in two twin German shepherd dog.

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Introduction: Cutaneous vasculitis represents an uncommon disorder in dogs; most of it is attributed to immunoreactive mechanisms, but they are poorly studied and characterized. Familial cutaneous vasculopathy of the German shepherd dog is a rare disease, with only few works discussing this disease. Its exact pathogenesis still remains unknown. However, an immune-mediated vasculopathy with an autosomal-recessive mode of inheritance was suggested.

Material and methods: We report two cases of twin German shepherd dogs, male, 4-months-old, with clinical signs of lethargy and fever, lameness with dropped carpi and tarsi, poor body conditions, stunted growth, poor coat quality, swelling of the bridge of the nose and all four footpads, most of which appeared depigmented.

Results: At necropsy, both dogs showed severe oedema of all four footpads, the bridge of the nose, mesentery and gastric submucosa, and a diffuse oedematous appearance of subcutaneous tissues of ventral thorax and abdomen, peritracheal and perioesophageal connective tissues. Bilateral multiple pale renal cortical foci were observed in both animals, while only one showed gastric ulcers and valvular endocardiosis of the left atrioventricular valve. Histopathological examination of nose and footpad samples revealed severe oedema and nodular to diffuse dermatitis, mainly presenting a perivascular pattern involving the deep dermis, panniculus and subcutis, associated with vascular damage, characterized by thickening of the vessel wall, hyaline degeneration, sometimes infiltration of inflammatory cells, and occasionally leukocytoclasia. A focal superficial dermatitis with hydropic degeneration of basal cells and pigmentary incontinence was observed in some footpads. We found a diffuse inflammatory infiltrate, characterized by monuclear cells and neutrophils, associated with collagenolysis and involvement of vessels in all oedematous tissues, as well as in deep fascia and epimysium, associated with myofiber atrophy, fibrosis and myositis.

Discussion: We diagnosed familial cutaneous vasculopathy of the German shepherd dog based on the characteristic cutaneous histological features. Our findings showed, that in addition to cutaneous lesions there were features of systemic vasculitis, involving small and medium-sized vessels.

The Tissue Microarray as a tool for evaluation of antibodies against Chlamydia-like organisms.

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Introduction: Over the last decade, numerous Chlamydia-like organisms have been identified, with evidence suggesting, that they are emerging into a possible public health threat. Simkania negevensis and Parachlamydia (P.) acanthamoebae are thought to be possible agents of pneumonia in humans, whereas Waddlia chondrophila was isolated from bovine abortion. In addition to highly sensitive molecular methods (i.e. real-time PCR) to diagnose these new infectious agents, immunohistochemistry (IHC) protocols using new antibodies against these Chlamydia-like organisms are needed to demonstrate the agent within lesions. We used the tissue microarray (TMA) technology to test antisera raised against two different Chlamydia-like organisms for their future use in IHC on animal tissue samples.

Material and methods: Acanthamoeba castellanii cultures were infected with P. acanthamoebae strain Hall' coccus, Waddlia chondrophila strain ATCC 1470, Simkania negevensis strain ATCC VR-1471, Rhabdochlamydia crassificans strain CRIB-01 and Protochlamydia amoebophila UWE25 (ATCC PRA-7). HEp-2 cell monolayers were infected with different human and animal Chlamydiaceae strains, such as Chlamydia (C.) trachomatis, C. suis, Chlamydophila (Cp.) pneumoniae, Cp. abortus, Cp. psittaci and Cp. pecorum. Pellets from all infected monolayers were formalin-fixed and paraffin-embedded. Uninfected amoebal and cell pellets were included as negative controls. A cell pellet microarray was constructed, containing cylindrical samples of 2 mm diameter. IHC was performed using a commercial monoclonal antibody targeting the chlamydial lipopolysaccharide of members of the Chlamydiaceae (mLPS), and two experimentally produced polyclonal mouse antibodies directed against Parachlamydia and Waddlia, respectively.

Results: Optimisation of the IHC protocol on experimentally-infected amoebal pellets demonstrated the species-specificity of Parachlamydia and Waddlia mouse antibodies. Neither antibody cross-reacted with other Chlamydia-like organisms. Moreover, no cross-reactivity was observed for the polyclonal mice antibodies on the HEp-2 cell pellets infected with the different Chlamydiaceae species. In contrast, the mLPS antibody reacted as expected with the different Chlamydiaceae infected HEp-2 cell pellets, but not with the amoebal pellets infected with Chlamydia-like organisms.

Discussion: Antibodies against Parachlamydia and Waddlia are suitable for IHC on formalin-fixed and paraffin-embedded samples. The TMA technology represents a useful tool for establishing IHC protocols for experimentally produced antibodies and for testing their specificity. The two antibodies were further used to investigate bovine placental specimens to support the role of Chlamydia-like organisms in bovine abortion.

Detection of Bovine Papillomavirus type 2 in urinary bladder tumours of cattle from Eastern Romania.

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Introduction: In cattle, tumours of the urinary bladder are commonly associated with a syndrome known as Chronic Enzootic Haematuria (CEH) due to prolonged ingestion of bracken fern. Bracken fern is believed to be the only higher plant proven to cause cancer naturally in animals; it contains immunosuppressive, mutagenic, clastogenic and carcinogenic chemicals. CEH occurs in several areas worldwide and is very common wherever the bracken is spread. Previous studies have pointed out a strong relationship between Bovine papillomavirus type 2 (BPV-2) and bracken fern. The high degree of association between bladder cancers and BPV-2 suggests, that this virus could play a role in bladder oncogenesis. The aim of this study is to investigate the presence of BPV-2 and the expression of its major oncoprotein in bovine urinary bladder cancer from Eastern Romania.

Material and methods: Ninty urinary bladder samples were collected at slaughterhouse from cattles grazing on bracken infested lands of Neamt County - Moldavia - Romania. The samples were formalin-fixed, paraffin embedded and the diagnoses were assessed on H&E stained sections according to the WHO Histological Classification of the Tumours of the Urinary System of Domestic Animals. PCR analysis was performed on a subset of paraffin blocks in order to detect BPV-2 DNA (see Borzacchiello et al., 2007). The tumour samples and normal controls were immunohistochemically investigated for the presence of BPV-2 E5 oncoprotein using a sheep anti-E5 antiserum as previously reported (Borzacchiello et al., 2006).

Results: Of the 90 urinary bladder samples collected, 40 were diagnosed as neoplasia; 5 cases showed dysplasia; 10 showed hyperplasia; 3 samples showed inflammatory lesions; the remaining were normal. 15 out of 40 tumour samples were mixed tumours with haemangioma or haemangiosarcoma being the most prevalent histotypes. DNA of PCR-quality was recovered from 27 tumour samples and 1 normal mucosa. A fragment of the expected size was amplified in all 27 tumour samples and in the normal ones. No association was found between the presence of viral DNA and a particular type of tumour. All neoplastic cells showed E5 expression intracytoplasmically. A typical juxtanuclear immunoreactivity was observed. No E5 expression was observed in normal samples.

Discussion: It has been known for a long time that the synergism between toxic and carcinogenic principles from Bracken fern and BPV-2 infection plays an important role in the carcinogenesis of the urinary bladder in cattle. It has been suggested that the virus is in a latent state in the bladder and that immunosuppressants from the Bracken activate the virus. Moreover, only recently the role of virus in vivo has been established due to the expression of its E5 oncoprotein. Few studies about the spread of the BPV-2 infection are reported, so far. BPV-2 infection in cattle has been reported in Italy and in Portugal as the only European countries. In this study we report, for the first time, the presence of BPV-2 in naturally occurring urinary bladder tumours of cattle in Romania, an eastern European country, where bracken fern is widely distributed confirming the role of the BPV-2 in the aetiopathogenesis of bovine bladder neoplasia.

Borzacchiello G, Russo V, Spoleto C, Roperto S, Balcos L, Rizzo C, Venuti A, Roperto F (2007). Bovine papillomavirus type-2 DNA and expression of E5 and E7 oncoproteins in vascular tumours of the urinary bladder in cattle. Cancer Letters 2007 May 18;250(1):82-91.
Borzacchiello G, Russo V, Gentile F, Roperto F, Venuti A, Nitsch L, Campo MS and Roperto S. Bovine papillomavirus E5 oncoprotein binds to the activated form of the platelet-derived growth factor b receptor in naturally occurring bovine urinary bladder tumours. Oncogene 2006 Feb 23;25(8):1251-1260.

Laser micromanipulation - a new dimension in life sciences.

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Introduction: Modern molecular biomedical research relies on the capability of pure sample preparation. Amongst various options to achieve homogeneous samples, only laser microdissection and micromanipulation offers high-resolution control of sample composition by selecting or rejecting individual cells.
Tissue preparation and extraction protocols allow the utilization of microsamples for qualitative and quantitative molecular and proteomic analyses like, e.g., PCR and RT-PCR amplification, microarray analysis, and MALDI/SELDI spectrometry. Investigation of cell-cell interactions as well as the study of tumour microenvironment are possible applications. Laser Microdissection and Pressure Catapulting (LMPC) results in an eminent increase in the specificity of downstream analyses.

Material and methods: The PALM MicroBeam combines laser technology with high quality robotic tools for precise microdissection of specimens, whilst the patented pressure catapulting feature allows for non-contact collection with no impairment to the recovery of DNA, RNA or protein. The integration of image analysis platforms to the LMPC technology fully automates screening, identification and finally subsequent high-throughput sample handling.

Results: An important innovation is the laser driven isolation of living cells out of a cell culture. Individual or small groups of cultured cells or stem cells can be used for direct molecular analysis or re-cultivation. This helps scientists to isolate cell clones and separate different cell types by morphology or fluorescent label. The focused laser allows to poke minute holes into cells and nuclear cell walls, which were closed by the cell itself within a few seconds or minutes. This enables injection of, e.g., drugs or genetic material without using viral vectors or chemical treatment of the cells. The work with selected living cells is extremely facilitated with this new approach and opens a wide field of new applications and research possibilities.
In reproductive medicine, polar body extraction is a safe and accurate technique. However, it may be critical for the oocyte as the zona pellucida has to be opened to get access to the polar bodies. The most convenient and gentle method is laser-assisted microdissection. Laser microdissection is used to open the zona pellucida in a completey non-contact way. Subsequently a blunt-ended pipette instead of a sharp one can be used for polar body extraction. This procedure may decrease the degeneration rate of oocytes. We will show some examples for the different applications.

Discussion: Non-contact laser microdissection and pure capture of the selected material as well as microsurgery are innovative tools for biomedical research.

DON (Deoxynivalenol) long-term intoxication in pigs: A study on the cellular immune response.

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Introduction: The stimulus to develop novel experimental protocols to demonstrate and frame the immunotoxic potential of mycotoxins in swine arises from field evidence demonstrating, that animals exposed to such toxins are more sensitive to various illnesses, and from conclusive proof in domestic species, namely the chicken. On this basis, this study was performed to evaluate the potential immunotoxic effect of DON (deoxynivalenol) on the cellular immune response of young pigs.

Material and methods: Seven 8 week-old piglets have been fed for 6 weeks with a diet supplemented with DON (0.5 ppm/pig of DON in the first week and 1 ppm/pig for 5 weeks afterwards) and seven piglets were kept as untreated controls. Haematological absolute counts of WBC, lymphocytes and neutrophilic granulocytes were determined, using an automatic haemocytometer. Percentage and absolute levels of NK cells ($CD3^-CD8\alpha^+$), γ/δ T lymphocytes ($TCR\gamma/\delta^+$), T helper lymphocytes ($CD4^+CD8\alpha^-$), memory T cells ($CD4^+CD8\alpha^{low}$) and cytotoxic T lymphocytes ($CD4^-CD8\alpha^{high}$) were determined in the peripheral blood, using flow cytometric analysis. The reactivity of immune cells was determined using a MTT lymphoproliferative assay and an IFN-γ ELISpot assay, respectively. MTT assay was performed by stimulating in vitro pig PBMC with 5-10 μg/ml PHA for 72 h, 37°C, 5% CO_2; ELISpot assay was performed stimulating PBMC in vitro with 1-2.5 μg/ml PHA for 20 h, 37°C, 5% CO_2, to determinate the levels of IFN-γ secreting cells.

Results: WBC counts did not show relevant changes whereas lymphocyte/neutrophilic granulocyte ratio increased during the experimental period in both groups; no statistical differences were found in flow cytometric data of DON-treated and control pigs. An age-dependent increase was also shown in cytotoxic and memory T cell levels (NK cells, γ/δ T lymphocytes, $CD4^-CD8\alpha^{high}$, and $CD4^+CD8\alpha^{low}$ cells) and in lymphocyte proliferation response to PHA. The levels of IFN-γ secreting cells in DON-treated and control pigs were not significantly different.

Discussion: In the period of age considered, an age-dependent increase of cytotoxic and memory T cells and of the lymphoproliferative activity was observed. This trend, testifying a phenotypical and functional maturation of cell-mediated immunity, was not influenced by the treatment with DON. Also the lymphocyte functional activity measured as IFN-γ secretion was not influenced by the treatment. Such responses could be due to a limited period of investigation or to a low ratio of mycotoxin/pig/day used (even though the experimental dose of DON is comparable to the quantity naturally present in contaminated food) or a combination of both. Further investigations focused on the phenotypical and functional analysis of other lymphoid organs and secretions such as lymph nodes, GALT (Gut Associated Lymphoid Tissue), BALF (BrochoAlveolar Lavage Fluid) using flow cytometry, immunoenzymatic and histochemical techniques, will permit us to study a potential toxic effect of DON on local immunity.

Cerebral vasculitis in pigs: pathological and serological investigations.

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Introduction: Vasculitis is an inflammatory lesions affecting blood vessels associated with direct infections of vessel walls or with immunologic mechanism not directly caused by the pathogens.

Material and methods: 214 brains collected from regularly slaugthered Nebrodi feral Black pigs aged from 6 to 24 months were submitted to histopathological investigations. Immunohistochemical studies were performed to characterize the inflammatory cells and to detect the presence of Aujeszky virus infection. Serological tests were performed for detection of antibodies against Leptospira spp., Mycoplasma spp., Salmonella spp., Toxoplasma gondii, Neospora caninum, Aujeszky virus, PCV2, PRRS, Influenza virus, tick-borne encephalitis virus (TBEV), Coronavirus and Parvovirus.

Results: 48% of the animals showed pathological lesions. The main lesions were inflammation (76.06%) comprising of diffuse encephalitis (10.32%), leptomeningitis (28.57%), multifocal or focal mononuclear perivascular cuffs (29.37%), arteritis (23.80%) and plexus choroiditis (7.94%). The most significant finding was an arteritis classified as periarteritis nodosa. The affected arteries were distributed expecially in cerebral meninges and less frequently in other areas of the cerebral cortex and in the basal nuclei area. The arteritis was characterized by fibrinous necrosis and severe infiltrates mainly composed by lymphocytes and monocytes. Malacic areas were also observed in three brains, sometimes associated to periarteritis. Immunohistochemistry for Aujeszky virus was negative in all samples.

Discussion: Periarteritis nodosa is reported in many species and seems to be an immune complex disease. In swine viral or Streptococcal infections are considered the possible causes of these lesions. Further investigations are in progress in order to better understand the aetiopathogenesis of this from of vasculitis.

Chemo-angiogenic profile of bovine urinary bladder tumours distinguishes urothelial carcinomas from haemangiosarcomas.

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Introduction: Angiogenesis and inflammation are two processes regulated by numerous common molecular mechanisms. Inflammation can stimulate angiogenesis, and angiogenesis can facilitate inflammation; both mechanisms have been shown to be involved in carcinogenesis. With this study we sought to gain a molecular understanding of the mechanisms involved in tumour angiogenesis and inflammation in urinary bladder tumours.

Material and methods: 26 urinary bladder tumours of urothelial and endothelial origin were collected from 14 Friesian cows with enzootic haematuria. Molecular analyses of angiogenic factors (VEGF, VEGFR1, VEGFR2 and Angiopoietin-2) and chemokine production (MCP1, Mip1α, Mip1β and the chemokine receptors CCR1, CCR2, CCR5, CXCR4) were evaluated using RT-PCR. Microvessel density (MVD), microvessel pericyte coverage index (MPI) and tumour cell proliferation and apoptosis were evaluated by immunohistochemistry and TUNEL analysis. The extent of tumour-infiltrating leukocytes (TILk) was also assessed. Differences between groups were evaluated using 2-tailed T-test and Pearson correlation coefficient was applied for comparison of the morphological parameters and gene expression.

Results: Chemokines and chemokine receptors, Angp2, VEGF, and VEGF receptors are expressed in bovine urinary bladder tumours. All chemokines and chemokine receptors are in fact up-regulated in urinary bladder haemangiosarcomas (HSA) and urothelial carcinomas (UC), when compared with normal urinary bladder ($P < 0.05$). These malignant bladder tumours also display higher TILk counts, and higher tumour cell proliferation and apoptosis when compared to papillomas and haemangiomas ($P < 0.005$). Specific chemokine and angiogenic profiles distinguish HSA from UC. While Mip1β ($P < 0.01$), CCR1 ($P < 0.05$) and VEGFR2 ($P < 0.01$) are up-regulated in HSA, VEGF transcript levels are significantly higher in UC ($P < 0.01$). As expected MVD is higher in HSA ($P < 0.01$), yet no significant difference in MPI was found between these and UC. Using Pearson correlation it showed that high CXCR4 levels correlated with high Angp2 and VEGFR2 and low vessel maturation index. Other than being related with the angiogenic profile of bladder tumours, CXCR4 was also found to be an important link to tumour-associated inflammation. CXCR4 levels positively correlated with TILk counts, Mip1β and CCR1.

Discussion: Hereby we provide evidence for the putative role(s) of inflammatory cells and chemokines to interfere with the angiogenesis pathway and differentially affect tumour cell-microenvironment interactions in urinary bladder carcinogenesis. The above data supports the hypothesis that malignant transformation is associated with the adoption of an immune phenotype by cancerous cells. Although up-regulation of all chemokines and chemokine receptors was generally seen in bovine urinary bladder tumours it seems, that these genes are being differentially regulated in urothelial carcinomas and haemangiosarcomas. We believe that this is a good model to study the importance and clinical application of the angiogenesis/chemokine pathways in oncogenesis with.

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Necrotizing pneumonia due to feline herpesvirus in kittens - an underdiagnosed disease?

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Introduction: Feline Herpesvirus - 1 (FHV-1) is an alphaherpesvirus which is an important cause of acute upper respiratory tract and ocular disease in cats. Sporadically, generalized infections and primary viral pneumonia have been reported, particularly in young and debilitated animals. Here we describe four cases of fatal necrotizing pneumonia due to an FHV-1 infection in kittens.

Material and methods: Lungs of four male kittens (Bengal, Selkirk Rex, 16 days of age; Siam, 25 days of age, European shorthair, 6 months of age) were investigated histologically. FHV-1 antigen was detected by immunohistochemistry using monoclonal antibodies and the avidin-biotin-complex-peroxidase method.

Results: In all four cases the lungs showed a diffuse necrotizing interstitial pneumonia, with an accompanying sero-fibrinous component with neutrophils and severe necrosis of bronchial and bronchiolar walls. In necrotic areas alveolar lumina were filled with a mixture of degenerate neutrophils, cell debris, sloughed epithelial cells and erythrocytes. Immunohistochemically, large amounts of FHV-antigen were predominantly detected within the necrotic lung tissue, in necrotic bronchial and bronchiolar epithelium, but also in the cytoplasm of intact alveolar epithelial cells and alveolar macrophages.

Discussion: Primary viral pneumonia induced by FHV in cats is rare and only very few reports describe FHV-1 induced pneumonias in kittens. The present four cases demonstrate that FHV-1-induced primary necrotizing pneumonia is not an extremely exceptional finding within feline necropsy cases. We suggest that in cases of fibrino-necrotic pneumonia in kittens FHV-1 infection is considered a relevant differential diagnosis and demonstration of viral antigen is attempted by IHC or another suitable technique.

Visceral spreading of acute influenza infection in mice.

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Introduction: Because of the great danger represented by influenza infection, an intense research activity is performed all over the world. Different strains of mice (Balb/c, CD1) and ferret (Mustela putorius furo) are used in experimental models. This model followed the way of injury expression in different organs of murine strains with different reactivity against influenza virus.

Material and methods: Four strains of mice between 6 - 8 weeks old have been used: Balb/c, TCR-HA, Ins-HA, and dTg. A/PR8/34 influenza virus strain from allantoic liquid was intranasally instillated, using 268.8 HAU/mouse. The animals were euthanized 48 hours after infection. Influenza infection was confirmed for each mouse using a quick test for virus identification. Histological investigation (Masson trichrome stain) of lung, heart, brain, kidney, liver and pancreas in all animals of the experiment has been performed.

Results: Lesions of bronchioles, interstitium, alveolar spaces and blood vessels were the objectives in lung investigation. Necrosis of bronchiolar epithelium and bronchointerstitial pneumonia dominated in Balb/c. Lesions were represented by necrosis of bronchiolar epithelium and bronchointerstitial pneumonia with obvious tendency of spreading in alveolar walls in Ins-HA mice. The intensity of reaction in peribronchial spaces was different for each mouse. Bronchointerstitial pneumonia was constantly complicated with lung consolidation foci in TCR-HA. All cases of dTg exhibited degeneration and necrosis of bronchiolar epithelium with lumen obstruction and cuff peribronchiolar reaction. Myocardium, kidney, liver and pancreas presented a preserved architecture, without inflammatory reaction. Hepatosis and insulitis were noticed in dTg, being a strain characteristic. Brain revealed isolated neuronal necrosis and sporadic gliosis.

Discussion: Alveolar walls were severely injured, peribronchiolar inflammatory reaction being discrete or absent. These findings suggest viral spreading in 48 hours after infection. TCR-HA (this strain has a T-cell clone with receptors for HA of PR8) exhibited a strong inflammatory reaction of lungs. A reverse correlation was observed between intensity of lung inflammatory reaction and clinical evolution of the disease. Lesions in sensitive strains are identical with those described in medical literature. We emphasize the role of inflammatory reaction in visceral spreading of influenza reaction.

Immunophenotyping and expression of VEGF in canine inflammatory mammary carcinoma.

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Introduction: Canine (IMC) and human (IBC) inflammatory mammary carcinoma is a rare form of rapidly advancing mammary cancer. It is a distinct clinical subtype of locally advanced breast cancer, which occurs with or without the presence of mammary nodules and is characterized by a particularly aggressive behavior and prognosis, with erythema, warmth and oedema of the breast. Its histological hallmark is the invasion of dermal lymphatic vessels by neoplastic emboli, which block lymphatic drainage causing oedema. It is characteristically angioinvasive; increased angiogenesis and lymphangiogenesis have been indicated in IBC by PCR gene expression quantification. The aims of this study were to characterize the neoplastic cell types found in IMC by immunohistochemistry and to evaluate the expression of the angiogenic factor VEGF by immunohistochemistry and RT-PCR in inflammatory versus non-inflammatory malignant canine mammary tumours.

Material and methods: Immunohistochemistry of AE1/AE3 cytokeratins, vimentin, α-actin, desmin, von Willebrand factor, CD31 and CD34 was performed on samples of 15 canine inflammatory mammary carcinomas with clinical and pathological diagnoses. VEGF immunohistochemistry and RT-PCR expression were assessed in IMC cases and in grade III non-inflammatory canine malignant mammary tumours (non-IMC).

Results: Immunophenotyping confirmed the epithelial origin of most of the IMC, including lipid rich tumours. Only one tumour was re-diagnosed as sarcoma.
There was no evidence of myoepithelial proliferation. In six IMC cases, highly malignant neoplastic cells resembling endothelial cells and forming small capillaries were frequently found. The immunostaining of these structures with endothelial markers was not conclusive. VEGF expression was significantly higher in IMC cases.

Discussion: The broad term inflammatory mammary cancer should be preferably used to designate this type of mammary tumour in order to include the possible presence of sarcomas. According to previous in vitro studies on human IBC, the transformation of the IMC neoplastic cells into endothelial-like cells or endothelial precursor cells is possible, although further studies should be done to confirm this hypothesis. Angiogenic factor VEGF might play an important role in this phenomenon.

Long-term morphological monitoring and feline coronavirus (FCoV) shedding in cats experimentally infected with FCoV type I.

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Introduction: Feline infectious peritonitis is a fatal and widely distributed disease of cats, induced by feline coronavirus (FCoV). Two FCoV serotypes have been identified on the basis of in vitro neutralization tests. Each type is capable of causing a spectrum of clinical signs, ranging from asymptomatic infections to diarrhea to FIP. Until now, the pathogenesis is unknown. Differing cell tropism of either enteritis- or peritonitis-producing viruses has been suggested as a possible explanation of different manifestations of the disease.
The goal of this study was, to evaluate the clinical signs and pathological lesions in the intestinal tract and in various organs of cats experimentally infected by FCoV type I and to correlate these with the viral load.

Material and methods: Twelve 19-20 week-old specified pathogen-free cats were included in this study. Two were used as controls. Ten were divided into five groups of two animals each and perorally infected with the same infectious dose of FCoV type I at day 0. For clinical follow-up, animals were daily assessed. Serology was performed on day 0, 7, 14, 28, 42 and 70. FCoV shedding was determined by measuring FCoV load in faecal swabs by RT-PCR. Faecal swabs were collected every two days during the first two weeks, then every two weeks. The cats were successively euthanized on day 7, 14, 28, 42 and 70. A complete necropsy was performed. Oesophagus, stomach, duodenum, ileum, caecum, colon, mesenteric lymph nodes, liver, spleen and thymus were collected for histopathological examination and for detection of virus by immunohistochemistry. Additionally, they were analyzed for FCoV viral load.

Results: Experimentally affected cats remained clinically healthy. They exhibited a mild inflammatory response exclusively located to the intestinal tract most prominent at day 28, which disappeared at day 70. The inflammatory response was associated with a villous intestinal atrophy. The virus was detected by immunohistochemistry in the mature enterocytes at the tip of intestinal villosities for duodenum, ileum, caecum and colon early at day 7 and at day 70 for the distal parts. It was also detected in macrophages located in the intestinal chorion and in the sinus of mesenterial lymph nodes as shown by double immunostaining using a macrophages labelling antibody. The FCoV load was early detected in all the intestinal tract at day 7 and until day 28. Then it was located in the distal parts except for the duodenum, which remained positive until day 70.

Discussion: These findings demonstrate the long-term infection of the intestinal tract, a specific tropism of the virus for the enterocytes and macrophages, the correlation between the intestinal microscopic lesions due to FCoV infection and the viral load.

Experimental and spontaneous infection with Helicobacter pylori in dogs.

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Introduction: Helicobacter pylori represents one of the most common and medically prominent infections worldwide in humans. Infection with this bacterium has an association with histological gastritis, gastric atrophy, gastric cancer, and mucosa-associated lymphoid tissue (MALT) lymphoma in the stomach. Dogs can be experimentally infected with H. pylori, but there are limited sources of GI pathology in dogs. The aims of this study are, (1) to see if it is possible to infect dogs with Helicobacter pylori and to evaluate the pathogenesis of the bacteria and (2) to evaluate the gastric lesions in one dog naturally infected with H. pylori.

Material and methods: (1) Experimental infection. Two puppies, male and female, three months old, mixed breed, were used for experiment. The puppies were treated for possible spontaneous infections with Helicobacter spp., using the standard therapy recommended for H. pylori eradication: seven-day treatment consisting of Amoxicilin (500 mg/day), Metronidazol (300 mg/day) and Nizatidin (150 mg/day). Seven days after the treatment the dogs were infected with a H. pylori culture obtained from human gastric biopsy. After one month the female and two months the male dog were euthanized and the gross examination was followed by the cytology and the histology of gastric mucosa. (2) Spontaneous infection. In one German Shepherd dog, male, ten-years-old, which was euthanized due to medullar compressive syndrome, necropsy showed multiple miliary antral acute ulcers. Gastric cytology revealed high numbers of bacteria, morphologically close to H. pylori (short spirals or coma, 3-3.5 μm length).

Results: Both experimental cases showed multiple dot-form haemorrhagic ulcers, only in the pyloric antrum zone. The exam of the touch smears, in both cases, showed the presence of only Helicobacter pylori-like bacteria. The microscopic findings were necrosis of superficial epithelium of antrum, neutrophilic superficial gastritis, ulcers and hyperplasia of lamina propria. In the spontaneous infection the lesions were similar.

Discussion: Our results suggest that (1) Helicobacter pylori are pathogen for dogs, in both experimental and naturally conditions, (2) the acute lesions caused in dogs are similar to those in humans - neutrophilic gastritis and ulcers, and (3) there is the possibility of human - dog transmission of the infection.

Experimental infection with Helicobacter pylori in rats.

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Introduction: In 1983, Warren and Marshall proposed the possible association of Helicobacter pylori with peptic ulcer disease and gastric cancer. In June 1994, the International Agency for Research on Cancer Working Group of the World Health Organization classified H. pylori as a group I, or definite, human carcinogen. Immediately following infection, H. pylori causes acute gastritis characterized by neutrophilic infiltration into the foveolar and surface epithelium and epithelial degenerative changes. H. pylori cause a persistent infection in the majority of infected individuals. The acute phase lasts 1 to 4 weeks and is replaced gradually by a chronic, mononuclear infiltrate in the lamina propria.

Material and methods: The aim of this study was to see if it is possible to infect rats with Helicobacter pylori and to evaluate the pathological gastric changes induced by the bacteria. We used an experimental group of 6 adult Whistar rats, and a control group of 4 rats. Each experimental rat was orally inoculated with Helicobacter pylori culture, two times at three weeks interval. Three weeks from the second inoculation the rats were euthanasied and examined (gross examination, bacterioscopy, urease rapid test and histology). In all experimental rats, not in the control ones, urease test was positive.

Results: The exam of the gastric smears in infected cases showed the presence of only Helicobacter pylori-like bacteria. The histological findings consisted of chronic active superficial gastritis. Apoptosis and necrosis of superficial epithelium of gastric corpus were encountered. Neutrophilic infiltration associated with superficial epithelial alteration, in addition to the inflammatory infiltrate with mononuclear cells and superficial fibrosis of lamina propria, characterizes the chronic active superficial gastritis. In addition, acute haemorrhagic ulcers in gastric corpus mucosa were noticed.

Discussion: Our results suggest that Helicobacter pylori is pathogen for rats, at least in experimental conditions. The lesions caused in rats are similar to those in humans but, in contrast to predominant antral localisation in humans, the localisation is in the gastric corpus.

Pathological features in the European Mink (Mustela lutreola).

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Introduction: The European mink (Mustela lutreola) is a small mustelid that is nowadays one of the most endangered mammals in Europe. Therefore, in Spain only a few populations remain in the North, so there is not much information about it. The reasons for its decline includes habitat loss, water pollution and most of all, the competition with the American mink (Mustela vison), which was introduced into Europe in 1926 for fur farming. This work describes the main pathological alterations observed in a small group of European minks found death during 2004 and 2005 in Navarra (Spain).

Material and methods: 23 European mink cadavers were submitted to the Pathology Diagnostic Service of the University of León (Spain) for necropsy in order to investigate the cause of death. After gross examination, samples were conventionally processed and stained with H&E, PAS, Grocott or Gram methods whenever required. In one case, an immunohistochemistry stain employing an anti-canine distemper virus monoclonal antibody was also carried out. Bacterial cultures were performed when necessary.

Results: Almost all the animals had traumatic and haemorrhagic lesions. Amongst pathological findings related to infectious processes, one animal showed interstitial pneumonia accompanied by lymphocyte depletion in spleen and demyelination in ponts and cerebellar peduncles. In this case, positive stain for distemper virus antigen was observed on immunohistochemistry. Furthermore, three cases of granulomatous pneumonia associated with the typical adiaspores of Chrysosporium spp. (Adiaspiromycosis) and two animals with parasitic pneumonia caused by Filaroides spp. were described. Another animal had multifocal necrotic hepatitis with many Gram negative bacteria and Sarcocystis spp. cysts were observed in six minks.

Discussion: Traumatic lesions were found in almost all the animals studied and were compatible with road traffic accidents, being then the main cause of death. However, infectious lesions were also observed. It is worth highlighting the presence of lesions associated to adiaspiromycosis, a pulmonary mycosis that occurs worldwide and affects many small mammals, among them the Mustelidae family, but as far as we are concerned, has not yet been described in the European mink. Notice as well the description of interstitial pneumonia associated to distemper virus in this species in wild conditions.

Ackowledgements: To the Gobierno de Navarra, for providing the mink cadavers which were used in this study.

Role of cytological investigation in identification of hepatopathies and infection with Toxoplasma gondii in abattoir slaughtered swine.

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Introduction: Hepatic lesions in swine produce important losses due to total rejection of liver and depreciation of carcasses quality, and are also a source of infection for people and animals. The aim of this study is to identify cytological changes in the liver and to evaluate the incidence of toxoplasmosis in slaughtered swine.

Material and methods: The liver of 60 abattoir slaughtered clinically healthy pigs was cytologically investigated. Pigs were slaughtered in 2 abattoir units (unit A and B with 30 pigs each) from Southern Romania. Imprints were May-Grünwald-Giemsa stained. Samples of serum and meat juice harvested from the same pigs were evaluated using ELISA to identify anti-Toxoplasma gondii antibodies.

Results: Samples from unit A revealed a normal morphology of hepatocytes. Only one case presented foamy cells (fat liver). In all cases a heterogeneous inflammatory cell population, represented by lymphocytes, macrophages and large number of eosinophils, occurred, being more abundant in T. gondii positive cases and discrete in the rest. One T. gondii positive case was dominated by numerous neutrophils. A large number of isolated or clustered mesenchymal cells, with fibroblastic characteristics has also been observed. Identification of trophozoits was revealed in ELISA T. gondii positive cases. The results of ELISA in pigs slaughtered in unit A exhibited 7 positive samples (23.3%) both in serum and meat juice, with 503-606 optic density (OD) in serum and 516-675 OD in meat juice. The imprints from liver of pigs slaughtered in unit B exhibited more severe lesions. In almost half of the cases a polymorphous flora was observed (bacilli, cocci, diplococci, fungi). In all cases hepatocytes had normal cytological features. Inflammatory population was dominated by mononuclear cells and fewer eosinophils and neutrophils. T. gondii trophozoits were identified also. 18 cases (68%) were positive to anti-T. gondii antibodies, with 475-736.5 OD in serum. 9 samples from 18 cases (30%) of meat juice were positive, with 487-818.5 OD.

Discussion: Cytological investigation exhibited early identification of some lesions without gross expression, despite the normal morphology of hepatocytes. The constant presence of eosinophils point towards parasitic diseases (toxoplasmosis and another). Mononuclear population and cells with fibroblastic morphology proved a subacute or chronic evolution of lesions (chronic hepatitis, interstitial hepatitis in pigs slaughtered in unit A and B). Presence of T. gondii trophozoits confirmed toxoplasmosis, being correlated with a high value of OD in serum, according to the reference values (negative reference serum <300 OD, positive reference serum >800 OD). Further research and testing are necessary to diagnose toxoplasmosis and for interpretation of values of ELISA on meat juice samples.

Immunohistochemical and biochemical studies on keratin expression in normal equine skin and skin of heavy draft horses with chronic pastern dermatitis.

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Introduction: Keratins (Ks) are components of the intermediate filament network of epithelial cells in humans and mammals. The 37 identified human epithelial Ks are highly differentiation-specific in their expression patterns. Therefore antibodies (Abs) to Ks are useful markers of tissue differentiation in human and veterinary diagnostic pathology. In contrast to canine skin, there is only limited knowledge about the keratin expression pattern of equine skin. Pastern dermatitis of heavy draft horses is a chronic idiopathic hyperplastic skin disease, involving the posterior aspects of the pasterns of draft horses. Previous studies indicated the occurrence of alterations in the expression of certain Ks in these horses. The purpose of this study is (1) to characterise the distribution of Ks in normal equine skin and (2) to examine the expression of proliferation-associated keratins in the skin of heavy draft horses with chronic hyperplastic pastern dermatitis.

Material and methods: 30 skin samples of the neck and the pastern of 10 warmblood horses without clinical skin lesions were examined immunohistochemically using 1 polyclonal and 11 monoclonal Abs directed against human K1, K5/6, K6, K9, K10, K14, K16, K17, K19 and K20. Skin samples from 5 of these horses were examined by gel electrophoresis and western blotting with 9 Abs of unknown cross-reactivity for horse tissues. Skin samples from neck and pastern of 47 draft horses with chronic pastern dermatitis and from 4 draft horses without clinical skin lesions were examined immunohistochemically with Abs against human K6 and K16.

Results: In normal horse skin the staining pattern of the Abs directed against K5/6, K6, K14 and K20 showed minor differences in comparison to man. K1, K10 and K16 were expressed in exactly the same way as in man. One of the Abs against human K9 (Ks9.70+Ks9.210) reacted mainly with basal cells of the outer root sheath, but not with suprabasal cells of palmoplantar epidermis like in man. Surprisingly, the antibody against K13 showed cross-reactivity with K15 in human and equine skin. The interfollicular epidermis of diseased skin from all heavy draft horses with chronic pastern dermatitis showed suprabasal expression of K6 and K16 with a significant increase in relation to the histological degree of epidermal hyperplasia.

Discussion: The study revealed that the expression pattern of keratins in clinically normal equine skin is largely identical to normal skin of man. However, not all commercial Abs with specificity for human keratins showed a specific cross-reactivity with keratins of equine skin. Results with antibodies against K6 and K16 in hyperplastic skin of draft horses with chronic pastern dermatitis demonstrated that these keratins, like in certain hyperproliferative skin diseases in man, are upregulated and are expressed in the suprabasal epidermis.

Avian influenza virus (H9N2) and renal apoptosis.

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Introduction: Avian influenza produces cell death in chicken and humans. Cell death can be caused by either necrosis or apoptosis. We investigated the types of cell death that occurs in chickens infected with avian influenza virus, A/chicken/Iran/772/2000(H9N2).

Material and methods: Twenty 3-weeks-old SPF chicken were divided into two groups. The first group was infected with $10^{7.5}$EID50 titer of the virus intravenously and the second group was treated with saline solution. After the following 72 h, kidney tissue was collected and fixed in 10% formaldedhyde. The prepared microscopic sections with the thickness of 5-6 μm were stained using TUNEL method.

Results: In comparison to the control group, there was a significant amount of apoptotic cells in renal tubular cells of the infected group ($P<0.005$). The number of apoptotic cells was counted in five microscopic fields of the sections.

Discussion: The role of viral proteins in the induction and regulation of apoptosis has been extensively studied, but relatively little is known about how such proteins contribute to apoptotic cell death due to influenza virus infection. NS1 is an attractive influenza viral protein in this regard, because of its homology with the cytoplasmic domain of the FAS antigen. We demonstrated that A/chicken/Iran/772/2000(H9N2) is able to induce apoptosis in renal tubular cells of chicken.

Brain fluorescence in equine hypoxic ischaemic encephalopathy.

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Introduction: Hypoxic ischaemic encephalopathy (HIE) is the single most important perinatal cause of neurologic morbidity in full-term and low-birthweight human infants. In veterinary medicine HIE is best known in the foal and was previously known as the "dummy" or "barker" foal. The equine syndrome is usually involved in the more generic condition, neonatal maladjustment and/or failure of passive transfer of colostrum. The HIE lesion in the brain is one of widespread laminar cortical neuronal necrosis. Usually, for lesions to be identified macroscopically, an affected foal needs to be clinically supported for several days and develop severe lesions.

Material and methods: A term, cloned foal was born unassisted. The placenta was submitted for examination by a pathologist. The foal was mildly dyspneic and developed haematuria. Clinical examination showed umbilical enlargement and on day 4 of life, the foal was sent to surgery for removal of an umbilical haematoma. When anaesthetized, the blood oxygen could not be elevated above 20mm Hg and surgery was aborted. During recovery, the foal began to seizure. Seizures became progressively worse and on day 7 of its life, the foal had a post-ictal cardiopulmonary arrest. It was necropsied.

Results: The placenta macroscopically and microscopically showed large areas of reduced microcotyledonary chorionic fronds. Macroscopically, umbilical haemorrhage and omphalophlebitis were noted. The lungs failed to collapse, but were not firm. Sections of the brain were oedematous and diffuse laminar cortical profiles of autofluorescence were noted when the brain was exposed to UV light. The neuronal cortical laminar necrosis was confirmed microscopically. Although histologically the umbilicus was inflammed, no significant pathogens were isolated and no signs of generalized sepsis were noted. Mild alveolar proteinosis and histiocytosis had caused the lungs to remain inflated.

Discussion: Autofluorescence of the brain of neonatal "hypoxic" foals has not been previously described. It is a simple and useful technique to identify lesions and is now used to scan the brains of all weak, neonatal foals necropsied in our laboratory. Often, unsuspected lesions are identified and specific foci are cut in for histological examination. In the case of this report, the condition had its origin in utero and was exacerbated by the brief, unsuccessful anaesthesia. The use of UV scanning of the brain is a well-known procedure used to diagnose polioencephalomalacia of cattle presumably related to thiamine deficiency, but not all forms of laminar cortical necrosis are autofluorescent. The phenomenon will be discussed.

Determination of equine uterine secretion products - a potential diagnostic tool for endometrial fertility problems?

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Introduction: Uterine secretions in mares have been proposed to play a particularly important role in maintenance of pregnancy and nutritional support of the conceptus. Therefore, deficiencies in uterine secretions may be a cause of fertility problems as a disturbed uterine microenvironment might lead to inadequate conceptus nourishment and embryonic death. The objective of this study was to investigate secretion patterns in uterine flushings and in endometrial biopsies of mares with endometrial alterations compared to those of gynaecologically healthy animals.

Material and methods: Uterine flushings were obtained from 20 mares with various endometrial alterations (endometritis, endometrosis, maldifferentiation) and from 6 gynaecologically healthy animals. The uterine secretion proteins were separated by SDS-PAGE. To immunolocalize the normal distribution of uterine secretory proteins (uterocalin [UC], uteroferrin [UF], uteroglobin [UG]) in the endometrium, biopsies were obtained from 3 gynaecologically healthy mares throughout the oestrous cycle and from 20 pregnant mares between days 16 and 309 of gestation. To characterize the uterine secretion patterns in altered equine endometria, biopsies with maldifferentiation due to (i) ovarian disorders (n=22), (ii) long term progestin application (n=4) and (iii) of unknown aetiology (n=29) as well as biopsies of 509 mares suffering from endometrosis were investigated. All biopsies were processed routinely and investigated by (immuno)-histology (UC, UF, UG).

Results: SDS-PAGE revealed deficient protein secretion patterns in uterine secretions especially of mares suffering from endometrosis (n=6) or from endometrial maldifferentiation (n=5). Via immunohistochemistry (IHC), UC, UF and UG were present throughout the oestrous cycle with strongest staining intensity during dioestrus. During pregnancy, UF remained detectable with a maximum staining from endometrial cup development until day 309. UC showed variable staining throughout gestation with most distinct intensity in early pregnancy and with endometrial cup development. UG secretion was virtually absent throughout gestation. Comparing biopsies with endometrial alterations with those of cyclical gynaecologically healthy animals, disturbed uterine secretory protein patterns were detectable in all cases of maldifferentiation and in all mares with endometrosis.

Discussion: UC, UF and UG were detectable (IHC) in the healthy, cyclical endometrium with maximum staining intensity during dioestrus. These findings indicate that these proteins play an important role especially in the nourishment of the preimplantated conceptus. During pregnancy, UF remained detectable throughout gestation, UC staining was variable and UG was virtually absent. Hence, UC and UF, but not UG, seem to play an important role in conceptus supply with essential nutrients and thereby contribute to the maintenance of pregnancy. In mares suffering from endometrial maldifferentiation or endometrosis deviations of the uterine secretion proteins were demonstrated. These results indicate that the disturbances of the uterine microenvironment might be one factor for reduced fertility in mares with such endometrial alterations. Finally, uterine flushings by means of SDS-PAGE do not reflect a deficient protein pattern due to endometrial alteration in every case whereas via endometrial biopsy and IHC a morpho-functional evaluation of the uterine secretion in mares suffering from fertility reducing endometrial alterations is definitely possible.

Survey on prevalence of pneumonia in condemned sheep and goats at the Ziaran abattoir.

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Introduction: Since 1969 data of condemned lung tissue from all abattoirs have been reported (Steward, 1969; Daniel et al., 2006). In this study pneumonia and pleuro-pneumonia due to Pasteurella spp. and Mycoplasma spp. infection have been isolated and the histopathological aspects were studied and recorded.

Material and methods: 10,129 sheep and 2039 goats were inspected at the Ziaran Abattoir within the years 2005 to 2006. Totally 282 lung tissue samples showed consolidation. They were collected and transferred to the Pathology Department at the Razi Institute. In order to study Pasteurella spp. the lung tissue was cultured on blood agar and McCankey´s agar plates, being incubated at 37°C and confirmed by using oxidase tests and other biochemical reactions. To isolate Mycoplasma ,tissue was cultured on PPLO Broth Media and positive colonies were detected every 48 hours. After 72 hours the first positive cases were confirmed. PCR studies were carried out on DNA samples from Pasteurella spp. and Mycoplasma spp.. Data from the Pasteurella and Mycoplasma slides were studied using histopathological techniques and the frequency of both bacteria were analyzed statistically by using chi square test.

Results: From the 282 condemned lungs Pasteurella spp. have been isolated from 125 pneumonic cases (4.25%). In 5 cases P. caballi could be isolated and the restwas identified as P. multocida. Mycoplasma spp. have been isolated from pneumonic lungs in 4 sheep and 2 goats. In one case both bacteria were recognized. 66 strains of P. multocida and all of the Mycoplasma spp. were confirmed positively by PCR. 73 out of 125 isolated Pasteurella showed little raised lesions and consolidation in tissue at the right cranio-ventral lobes. The relatively occurring frequency of pneumonia in sheep comparable with goats were 2.54% versus 1.22%. Pasteurella cases 11.9 versus 2 in 1000 I.U. and Mycoplasma 3.98 versus 9.8 in 10000 I.U. Statistical data gained from the Pasteurella cases studied, were analyzed by using chi square test showed, that the frequency of outbreaks in the various seasons within a year were significantly different $P<0.001$. Histopathological observations of Pasteurella cases showed: purulent interstitial bronchopneumonia (19.2%),purulent bronchopneumonia (28.8%),purulent bronchitis/bronchiolitis (9.6%), purulent fibrinous pneumonia (4%), purulent pleuropneumonia (7.2 %), and progressive pneumonia (4.8%). Lungs with Mycoplasma infection revealed purulent interstitial bronchitis (33.33%), purulent bronchopneumonia (33.33%), purulent fibrinous pneumonia (16.6%), and progressive pneumonia (16.6%).

Discussion: In this study the frequency of reported outbreaks in cases of pneumonia was observed and detected as follows : 83.25 in sheep and 16.75 in goats, Pasteurella cases: 11.2 in sheep and 2 in goats in 10000 I.U. The study revealed seasonal variations, showing that the relative frequency in infected sheep Pasteurella was the highest in summer time (2.55 %). The most important histopathological lesions in Pasteurella cases were Oat Shaped Leukocyte in bronchopneumonia and also peribronchial lymphoid cells in Mycoplasma cases. Isolating and identification of strains of Pasteurella should be used in prospective studies and can be useful for vaccine production.

PigMAP detection at the onset of clinical signs in experimental viral diseases of swine.

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Introduction: The acute phase response (APR) is a prominent systemic reaction of the organism to local or systemic disturbances in its homoeostasis caused by infection, tissue injury, trauma or surgery, neoplastic growth or immunological disorders. C-reactive protein (CRP), SAA, haptoglobin (Hp) and some other acute phase proteins (APPs) have been described as useful for assessing health in human patients and in various domestic animals. PigMAP is, together with Hp and CRP, one of the most important APPs in pigs. PigMAP (PigMajor Acute-phase Protein) levels increase quickly to peak levels in 1-2 hours after stimulation and this serum level can remain until 14 days afterwards.

Material and methods: The level of PigMAP has been measured in pigs used for experimental inoculation with Classical swine fever, African swine fever and Foot and mouth disease viruses. 40 Large White x Landrace female pigs, 8-weeks-old were used. Serum samples were taken from jugular vein after anaesthesia with tiletamine and zolazepam. PigMAP levels were measured using a commercial ELISA Kit. Animals were inoculated in a biocontainment level 3 animal facility with virulent strains/isolates of CSF (Alfort 187) ASF (E-70) and FMD (Cs8c1) virus to study the pathogenesis of these diseases. Blood samples were taken from them at 1-24, 1-7 and 1-17 dpi respectively.

Results: Serum levels from animals before inoculation were ranging from 0 to 1.1 mg/ml. After inoculation, the serum levels of PigMAP were increased (ranging from 1.5 to 5 mg/ml) in animals coinciding with the onset of clinical signs of these devastating viral diseases of swine.

Discussion: The use of conventional pigs to evaluate pathogenesis of infectious diseases and vaccine trials is an important tool for animal health research, what can simulate the field conditions. A disadvantage of using conventional pigs, is the persistent infection with Porcine Circovirus type 2. Two animals have been discarded for experimental inoculation at the BCL-3 facilities due to the development of porcine dermatitis and nephropathy syndrome (PDNS) or postweaning multisystemic wasting syndrome (PMWS). Those animals showed 1.5 and 1.7 mg/ml of PigMAP before being inoculated. With these results we can conclude that PigMAP is directly related to the appearance of clinical signs in these viral diseases and is a valuable tool to evaluate the immunological stress of pigs before using them for experiments.

Forced exogenous expression of equine MyoD in cultured equine fibroblasts transforms them into multinucleated myotubes.

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Introduction: Recurrent exertional rhabdomyolysis (RER) is a heritable disorder thought to affect approximately 5% of the Thoroughbred racehorse population that is manifest as poor performance, painful muscle cramping and occasionally death. Evidence suggests the disorder is associated with defective intracellular muscle calcium regulation similar to human malignant hyperthermia, although candidate genetic approaches and linkage studies have ruled out the involvement of RYR1 (ryanodine receptor) and other genes mutated in the human disorder.

RER can only be reliably definitively identified by an invasive in vitro muscle contraction test: an alternative approach for the diagnosis or RER that obviates the need for muscle biopsy would be ideal and an aid in genotyping studies. Since human fibroblasts can be transformed into myotubes after transfection with the muscle-specific transcription factor, MyoD, subsequently enabling phenotyping of human patients with RYR1 mutations through calcium fluorescence experiments (Zhou et al., Hum. Mol. Genet. 2006;15 (18):2791-803), we hypothesized that a similar approach could be applied to equine fibroblasts.

Material and methods: Preliminary experiments showed that an adenovirus expressing human MyoD failed to transform equine fibroblasts despite its expression. The equine MyoD genomic sequence was therefore derived from equine genomic BAC screening and subsequent PCR sequencing. Total mRNA was extracted from a sample of foal skeletal muscle and eqMyoD cDNA cloned into pCR-TOPOII (Invitrogen) following RT-PCR, and subcloned into pIRES2-EGFP (Clontech). Expression of equine MyoD was examined in transfected NIH-3T3 cells. Equine dermally-derived fibroblasts were transfected with the construct and following 48 hours, were incubated in a differentiation medium for 5 days. Myotube formation was assessed by immunocytochemistry for muscle-specific proteins and through Hoechst labelling of nuclear DNA.

Results: Immunocytochemistry and western blot verified expression of equine MyoD. Transformation of equine skin-derived fibroblasts into multinucleated myotubes was confirmed by immunofluorescence.

Discussion: Equine fibroblasts can be transformed into myotubes by transfection with equine MyoD. Future production of an adenovirus expressing the eqMyoD cDNA may be a valuable tool enabling further investigation of the calcium regulatory abnormalities in horses with RER and accurate phenotyping of horses for genetic investigation.

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Pathological effects of natural T-2 toxicoxis in sheep.

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Introduction: T-2 toxin (T-2) is a potent type-A trichothecene mycotoxin produced primarly by Fusarium sporotrichioides and F. poae. Experimental studies have shown that T-2 intoxication causes retard in growth rate, necrotizing lesions in alimentary tract and lymphoid depletion. Spontaneus poisoning with T-2 is rarely reported and has been associated with alimentary toxic aleukia in humans and the "mouldy corn toxicosis" of cattle. This study describes the pathological features observed in sheep naturally exposed to T-2 toxin in diet.

Material and methods: Six adult crossbreed Awassi sheep were submitted to the Pathologic Diagnostic Service of the Veterinary Faculty of León for necropsy. Serum samples of 4 sheep were collected for biochemical analysis. After gross examination, samples from several organs were processed for histology. Samples from the suspicious diet were analyzed for identification of different mycotoxins.

Results: In the acute stage of the intoxication, eighty-four out of 440 sheep (19%) died, showing feed refuse, anorexia, adipsia, ruminal atony, soft faeces and apathy. The main lesions observed in necropsied animals at this stage were rumenitis and ulcerative abomasitis, depletion of lymphocytes in the Peyer´s patches, lymph nodes and spleen, myocardosis and also intense oedema in different organs (skin, encephalon). Sheep in the chronic stage of the poisoning mainly showed weigth loss and reproductive inefficiency (123 abortions). In the four animals studied at this stage, the main pathological features were characterized by chronic inflammatory lesions in the gastrointestinal tract, myocardial fibrosis as well as suppurative necrotic lesions in oral cavity (glossitis, pharyngitis, tonsillitis, adenitis) with extension to skeletal muscle and bone. Other opportunistic infections by Aspergillus spp., Balantidium spp, Eimeria spp. and Muellerius spp. were also diagnosed. Increase of serum lactate-dehydrogenase and creatin-kinase was observed, possibly related to heart lesions. T-2 toxins were detected in all diet samples studied.

Discussion: This study indicates that, in natural conditions, T-2 induces lesions in gastrointestinal tract and lymphoid tissues, as well as reproductive failures and immunosuppression, similar to previous descriptions in experimental studies. Heart lesions observed in these animals also suggest a cardiotoxic effect of T-2 in sheep, as has been shown to occur in other species (rats and pigs). It is worth highlighting that spontaneous T-2 toxicosis in sheep was not previously reported in the literature.

Results of histopathological examination from endoscopical biopsies of gastrointestinal tract in 29 dogs.

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Introduction: In the years 2006 and 2007 we examined endoscopical biopsies of gastrointestinal tract in 29 dogs.

Material and methods: Tissue samples were fixed in 10% formaldehyde, processed by using paraffin technique and stained with H&E. In one sample we used Van Gieson staining method. In some of them the immunohistochemical examination was performed. We examined 26 gastric biopsies, 26 duodenal, 1 jejunal, 5 ileal, 1 caecal, 12 from the colon and 4 rectal biopsies.

Results: Subacute and chronic lymphoplasmacytic superficial gastritis was detected in 13 specimens, subacute and chronic diffuse gastritis in 10, subacute and chronic eosinophilic gastritis in 2 and chronic hyperplastic gastritis in 1 tissue specimen. We diagnosed intermediate grade lymphoma in 2 and gastric tubular adenocarcinoma in one sample. In 22 duodenal biopsies subacute and chronic lymphoplasmacytic inflammation was detected, secondary lymphangiectasia in 3, subacute and chronic eosinophilic duodenitis in 3 and intermediate grade lymphoma in 1 sample. In one tissue sample of jejunum chronic lymphoplasmacytic jejunitis was diagnosed. Chronic lymphoplasmacytic ileitis was found in 5 samples. One case of papillary adenocarcinoma was diagnosed in the caecum. We detected chronic lymphoplasmacytic colitis in 7 cases, chronic eosinophilic colitis in 1, a papillary polyp in 2, an adenomatous polyp in 1 and a villous adenoma in 1 case. Papillary adenocarcinoma of the colon was diagnosed in 1 sample. Papillary polyp was detected in 2 samples of the rectum, papillary adenocarcinoma in 1 and leiomyoma in 1 case.

Discussion: Detected histopathological findings display a high incidence of lymphoplasmacytic inflammation of gastrointestinal tracts in dogs. Their occurrence is most frequent in stomach and duodenum of examined animals.

Migration of Feline immunodeficiency virus infected lymphocytes across an invitro feline blood brain barrier model is promoted by the basolateral presence of FIV and TNF.

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Introduction: Feline Immunodeficiency Virus (FIV) infection of cats is an established model of HIV-1 infection in man. Like HIV, FIV is neurotropic. Virus enters the brain early in infection, and is associated with prominent leukocyte trafficking across the blood-brain barrier (BBB). Cytokines, including TNF, are elevated within the brain in acute infection, and this is known to influence the methods of entry of viruses and cells into the brain at the level of the BBB. The aim of the present study is, to evaluate the magnitude of cell-free FIVGL8 and FIV-infected lymphocyte cell migration across an in vitro model of the feline BBB, to evaluate the potential extent to which this is influenced by the presence of TNF in serum or CNS, and the presence of virus within the CNS.

Material and methods: FIV was cultured in MYA-1 cells and the feline BBB was developed as described previously (Fletcher et al., 2006 Vet. Immuno. Immunopathol.). Barrier was exposed either to cell free (500 TCID50/ml FIV) or cell associated virus (10^6 infected cells) with or without TNF (10ng/ml). Cell and virus migration was assessed after 24h by cell viability analysis and by RT assays, respectively. Barrier tight junction integrity was assessed by the paracellular transport of FITC-labelled dextrans FD-4 and FD-40.

Results: Cell-free FIV migrated through the blood-brain barrier model in statistically insignificant quantities, which were not significantly increased in the presence of TNF. Furthermore, the presence of cell-free FIV did not affect the integrity of the blood-brain barrier tight junctions. In contrast, cell-associated FIV readily transmigrated across the BBB in a similar magnitude to uninfected, activated cells, with neither cell population altering BBB integrity. However, in the presence of scenarios to mimic elevated serum and/or CNS TNF concentrations, a statistically significant increase in transmigration of both cell populations was observed and accompanied by a moderate, but not statistically significant, disruption of barrier integrity. Further enhancement of migration occurred, when scenarios to mimick infected cells and TNF within the brain were undertaken. This induced the most significant disruption of BBB tight junctions suggesting that, in vivo, small quantities of virus in the brain with the potential to trigger TNF production may attract greater viral entry into the CNS.

Discussion: FIV interacts with the feline BBB in a similar manner to HIV interactions with the human BBB and the trafficking of infected lymphocytes is influenced in similar ways to that of HIV-infected monocytes. Furthermore, findings suggest, that cell migration and altered BBB integrity is greatly influenced by the preexistance of virus and TNF within brain. This might implicate in the design of therapies to reduce or eliminate CNS reservoirs of infection, or for controlling the consequences of CNS infection.

Immunohistochemical study of Epidermal Growth Factor Receptor (EGFR) expression in canine mammary gland tissues.

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Introduction: EGFR overexpression has been found in a variety of human tumours, generally associated with a more aggressive behaviour. Receptors for EGF have been demonstrated on several breast cancer cell lines and in human and canine primary tumours; however there is no agreement on the clinical relationships and prognostic value of EGFR in human breast cancer. The aim of the present study was to assess EGFR expression pattern in canine mammary tissues and to investigate a possible correlation with clinicopathological parameters and survival, in order to provide information on its potential diagnostic use and biological significance.

Material and methods: A series of 131 canine mammary tumours (47 benign and 84 malignant) was classified according to the World Health Organisation diagnostic criteria. Representative areas of normal and hyperplastic mammary glands in tissue adjacent to benign lesions were also examined. The expression of EGFR was evaluated by immunohistochemistry using a mouse monoclonal antibody anti-EGFR (1:50, Clone 31G7, Zymed Laboratories) and Herceptest scoring system was applied (negative=no membrane staining or <10% of cells stained; 1+ = incomplete membrane staining in >10% of cells; 2+ = >10% of cells with weak to moderate complete membrane staining; and 3+ = strong and complete membrane staining in >10% of cells). Statistical analysis was performed with SPSS software.

Results: In normal and hyperplastic canine mammary glands, EGFR expression was consistently observed in myoepithelial cells. Luminal epithelial cells were usually negative, with the exception of some normal and hyperplastic ducts. Perilobular stroma was frequently positive. In tumour tissues, we found EGFR positivity (grade 2+ and 3+) in 9 benign (19%) and 26 malignant (31%) lesions. No association between EGFR expression and clinicopathological features (age, breed, size, histological type, location, ulceration, necrosis, type of growth, stromal invasion, lympho-vascular invasion, lymph node and distant metastasis) was observed. In addition, EGFR expression showed no association with disease-free survival or overall survival.

Discussion: Our study shows that myoepithelial cells in normal, hyperplastic and benign lesions constantly express EGFR, which appears to be of diagnostic use for myoepithelial cell identification. In the present study, no association was found between EGFR expression and clinicopathological parameters or survival, which is in accordance to some human breast cancer studies and in contradiction to others. So, further studies are warranted to analyse the prognostic impact of EGFR overexpression in larger series of canine malignant mammary tumours and to explore EGFR tyrosine kinase inhibitors as potential therapeutic agents.

Histopathological survey of the uterus of Holstein dairy cows without clinical signs of reproductive system disorders.

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Introduction: The clinical symptoms of reproductive abnormalities may not be demonstrated by clinical examination such as: rectal palpation or vaginal examination. Delayed conception in dairy cows often causes economical losses. The objective of this study was to evaluate the uterine histopathological changes in Holstein dairy cows without any clinical signs of reproduction system abnormalities.

Material and methods: 115 lactating Holstein cows without any clinical symptoms of reproduction system diseases were examined. Cows were inseminated artificially (n = 83; 72.2%) for 3 times after the last calving, or had not been recorded with any oestrus signs since 3 months after their last calving (n = 32; 27.8%). Biopsies of the endometrium and submucosa were obtained, by using a sterile alligator-jawed (rounded) biopsy forceps, from the medial wall of the uterine horns. They showed various stages of the oestrus cycle. The samples were placed in 10% formaldehyde. After the related processing of the samples, they were evaluated for microscopic pathological changes.

Results: The results showed that 89 (77.4%) dairy cows had no pathological lesions. However, 26 (22.6%) cases had pathological changes such as: chronic endometritis (n = 18; 15.7%), follicular chronic endometritis (n = 3; 2.6%), and acute endometritis (n = 5; 4.3%).

Discussion: Evaluation of uterine biopsies is not common in cows. Endometritis can be a common cause for subfertility and infertility in dairy cattle. This study showed that 1) an endometrial biopsy can be useful for evaluation of reproduction performance of dairy cows; 2) non-pathological effects such as management of dairy cattle herds, heat detection, artificial insemination in wrong time and so on may be the reasons of unsuccessful conception; 3) cows without any clinical signs of reproductive disorders may be infected; 4) dairy cows with high milk production may not show oestrus signs.

Computed tomography guided fine needle aspiration and / or tissue core biopsy of intrathoracic masses in the dog.

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Introduction: Intrathoracic masses are very difficult to diagnose without a guided diagnostical imaging support. Aim of the study is: (1) to asses diagnostic accuracy of percutaneous computer tomography (CT) guided fine-needle aspiration (FNA) and / or tissue core biopsy (TCB) of intrathoracic masses of dogs; (2) to evaluate pneumothorax risks. 49 dogs of different sex, breed, size and age underwent CT-guided FNA and / or TCB (41 pulmonary, 8 mediastinal).

Material and methods: Cytodiagnosis was performed on FNA. Histology and immunohistochemistry were performed on TCB. TCB formalin fixed paraffin embedded sections were stained for histological routine methods and for immunohistochemistry using an antibody panel. (Vimentin, CD31, CK19, CK AE1/AE3, CD3, CD79, CD44, ICAM-1, Ki67, smooth muscle actin). 38 dogs were investigated with CT-guided FNA (33 lungs, 5 mediastinum) with a 73.7% of diagnostic accuracy.

Results: Lung neoplasia was diagnosed in 20 dogs (76.9%), inflammation in 6 animals and 7 specimens were non-diagnostic. No clinical pneumothorax was observed in 4 dogs. Five mediastinal masses were diagnostic in 3 cases and 2 were tumours. Five dogs were investigated with CT-guided FNA and CT-guided TCB with a 100% diagnostical accuracy. All masses were localized in the lung, 4 were neoplasia, and 1 pneumonia. No clinical pneumothorax risk was recognized in 4 dogs. Six dogs were investigated with CT-guided TCB with 83.7% diagnostical accuracy. Three masses were localized in the lung, the other 3 in the mediastinum. Two of the 3 lung masses were neoplastic. No clinical pneumothorax was observed in 2 dogs. The 3 masses localized in the mediastinum were all diagnostic and neoplastic. No clinical pneumothorax was observed in 1 dog.

Discussion: In conclusion CT-guided TCB has a higher diagnostic accuracy (81.8%) in comparison to CT-guided FNA (74.4%), but has a higher risk of the development of a non-clinical pneumothorax (pulmonary CT-guided TCB (63.6%), pulmonary CT-guided FNA (10.5%)). The absence of a pneumothorax was observed in mediastinic CT-guided TCB and CT-guided FNA.

The role of KIT in canine mast cell tumours: a biopathological study.

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Introduction: Cutaneous mast cell tumours (MCTs) are common neoplasms in dogs, particularly in certain breeds such as boxers. MCTs are often associated with c-kit exon 11 deletions and duplications. C-kit is a proto-oncogene codifying KIT, a transmembrane receptor tyrosine kinase expressed by mast cells. C-kit exon 11 mutations cause constitutive KIT activation and have been associated with aberrant, cytoplasmic KIT expression. Cytoplasmic KIT expression was recently recognized in canine MCTs and correlated with higher histological grade and reduced post-surgical survival. Although proliferation markers have long been studied in MCTs, no correlations between these and KIT expression patterns have yet been described. No explanations have been advanced for the predisposition of certain breeds for developing MCTs. It is possible that, as in human familial mastocytosis, hereditary c-kit mutations are implicated.

Material and methods: 103 MCTs from 67 dogs were histologically graded and studied for presence of necrosis, ulceration and mitotic index. AgNORs were counted in 100 cells and Ki67 expression was studied immunohistochemically using a monoclonal antibody (MIB-1, Dako) in 1000 cells. KIT expression was studied immunohistochemically using a polyclonal antibody (Dako). Correlations between these variables were studied using Kruskal-Wallis and chi^2-tests. 15 MCTs and blood samples from 14 animals were also used for genetic analysis. C-kit exon 11 was amplified using a standard PCR reaction with previously published primers, followed by direct sequencing.

Results: Highly significant statistical correlations ($p<0.001$) were established between cytoplasmic KIT expression, higher Ki67 (32.9% vs 92.0%) and AgNORs (1.9 vs 1.3 AgNORs per nucleus) values, when compared with membrane-associated expression. Equally significant correlations were established between cytoplasmic KIT expression, higher histological grade, presence of necrosis, epidermal ulceration and mitotic index. Interestingly, no significant differences were found between cytoplasmic focal (Golgi-like) and diffuse patterns when these were considered separately. Both proliferation markers were linearly correlated and showed highly significant associations ($p<0.001$) with histological grade, presence of necrosis, epidermal ulceration and mitotic index. Only 4 out of 15 MCTs used for genetical analysis could be amplified and sequenced. One grade I MCT from a boxer dog showed a single cytosine insertion in the terminal portion of exon 11. The same insertion was observed in the blood sample of another boxer, whose MCT was not sequenced. A single thymine insertion was detected in the same area, in the blood sample from a third boxer.

Discussion: Cytoplasmic KIT expression correlates with increased cell proliferation, tumour necrosis, ulceration and histological grade, in accordance with previous findings and known KIT functions. Cytoplasmic focal and diffuse expression patterns seem to represent similar phenomena or perhaps a progressive cytoplasmic KIT accumulation. Single base, hereditary c-kit mutations were detected and associated with grade I MCTs. Such hereditary mutations may help to understand the predisposition of certain breeds for developing MCTs.

Histological and immunohistochemical characterization of a renal cell carcinoma in a pig.

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Introduction: Although infrequent, renal carcinomas are the commonest primary renal tumours in most domestic species. Human renal cell carcinomas have been demonstrated to stain positively for both vimentin and low molecular weight cytokeratins and can thus be distinguished from urothelial carcinomas. However, comparative studies in animals have not yet been done. Furthermore, despite the importance of pigs as animal models for oncological research, no histological descriptions or immunohistochemical studies of renal carcinomas in pigs are yet available. This report describes the histological and immunohistochemical features of a renal carcinoma in a pig.

Material and methods: A unilateral renal mass replacing part of the cortex and most of the medulla was incidentally found on a 6-months-old, male, cross-bred pig presented for slaughter in an abattoir in northern Portugal. The tumour was fixed in 10% formaldehyde, processed and paraffin-embedded. H&E staining and immunohistochemical analysis were performed. A standard avidin-biotin-complex-peroxidase protocol and anti-vimentin (V9, Zymed) and anti-cytokeratin (AE1/AE3, Dako) mouse monoclonal antibodies were used for immunohistochemical analysis.

Results: Tumour cells were cuboidal, disposed in sheets and tubules, and presented a lightly eosinophylic cytoplasm with round, central, eucromatic nuclei. Immunohistochemically, tumour cells showed intense multifocal cytoplasmic staining for cytokeratins (AE1, AE3) and weak to moderate diffuse cytoplasmic staining for vimentin.

Discussion: These results parallel observations in human renal cell tumours and may contribute for a better understanding of renal neoplasms in domestic species.

In vitro and in vivo studies of the expression of carbohydrates in a canine mammary carcinoma cell line.

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Introduction: Spontaneous mammary tumours are the most common neoplasia in the female dog and have a high biological and histomorphological heterogeneity. Approximately one-half of all mammary tumours in dogs are malignant and have a great potential to metastasize to the regional lymph nodes or other organs such as the lungs. Malignant transformation is associated with abnormal glycosylation, resulting in the synthesis and expression of altered carbohydrate determinants. Although the majority of cancer research in humans is conducted using established cell lines, the interaction between the tumour and the host organism must be preserved, so the results have to be confirmed using animal models. In order to study the biology of canine mammary tumours we used a previously established canine mammary cell line and compared the information with an in vivo model.

Material and methods: The CMT-U27 cell line, derived from a ductular carcinoma, was cultured with RPMI 1640 medium with HEPES and Glutamax-1 supplemented with 10% foetal bovine serum and gentamycin and kept at 37ºC in 5% CO_2 atmosphere. Cells were previously fixed with acetone or 4% paraformaldehyde and stained for expression of carbohydrates by immunofluorescence and observed in a fluorescence microscope. In vivo experiments were performed using mice of the N:NIH(s)II-nu/nu strain 6 weeks old. Tumours and organs which had been removed from these mice were fixed in 10% neutral-buffered formalaldehyde and embedded in paraffin. We made H&E staining of each one and immunohistochemistry for carbohydrates and intermediate filaments were performed using the modified avidin-biotin-complex-peroxidase method.

Results: The CMT-U27 cells adhered to the bottom of the flask in single or paired cells as a compact thin monolayer. Immunofluorescence for carbohydrates showed reaction for anti-SLex, anti-Lex and anti-Lea antisera. The staining for Lex and Lea was only positive when the cells were fixed with 4% paraformaldehyde. The CMT-U27 cells grew when inoculated subcutaneously in the fat mammary pad of female nude mice. Tumour masses were histologically identical to the mammary tumour lesions they derived from, and when heterotransplanted tumours were cultured, the expression of carbohydrates was not altered. These cells metastasized to lymph nodes, lungs, heart, spleen and liver. To look for metastatic target tissues we performed an intravenous injection in the tail vein of the mice.

Discussion: The pattern of expression of carbohydrates in the canine mammary carcinoma cell line suggests that these antigens could be useful as a prognostic tumour marker in mammary gland tumours in dogs. The aberrant expression of carbohydrates may also play a fundamental role in the molecular mechanisms of metastization to distant organs and facilitate positive interactions within the target organ.

Comparison of diagnostic tools in porcine tuberculosis.

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Introduction: Tuberculosis is a bacterial disease characterized by granulomatous lesions which are nondifferentiable grossly from pyogranulomatous processes caused by other bacteria.
In this study, several diagnostical tools for Mycobacterium genus bacilli detection were evaluated with a double aim, to determine the real incidence of the disease in the South of Spain and to determine which technique would be useful for tuberculosis diagnosis.

Material and methods: From samples condemned at slaughterhouse, a histopathological study to confirm the existence of granulomatous lesions was carried out. In the samples with granulomatous lesions, Ziehl-Neelsen (ZN) staining, immunohistochemical techniques with a polyclonal antibody against M. bovis BCG and real time PCR were performed.

Results: 10.3% of the animals (3/29) were positive to ZN technique, 93.1% (27/29) were positive to immunohistochemical staining and 69.0% (20/29) were positive to real time PCR technique. Extracted DNA in real time PCR assay belonged to M. avium in 60.0% of the cases (12/20), while the remaining cases were identified as M. bovis or mixed infections.

Discussion: The higher incidence of M. avium proves wild animals as an important point in tuberculosis transmission than domestic livestock.
The difficulty of achieving a correct tuberculosis diagnosis was proven by all three methods, grossly and using immunohistochemical and molecular techniques.

IFN gamma expression in an early infection with an European pathogenic PRRS strain.

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) causes a prolonged active infection followed by a persistent infection in lymphoid tissues, lasting for several months. Pigs develop both an antibody and cell-mediated immune (CMI) response following PRRSV infection (Batista et al., 2004). In the CMI response, the gamma interferon (IFN-gamma) plays an important role in the induction of cellular or Th1 immunity, being produced mainly by macrophages, natural killer (NK) cells, gamma-delta lymphocytes and $CD4^+$ T lymphocytes (Olin et al., 2005; Wesley et al., 2006). The purpose of this experiment was to study the expression of IFN-gamma in peripheral blood leukocytes (PBL's) and lymphoid organs in an early state of experimental infection with PRRSV.

Material and methods: Eight pigs were challenged via the intramuscular route with PRRSV isolate, originated from a Spanish farm which had severe respiratory signs in piglets. Four animals remained as non inoculated animals. Animals were painlessly slaughtered in batches of 4 at 7 and 21 days post inoculation and control animals were slaughtered at the end of the experiment. Samples from blood (PBLs), spleen, retropharyngeal and tracheobronchial lymph nodes were taken and analysed by means of ELISPOT to study IFN-gamma expression.

Results: Inoculated animals did not show any significant respiratory or other clinical signs. At necropsy, lung lesions compatible with interstitial pneumonia were observed along with an enlargement of medial retropharyngeal, tracheal-brochial and mediastinal lymph nodes. Serum concentrations of IFN-gamma displayed a high increase at 21 dpi compared with basal levels (control animals), showing just a light increase at 7dpi. Leukocytes from spleen and the lymph nodes also displayed a significant increase.

Discussion: The early production of IFN-gamma in PRRSV-infected pigs might result from activation of Th1 cell response (Wesley et al., 2006), being an interesting cytokine for the study of the development of immunity against PRRS virus and for the development of efficient vaccines (Díaz et al., 2006).

References:
Batista et al., 2004. Can J Vet Res. 68(4):267-273
Díaz et al., 2006. Virology 351(2):249-259.
Olin et al., 2005. Viral Immunol 18(3):490-499
Wesley et al., 2006. Can. J. Vet. Res. 70(3): 176-182.

Retiform Sertoli Leydig cell tumour in a bitch. Morphological and immunohistochemical features.

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Introduction: Epithelial cell tumours and sex cord-stromal tumours are the most common ovarian tumours in dogs (Klein, 2001). Among the later, three different subtypes are recognized by the WHO histological classification of ovarian tumours of domestic animals (Kennedy et al., 1998): granulosa cell tumours, which are the most frequently found ones, thecomas, and interstitial cell tumours. A fourth subtype is recognized in the WHO histological classification of tumours of the ovary in women: the retiform Sertoli-Leydig cell tumour (RSLCT) (Tavassoli et al., 2003). We describe here the clinical, morphological and immunohistochemical features of a case of RSLCT in a dog.

Material and methods: An 11 year-old, intact, nulliparous, crossbred female dog had been presented to the referring veterinarian for the investigation of vaginal oedema or hyperplasia. On vaginal exam a firm, piriform mass with smooth surface measuring 3cm in its largest diameter was noted bulging through the vaginal introitus. Surgery was the treatment of choice. The vaginal lesions regressed 3-4 days after surgery. The surgical specimen was fixed in 10% buffered formaldehyde and tissue samples were embedded in paraffin wax, sectioned and stained with H&E, periodic acid-Schiff's (PAS) reaction, Congo red and Giemsa stainings. In addition, the immuno-phenotype of the tumour cells was analysed using a pannel of selected antibodies.

Results: On gross examination, the uterus had no changes but both ovaries were enlarged and cystic. Both ovaries had several bluish cysts, the largest measuring 1.5 cm in the left ovary and 1.0 cm in the right ovary. On cut section, the cysts content was amber jelly and the wall with or without nodular growths. Microscopically, both ovaries presented a neoplasia with two patterns of growth: tubular pattern and cystic pattern. Hyalinized papillary cores were frequently observed. No positive reaction was observed with PAS, congo red and Giemsa histochemical stainings. The tumour cells expressed alpha-inhibin, vimentin, cytokeratins, oestrogen receptor-alpha and progesterone receptor, while endothelial membrame antigen (EMA), von Willebrand Factor (F VIII-ra) and S 100 protein were not detected.

Discussion: The microscopical features of the case presented here are compatible with the diagnosis of canine RSLCT according to both the human description and the single case already published in one dog (Patnaik et al., 1988; Tavassoli et al., 2003). However, this subtype of sex-cord stromal tumours must be differentiated from other types of ovarian tumours such as serous tumours. Accordingly, the possibility exists that RSLCT has been overlooked in the dog. The immunophenotype presented here may be of helpful for the diagnosis of this tumour.

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Pathological lesions related with helminthosis in different species of captive wild ruminants.

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Introduction: Nematodiasis is often found at post-mortem examinations of captive wild ruminants and is considered to be one of the major causes of death and ill thrift in zoological collections. However, in-depth research on the correlation between the presence of nematodes and pathological lesions is rare and usually confined to case studies. In this abstract, we present the preliminary results of a long term study on the correlation of total worms counts, feacal egg counts and pathological signs as assessed in ruminants of a Belgian zoological collection.

Material and methods: From 2001 to 2005, a research was conducted to determine the level of infection with nematodes of the herds of the Animal Park Planckendael (Muizen-Mechelen, Belgium). Post mortem examination was performed on all ruminants of the observed herds that died or were culled. This examination included scoring for macroscopic abnormalities, sampling for coprological and histological examination and processing abomasa and intestines for helminth recovery. In total, 2% of the worm burden was counted and adult worms were identified at species level. Total worm counts were determined in 15 exotic Bovidae, 12 Caprinae and 11 Cervidae. Histological samples were taken of the abomasum, the jejunum, the ileum (near the ileo-caecal junction) and the caecum, formalinized, embedded in paraffin wax, sectioned at 5 µm and stained with H&E and Giemsa. Afterwards, the parasitological results were compared to the histopathological evaluation of the samples in order to evaluate the pathological importance of the different gastrointestinal parasites.

Results: As known from previously conducted studies in bovids, no good correlation exists between faecal egg counts and total worm counts. The correlation between total worm counts and the severity of the pathological lesions, however, seemed to be significant in this study. In Antilopinae and Hippotraginae, the dominant nematode species found in the abomasum was identified as Camelostrongylus mentulatus. In Caprinae, Teladorsagia circumcincta was predominantly present in the abomasa; in the abomasa of the Cervidae, mainly Spiculopteragia spiculoptera was observed. Generally, mild multifocal - mainly eosinophilic - inflammation was seen in correlation with an abomasal worm count higher then 2500. Severe destruction of abomasal glands was already seen at worm counts higher then 5000. In the small intestines, mostly Trichostrongylus spp. and Nematodirus spp. were found. Mild eosinophylic till diffuse inflammation with atrophy of the villi was observed with worm counts of Trichostrongylus spp. ranging from 150 till 5800, respectively. Nematodirus spp. seemed only to be correlated with mild inflammation. Moderate lymphocytic and eosinophilic multifocal inflammation was observed in the large intestines with more than 500 Trichuris spp.

Discussion: In the abomasa and especially in the small intestines, even low burdens of nematodes can cause significant lesions. The type and the severity of the lesions depend on the nematode species, the worm burden and host dependent factors.

Mast cell tumour in dogs. Incidence and histopathological characterisation.

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Introduction: Incidence of the mast cell tumours, their distribution according to sex, breed, age and localization in Croatia is not established yet. Also, the statistical significance of the various histopathological parameters according to Patnaik's scheme, in the diagnostics of the tumour grade was yet not performed.

Material and methods: Investigation analysed mast cell tumours, histopathologically characterized at the Department of General Pathology and Pathological Morphology of the Veterinary Faculty Zagreb from January 1st 2002 to Dezember 31st 2006. Sex, age, breed, localization and tumour grade of each animal were recorded. Statistical evaluation was performed using nonparametric chi square test with one or two sample. Each histopathological finding (localization, growth, cellularity, size and shape of cells, cell borders, granules, shape and localization of the nuclei, nucleolus, hyperchromasia, anisocytosis, karyomegaly, anisokaryosis, mitoses, eosinophilic infiltrate, collagenolysis, necrosis, haemorrhages, mineralization and ulceration) was scored 0-no change, 1-mild, 2-moderate, 3-severe and statistically compared with tumour grade. Normality of distribution was checked with Kolmogorov-Smirnova test and significance was calculated with unifactorial one-way ANOVA or Kruskal-Wallis variance analysis.

Results: In the analysed period, totally 1,630 dog tumours were analysed and mast cell tumours were found in 106 animals or in 6.5 % of all cases. With statistically significant difference, this tumour was recorded in more cases in male dogs (63.2% males, 36.8% females). Average age was 6.96 years in the range from six months old terrier to 14 years old schnauzer. Boxers and retrievers were most frequent breeds and localization was mostly on legs. Grade I tumour was found in 15.09%, grade II in 44.34%, and grade III in 28.30% of animals. There was no significant correlation between tumour grade and age, breed, sex or localization, although male animals tend to have grade I and females grade III tumours more often. Considering the lesion scores, statistically significant differences were found in cell shape, number of nucleoli, anisocytosis, anisokaryosis, karyomegaly, mitoses, necrosis, haemorrhages, cellularity, cell borders, and collagenolysis.

Discussion: This findings differ from the literature data considering the sex predisposition and incidence. The incidence is usually higher than in our data and all literature states, that there is no sex predisposition considering mast cell tumours in dogs. The reason for this may be the overally higher number of male dogs kept as pets in Croatia, which influence our results. Considering histopathological findings, results showed that there is no uniform influence of the various parameters on tumour grade. This significantly changed parameters (higher scores in the more malignant mast cell tumour) should be evaluated more carefully during routine tumour analysis.

Persistent cloaca, fused kidneys, female pseudohermaphroditism, and other anomalies in a Simmental calf.

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Introduction: A number of different congenital anomalies are known to occur in domestic animals. Simultaneous congenital defects of urogenital, gastrointestinal and skeletal systems have been reported rarely in cattle. The aim of this study was to evaluate persistent cloaca, fused kidneys, female pseudohermaphroditism (FPH), gastrointestinal and skeletal anomalies in a newborn Simmental calf.

Material and methods: A 5-day-old Simmental calf was referred to our department for atresia ani and postural abnormalities caused by skeletal deformities. The calf had an appearance of male sex because of the prepuce and penis. There was no such an anomalous condition in the maternal or pedigree history.

Results: At the necropsy, the kidney, which showed a horseshoe appearance, fused at their caudal poles and was located at the right side. It had three arteries, two veins and one common ureter communicated with two ureters originated from right and left pole. The common ureter, large intestine and bilateral uterine horns were connected to a dilated common cloaca having two sacs, which was filled by a yellow-brownish viscous fluid admixed with meconium and urine. Each uterine horn had one ovary and only the right horn was communicating with the cloaca by a wide orifice and included similar fluid. A urethra originated from the cloaca ended with external urethral orifice of the penis. The penis had a S-shaped curve (sigmoid flexure) and was covered by the typical prepuce. The urinary bladder also opened to the beginning of the urethra. The calf had no testes or male accessory sex glands and also no female external genitalia. Both adrenal glands were detected in the abdominal cavity at the level of the lumbar vertebrae. The intestinal tract had no rectum and anus and ended with the cloaca. Skeletal system had some deformities including scoliosis, eight lumbar vertebrae with partial synostosis between L4 and L5, with the distorted rudimentary sacrum and fused coccygeal vertebrae and narrowed pelvic cavity. Histologically, the kidney showed dysplasia with asynchronous differentiation of nephrons surrounded by loose mesenchymal stroma. The ovaries included various developmental stages of follicles. The cloacal mucosa waa very similar to uterine mucosa, with exception for colonic mucosa at the connection area with the intestine. The connection area of the urethra and the urinary bladder included squamous epithelium. The penis had a centrally located urethra covered by many disrupted primitive trabeculae and cavernous structures.

Discussion: This report describes rare multiple congenital abnormalities in a calf, affecting urogenital, gastrointestinal and skeletal systems. Any defects in mesodermal proliferation early in embryogenesis may result in this rare combination of malformations. We suggest that the urorectal septum malformation sequence consisted of atresia ani in association with ambiguous genitalia, urogenital, colonic, and lumbosacral anomalies. Main criteria for the diagnosis of FPH are normal female karyotype, masculinization of external genitalia and no palpable testes. Even though a karyotype was not performed FPH was diagnosed due to the presence of ovarial tissue in connection with the male external genitalia.

Expression of alpha basic crystallin in canine mammary tumours.

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Introduction: Heat shock proteins (HSP) or stress proteins are the products of several distinct gene families, which play an essential role in the maintenance of cellular homoeostasis, under both physiological and stress conditions. Alpha basic-crystallin (αB-crystallin) belongs to a family of small HSPs, and is induced in response to different stresses including heat shock, oxidative stress, metal ions and cytokines. αB-crystallin has chaperone like properties, preventing aggregation of damaged or misfolded proteins induced by cell stress. The aim of this study was to evaluate the immunohistochemical expression of αB-crystallin in canine mammary tumours and to evaluate the role of this protein in the carcinogenesis of the mammary gland and establishing their potential prognostic and/or diagnostic implications for this common neoplastic condition of the dog.

Material and methods: Formalin-fixed, paraffin-embedded blocks of 51 (11 benign and 40 malignant) naturally-occurring canine mammary tumours and eight normal canine mammary gland tissues were used as control. Section from all the tissue samples were stained with H&E and additional sections were stained by streptavidin-biotin-complex-peroxidase technique using mouse monoclonal antibody anti-alpha basic crystallin. The percentages of the total area of the immunohistochemically positive cells were assessed by using a microscopy image analysis system. A total of 10 high-power fields was randomly chosen and analyzed at 400x magnification. The findings were categorized as follows: (0) no positively staining tumour cells; (1) 5-25%; (2) 26-50%; (3) >50% of tumour cells positive. Statistical analysis was carried out using Mann-Whitney U test. The results were considered statistically significant if the $P<0.05$. All data are ex-pressed as mean ± standard deviation.

Results: In the control mammary tissues a few luminal epithelial cells were positive, but myoepithelial cells were negative. In benign or malignant tumours of simple type, αB-crystallin expression was observed in luminal epithelial cells. The myoepithelial basal cells were negative. In benign or malignant tumours of complex type, positive staining was noted predominantly in the cytoplasm of epithelial cells and adjacent extracellular matrix. Immunoreactivity of αB-crystallin was also found in neoplastic myoepithelial cells. A positive signal of αB-crystallin mesenchymal elements in mixed tumours or carcinosarcomas, such as cartilage or bone, was not observed. Muscles were always positive and used as a positive internal control. Statistically, cell number of αB-crystallin immunolabelling was found significantly different among the normal canine mammary gland, benign and malignant tumour ($P<0.05$).

Discussion: αB-crystallin is a member of the mammalian small HSP superfamily. To our knowledge, the current report is the first to document a connection between αB-crystallin and mammary tumours in dog. The data obtained in the current study revealed a strong association between high expression levels of αB-crystallin and primary mammary gland tumours of the dog.

Marek's disease in broiler flocks of Tehran province, Iran.

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Introduction: Marek's disease (MD) is a common lymphoproliferative disease of chicken, usually characterized by mononuclear cellular infiltration in peripheral nerves and various other organs and tissues including iris and skin. Prior to the use of vaccines, MD constituted a serious economic threat to the poultry industry causing up to 60% mortality in layer flocks and 10% condemnation in broiler flocks. MD has been a common, important problem for poultry industry in our country, Iran. The objective of the present study was to have an evaluation of importance and incidence of MD (visceral - nerves and cutaneous forms) in broiler flocks of four regions (Savojbolagh, Karaj, Shahriar and Varamin) in the Tehran province.

Material and methods: During 35 visits of 4 poultry slaughter houses in the Tehran province, samples of tissues consisting of liver, spleen, skin, proventriculus and sciatic nerve of 80 broiler chicken, which had been suspected to be infected with Marek's disease at meat inspection, were collected. Tissue samples were processed to histological slides and examined for histopathological lesions.

Results: Gross and microscopic examinations revealed that 24 out of 80 flocks (30%) have been infected and showed different forms of the MD. The rate of incidence of cutaneous, visceral and mixed cutaneous and visceral MD forms in these regions (four regions) was determined as 16.2%, 3.8% and 10.0%, respectively. No cases of nerve and ocular forms were seen in this study.

Discussion: These data indicated that MD has a relatively high occurrence in broiler flocks of the four regions in the Tehran province. The cutaneous form of MD is the commonest form of the disease in these regions. Further confirmatory tests for association of Marek's disease virus (MDV) with tumour cells was suggested.

Preliminary observations on the experimental transmission of chronic wasting disease (CWD) from elk and white-tailed deer to fallow deer (Dama dama).

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Introduction: Chronic wasting disease (CWD) is a prion disease which has been experimentally transmitted by intracerebral inoculations to a variety of domestic, wild and laboratory animal species. CWD has not been reported in fallow deer (Dama dama).

Material and methods: Thirteen fallow deer fawns were inoculated intracerebrally with CWD brain suspension from elk (n = 6) or white-tailed deer (n = 7). Three other fawns were kept as uninfected controls. This communication documents the first 4 years into the experiment.

Results: Three CWD-inoculated deer were euthanized at 7.6 months post inoculation (MPI). None revealed presence of abnormal prion protein (PrPd) in their tissues. At 24 and 26 MPI one sick deer died and one non-clinical deer was euthanized, respectively. Both animals had a small focal accumulation of PrPd in their midbrains. Between 29 and 37 MPI, three other deer became sick and were euthanized. All had shown gradual decrease in appetite and some loss of body weight. Microscopic lesions of spongiform encephalopathy were not observed but PrPd was detected in tissues of the central nervous system by immunohistochemistry, Western blot and by two commercial rapid tests.

Discussion: This study demonstrates that intracerebrally inoculated fallow deer amplified CWD PrPd from white tailed-deer and elk in absence of SE lesions. Similar observations have also been shown to occur in cattle inoculated with the scrapie and CWD agents; however, PrPd amplification in fallow deer was minimal in comparison to scrapie- and CWD-affected cattle. Four years after the CWD inoculation, the remaining five inoculated and two control deer are alive and apparently healthy. Although these preliminary findings demonstrate that it is possible to transmit CWD to fallow deer by intracerebral inoculation, past experience with TSE cross-species transmission studies indicate a low probability for CWD to develop following oral transmission to fallow deer in the species' normal life span.

Naturally occurring old dog encephalitis from Brazil: confirmation of canine distemper virus involvement by molecular biology.

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Introduction: Canine distemper virus (CDV) is a morbillivirus that produces encephalitis in dogs and other susceptible animals. Neuropathological CDV syndromes in dogs are related to viral strain, age at infection, and the anatomic location of the neurological lesions. These syndromes include: distemper encephalitis in immature dogs, multifocal encephalomyelitis in mature animals, and old dog encephalitis (ODE). Naturally occurring cases of ODE have not been described recently; therefore we present a case of spontaneous ODE in a dog from Brazil.

Material and methods: A 7-year-old, female, miniature Schnauzer dog who had been vaccinated against distemper as a pup, and showed a history of progressive behavioral changes, motor incoordination, and compulsive walking, was admitted to the Veterinary Teaching Hospital, Universidade Estadual de Londrina, Brazil; the animal's state detoriated and it was euthanized. Necropsy was performed soon after death; tissues were fixed in 10% buffered formaldehyde solution and routinely proces-sed for histopathological evaluation. Selected formalin-fixed and paraffin-embedded tissues were used for molecular detection of CDV by RT-PCR assay. RT-PCR was performed using the primers CDV1 and CDV2, specifically designed to amplify an amplicon of 287 bp of the CDV nucleoprotein (NP) gene; tissue from a case of acute distemper encephalitis served as positive control tissue, and from a healthy dog as negative control.

Results: Histological alterations were restricted to the brain and the lungs; all other tissues were normal. Nervous lesions were confined to the cerebrum; the cerebellum and brain stem were spared. Neurological alterations included extensive perivascular cuffs involving the white and grey matter, astrocytosis, astrogliosis, discrete demyelination and neuronal necrosis, and rare intranuclear eosinophilic inclusion bodies in astrocytes. Pulmonary lesions were characterized by oedema, haemorrhage, and emphysema. The RT-PCR amplified an amplicon of 287 bp size from fresh and formalin-fixed brain samples. Molecular analysis revealed a 287 bp CDV-specific amplicon; nucleotide sequences have been deposited in GenBank (Accession No EF197736).

Discussion: The diagnosis of ODE was based on characteristic cerebral lesions associated with the absence of CDV-induced alterations in other tissues. These findings are consistent with those previously described in naturally occurring and experimentally induced cases of ODE. Involvement of CDV was confirmed by RT-PCR amplification of amplicons of the NP gene of the virus. To the authors' knowledge, this case represents the first description of CDV-induced ODE in a Brazilian dog, and is the first molecular characterization of CDV with typical ODE lesions. These findings also suggest that CDV may participate actively in the aetiology of ODE.

Immunohistochemical characterization of N. helminthoeca in Brazilian dogs.

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Introduction: Neorickettsia helminthoeca is the cause of salmon poisoning disease (SPD) that is endemic in specific geographical regions of the United States. However, during 2001 - 2005, 20 dogs of both sexes (5 mongrels; 15 beagles) with lesions consistent with this infection were observed in Maringá, Paraná, Southern Brazil. Additionally, intestinal tissue from one of these was positive by three different genes by PCR. Further, by cloning, sequencing, and phlyogenetic analyses an organism that was very similar to N. helminthoeca was identified, and consequently named N. helminthoeca Maringá strain. This paper presents the immunohistochemical (IHC) findings of N. helminthoeca in new cases of SPD in Brazilian dogs.

Material and methods: During 2006, five 2-year-old Beagles (all males), from the same geographic area in Southern Brazil, demonstrated lesions consistent with SPD. Necropsy was performed soon after death; tissues were routinely fixed for histopathological evaluation. Selected formalin-fixed-paraffin-embedded sections were processed for IHC analysis. IHC using the streptavidin-biotin-complex technique was done in an automated detection system. Anti-N. helminthoeca antiserum (1:100 dilution) produced in two immunized rabbits served as the primary antibody. Pure cultures of N. helminthoeca that were grown and maintained in DH82 cells until more than 80% of cells were infected and consequently fixed in an agarose block, served as positive control. For negative control the primary antibody (rabbit serum) was substituted with PBS during the reaction. Rabbit immunization and production of N. helminthoeca were done at the Johns Hopkins Medical Institutions, USA; while IHC staining and analyses were done at the University of Helsinki, Finland.

Results: Immunohistochemical staining was uniform throughout all tissues, but background staining affected distinct visualization of intracellular bacterium. Nevertheless, antigens of N. helminthoeca were identified within the cytoplasm of reticuloendothelial cells of the intestine and lymph nodes of all dogs. These intracytoplasmic bodies are similar to those observed in tissues of dogs found to contain N. helminthoeca DNA by PCR and also found to contain bacteria morphologically consistent with N. helminthoeca by Giemsa stains. The negative control remained unstained; positive control revealed similar results as described in the dogs.

Discussion: This is the first successful demonstration of N. helminthoeca antigens in canine tissues by IHC. Although there was undesired background staining, intracytoplasmic bodies consistent with this organism were identified in target cells; further optimization of the method, including purification of the primary antibody should improve detection and recognition of the distribution of the pathogen in canine tissues. In Brazil, the prevalence of N. helminthoeca within the canine population is unknown, so this method, in addition to a serological study, is being implemented to evaluate the degree of infection in urban populations and may provide the tools needed to evaluate the bacterium's impact on canine health.

Colorectal cap polyposis in a dog.

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Introduction: Cap polyposis is a rare, unique, human benign colorectal disease described in 1985 by Williams et al.. It has distinctive clinicopathological features. Never described in veterinary medicine, cap polyposis is here presented in a five-year-old Bearded Collie.

Material and methods: The dog presented dyschezia, tenesmus and mucoid faeces with blood traces fro an unknown period. A rectal touch revealed a ring of polypoid masses 5 cm from the anus. The faecal examination was unremarkable. Diet restriction, metronidazole and spasmolytics had been administrated without improvement. At endoscopy, multiple sessile, 2 to 6 mm, red polyps were observed, located at the apices of enlarged transverse mucosal folds, covered by normal intervening mucosa. They were covered by an erythematous to whitish fibrinous cap.
A colostomy and routine histological preparation of the surgical specimen was performed. A transient improvement followed the surgery, but the patient died of unknown cause seven days later.

Results: The histopathological examination of the surgical specimen revealed multiple polypoid expansions on the surface of the mucosa. These polypoid structures were lined by monostratified cuboidal to flattened epithelium, identical to the normal glandular structure. The superficial portion of the polyps was often ulcerated and covered by a cap of mucoid and fibrinopurulent exudate. The core of the polyp was oedematous and infiltrated by proliferated vessels and by leukocytes. Occasionally, osseous metaplasia occurred. The underlying mucosa showed interstitial lymphoplasmacytic infiltration, mucous hypersecretion and gland dilatation, with occasional microcyst formation.

Discussion: In human patients, the main clinical presentation of cap polyposis is rectal bleeding (80%) that can be associated with mucous diarrhoea (50%), chronic straining of stool and constipation, abdominal pain and tenesmus. Mucous diarrhoea can be severe enough to induce a protein loosing enteropathy. Polyps may be palpable on digital rectal examination. The commonest site of involvement is the lower rectum but it may involve also the sigmoid and the transverse colon.
The pathogenesis is unknown. Abnormal colonic motility leading to mucosal prolapse may be an important cause. Helicobacter pylori have been associated in some cases, but no other infectious agent. Tumour necrosis factor-alpha and inflammatory processes may play a role. The treatment of this condition remains empiric. Metronidazole and steroids have been effective in some cases, Infliximab, an antibody directed toward tumour necrosis factor, in others. But in resistant cases polypectomy, recto sigmoid resection or panproctocolectomy may be required to control the diarrhoea. Rare cases of spontaneous resolution have been observed.

Tuberculosis in three Squirrel Monkeys (Saimiri scireus) caused by Mycobacteria typical for voles.

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Introduction: Three squirrel monkeys from a local zoo were presented for necropsy, two of them showing signs of a consumptive disease with involvement of the lung and one reported to drag the rear legs. All animals were wild born individuals imported from French Guiana several years ago. Due to the poor condition the animals were euthanized.

Material and methods: Necropsy was performed and fixed tissue specimens were prepared for histology by standard protocol. To detect acid-fast bacilli the slides were additionally stained with Ziehl-Neelsen stain. Further organ material was routinely preserved for microbiology. Mycobacteria were cultivated from different organs and further characterized by molecular biology. PCR was performed to assure the affiliation of the isolates to the Mycobacterium tuberculosis complex. Spoligotyping (spacer-oligo-typing) was carried out in order to discriminate the different members of the complex.

Results: In the animals showing weakness and signs of pulmonary disease, there were granulomas scattered within the parenchyma of the lung. Histologically, these lesions showed the typical structure of tuberculous granulomas, characterized by central necrosis with fibrin and degenerated neutrophils surrounded by epithelioid macrophages, large numbers of lymphocytes and neutrophils, small numbers of plasma cells and occasional multinucleated giant cells, predominantly of the foreign body type but some also of the Langhans type. The granulomas were surrounded by a peripheral rim of fibrous tissue. The majority of the nodules in one of the animals showed a more pyogranulomatous component, characterized by higher numbers of neutrophils and broader areas of necrosis. The latter animal showed no other lesions besides moderate acute enteritis. In the second animal, nodular lesions could also be found in liver, kidneys, spleen, mesenteric lymph nodes and mesenterium. The animal dragging the rear legs showed a fracture of the thirteenth thoracic vertebra. The spinal cord in this area was compressed and showed extensive haemorrhages. Additionally, there was an abscess-like excavation dorsal of the seventh cervical and first thoracic vertebra with signs of inflammation of the surrounding muscles. Nodular lesions similar to those described in the other two monkeys were present in mesenteric and mediastinal lymph nodes. Furthermore, an erosive to ulcerative gastritis as well as a small circumscribed haemorrhage medio-dorsal of the right knee could be found. Histologically, a pyogranulomatous inflammation was present in the muscles dorsal of the last cervical and first thoracic vertebra. Lymph nodes showed tuberculous granulomas as described above. Ziehl-Neelsen stain of the granulomas revealed acid-fast bacilli in the central necrosis and in the cytoplasm of the surrounding macrophages. PCR verified the affiliation of the isolates to the Mycobacterium tuberculosis complex and spoligotyping identified the isolates as Mycobacterium microti.

Discussion: M. microti belongs to the M. tuberculosis complex and is usually found in small rodents causing the so called “vole tuberculosis”. It is reported to produce sporadic tuberculosis in a variety of other species including domestic animals as well as humans. Here we report the first case of an infection of squirrel monkeys with M. microti, but it remains unclear if this infection was imported from French Guiana with delayed manifestation of the disease or if the animals were infected by contact with infected rodents in the zoo.

Clinical and pathomorphological characterization of diabetic mice generated in the Munich ENU mouse mutagenesis screen.

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Introduction: N-ethyl-N-nitrosurea (ENU) has been used in various mouse mutagenesis programs for the production of random mutations.

Material and methods: Three diabetic mutant mouse lines, generated in the Munich ENU mouse mutagenesis screen, were used for clinical and pathomorphological investigations. Further, linkage analyses for chromosomal localization of the mutation were performed.

Results: Linkage analysis of the line GLS001 revealed a strong linkage of the mutation to a polymorph marker on chromosome 11. Sequence analyses of the glucokinase gene, which is lying in this chromosomal region, are currently being performed. GLS004 mice exhibit a T to A transversion in the insulin 2 gene (Ins2) at nucleotide position 1903 in exon 3, which leads to the amino acid exchange C95S and loss of the A6-A11 intrachain disulfide bond. According to the mutation this line was named Munich Ins2C95S. From 1 month of age onwards, heterozygous Munich Ins2C95S mutant mice showed overt diabetes mellitus. The fasted and postprandial serum insulin levels of the heterozygous mutants were indistinguishable from those of wild-type littermates. However, serum insulin levels after glucose challenge were largely reduced, indicating disturbed first phase insulin secretion. Pancreatic insulin content and homoeostasis model assessment (HOMA) of beta cell function index of heterozygous mutants was significantly lower than those of wild-type littermates. Initial blood glucose decrease during insulin tolerance test was lower and HOMA of insulin resistance index was significantly higher in mutant mice, demonstrating a dominant negative mechanism of diabetes development in mutant mice. The total islet volume, the volume density of beta cells in the islets and the total beta cell volume of heterozygous male mutants was significantly reduced, as compared to wild-type mice. Electron microscopy of the beta cells of male mutants showed signs of glucotoxicity: virtually no secretory insulin granules were visible, the endoplasmic reticulum was severely enlarged and mitochondria appeared swollen.

Discussion: Munich Ins2C95S mutant mice are the first in vivo model for studying the consequences of the expression of a mutated Ins2C95S on beta cell function and survival. This novel diabetic animal model represents an excellent tool for studying the mechanisms of beta cell dysfunction and death, and for therapeutic intervention studies.

A case of rhinosporidiosis in a horse in Germany.

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Introduction: In this case of rhinosporidiosis in a 17-year-old mare (polopony), the owner noticed noisy breathing and, on inquiring, found an approximately 2x2x3 cm sized mass, originating from the left inner nostril, that could not have been growing there for longer than a month. The lump was excised and sent to the Institute of Veterinary Pathology, Justus-Liebig-University, Giessen, in April 2007.
The mare had been imported from Argentina 8 years before and has not left Germany since. It had been used in polo sports for four years, then been sold to its present owners. Since then there is evidence of regular vaccination and deworming. Except for a hoof abscess in October 2006, no affection has been reported.

Material and methods: The diagnosis was confirmed by means of histologic morphology using H&E stain and special staining (Grocott, PAS, Mucicarmin).

Results: The histologic morphology of the causative agent found in the mares nasal tissue is consistent with that of Rhinosporidium seeberi as it is described in the literature.

Discussion: Rhinosporidiosis is an uncommon disease in horses caused by Rhinosporidium seeberi. This organism belongs to a new clade of fish and amphibian parasites called Mesomycetozoa, which is settled at the lowest phylogenetic branch of the kingdom Animalia, between the animal-fungal divergence.
Apart from some sporadic cases in Europe (Germany, UK), the USA and Canada (mule) there are only two areas that could be regarded endemic for rhinosporidiosis in horses. In Rio Grande do Sul, a state of the Southern part of Brazil, 11 cases of rhinosporidiosis in horses and two in mules have been reported between 1941 and 1976. In the East Center area of the Chaco province, Argentina, which is situated in the same latitude as Rio Grande do Sul, 33 cases of rhinosporidiosis in horses have been reported until 1964 (period of time unknown).
Interestingly, the only known cases of rhinosporidiosis in horses in Europe (Germany 1979, UK 2007, presented case) all occurred in poloponys imported from Argentina. In this case, unlike the other two, where the horses had left Argentina only recently, the surprising fact is, that the mare came from Argentina 8 years ago and has not ever left Germany since. Recent findings in the study of rhinosporidiosis indicate, that humoral immunitiy is not sufficient to prevent the disease. Cellular response in humans decreases during the course of the disease.
The meaning of immune response for the late onset of the disease at least 8 years after infection remains to be elucidated in this case.

Diagnosis of sarcomatoid transformation of cholangiocarcinoma in a cross breed dog.

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Introduction: Here we present a case report of sarcomatoid (undifferentiated) cholangiocarcinoma (CCA) in a 12-year-old male mixed breed dog. Immunohistochemical analysis was performed using stem cell marker CD34, epithelial marker cytokeratin19 and muscle marker desmin. This pattern of tumour and cell morphology has not been reported in veterinary science yet.

Material and methods: H&E and IHC staining technique: sections from formalin-fixed, paraffin-embedded tissues were cut in 5.0 μm sections. After drying and incubation in a 60°C convection for 30 to 60 min, slides were deparaffinized in xylene, and hydrated through a series of graded alcohol to distilled water. Sections were stained with H&E other sections were transferred to APK buffer. In an automated immunohistochemistry stainer antibodies against cytokeratin 19, desmin, and CD34 were applied.

Results: At necropsy, there were more than 1000 mL of serosanguineous ascites. The liver weighed 1,350 g. An estimated 55% of the liver, 20% of the kidneys, 45% of the lungs parenchyma were replaced by multiple, diffuse tumour nodules. The right liver contained the largest mass (9.0 x 11.0 x 4.50 cm). Large soft tan masses contained firmer gray-tan nodules, areas of necrosis. Histologically, the tumour was centrally haemorrhagic and necrotic and was composed of an adenocarcinoma with trabecular pattern and a round-oval cell component, which had a hyperchromatic round-oval centrically located nucleus and basophilic cytoplasm without mucin production. Numerous mitotic figures were not observed and there was no capsule formation.
Immunohistochemically, the adenocarcinoma cells expressed cytokeratin 19. Desmin was not detected in their cytoplasm and staining for CD34 was also negative.

Discussion: Dissemination of cholangiocarcinoma has been reported. However, sarcomatoid transformation of cholangiocarcinoma has not been reported previously, to our knowledge. The benefit of IHC staining is not only, that it creates a marked contrast difference between normal cells and cells positive for the immunostain, increasing the pathologists' ability to detect micrometastases, but that it can distinguish undifferentiated carcinomas of bile duct and gallbladder, which are difficult tumours to treat with curative resection, from sarcomas. Nevertheless, complete surgical resection remains the mainstay of the treatment.

Cryptosporidium saurophilum infection in Leopard Geckos (Eublepharis macularius).

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Introduction: Cryptosporidiosis is one of the most frequent intestinal protozoal parasitoses observed in breeding colonies of Leopard Geckos. For the majority of affected geckos the infection is fatal as they develop a chronic proliferative enteritis, leading to cachexia and death. Since therapy is protracted and often unsuccessful, cryptosporidiosis has severe economical impact on breeding colonies worldwide.

Material and methods: A group of 30 subadult and adult Leopard Geckos (Eublepharis macularius), naturally infected with Cryptosporidium (C.) saurophilum was investigated by light, scanning and transmission electron microscopy. Species determination for C. saurophilum was achieved by nested PCR, detecting a 830bp segment of the small sub-unit ribosomal RNA gene (SSU rRNA gene), followed by sequencing.

Results: The body condition of infected geckos ranged from mild emaciation to cachexia. Severely affected geckos exhibited ascites, hepatic atrophy and diffuse, mild to moderate thickening of the intestinal wall. Cryptosporidial enterocytic infection was seen in small intestine, large intestine and cloaca, causing a mild to moderate, diffuse, proliferative and lympho-plasmacytic enteritis. The rate of cryptosporidial infection increased from the proximal to the middle part of the small intestine and decreased in the large intestine and cloaca. Ultrastructurally, different developmental stages, e.g. sporozoites, trophozoites, meronts, merozoites, macrogamonts and oocyts of C. saurophilum were demonstrated within the small intestine. Apart from mild hypertrophy, a moderately increased mitotic rate was observed within enterocytes of the small intestine.

Discussion: Intestinal infection of Leopard geckos with C. saurophilum causes chronic, proliferative enteritis with consecutive emaciation / cachexia. The predominant intestinal site of cryptosporidial infestation is the middle part of the small intestine, whereas the large intestine and cloaca are minor sites of enterocytic infection. The cloacal infection suggests that the egg shells could become infected during the egg laying process. This issue needs to be adressed in further experimental studies.

Comparative pathological study of the CNS during experimental infection with classical rabies and European bat lyssavirus in mice.

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Introduction: Rabies virus causes severe encephalitis that is invariably fatal for the victim. The extent of the inflammatory response in the central nervous system (CNS) is highly variable, depending on the virus strain and host. As the roles of the virus and the host´s immune response in the degradation of the central nervous system have yet to be fully characterised, we have studied lymphocyte recruitment, in a murine model, after infection with wild type rabies virus (wtRABV), European bat lyssavirus type 1 (EBLV-1), or European bat lyssavirus type 2 (EBLV-2).

Material and methods: OF-1 mice were peripherally inoculated (foot pad) with virus culture supernatant of either wtRABV isolated from a human bitten by a dog in India (1987), EBLV-1 isolated from a naturally infected Serotine bat from Germany (1997), or EBLV-2, isolated from a naturally infected Daubenton bat in the United Kingdom (2002). After developing clinical signs the mice were culled and samples from the CNS were fixed and routinely processed for histopathology. The number, severity and distribution of perivascular cuffing among the different brain regions was assessed. Immunohistochemistry was used to detect lyssavirus viral antigen, CD3 T lymphocytes and CD45/R220 B lymphocytes.

Results: Inoculated animals developed classical signs of lyssavirus infection, minimal to severe non-suppurative encephalitis and viral antigen was detected by immunohistochemistry. The severity of the histological lesions was more prominent in EBLV-2 inoculated animals and almost absent in wtRABV infected mice. In all three genotypes the highest number of perivascular cuffing occurred in pons and medulla, being more numerous and composed of a higher number of cells in EBLV-2 infection. Despite widespread antigen detection throughout the brain no correlation was found corresponding to the level of cuffing in the infected regions. Focal and diffuse gliosis, mainly associated to areas containing perivascular cuffing, was also more prominent in EBLV-2 infected animal. The lymphocytic component of the perivascular cuffs and the infiltrates in grey and white matter were almnost exclusively composed of T cells.

Discussion: A significant difference in the severity of pathological changes was observed when wtRABV (genotype 1) was compared with EBLV, particularly EBLV-2, infection. Differences in viral properties may be responsible for an increased immune awareness to EBLV-2 virus resulting in T cell occurrence in the perivascular spaces, which, if activated, can proceed into the parenchyma. A potential by-product of this recrutiment may be an increase in the permeability of the blood-brain-barrier, which may play a protective role in abortive rabies virus infection. However, despite this genotype related difference in inflammatory response the infection is still ultimately fatal.

Comparative Genomic Hybridization (CGH) analysis of radiation induced thyroid tumours in a mouse model.

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Introduction: Thyroid cancer is the most common endocrine malignancy in man. It is broadly subclassified into follicular and papillary thyroid carcinoma (FTC and PTC). The association of the PTC with a history of irradiation is well documented (e. g. Chernobyl nuclear accident in 1986). Although rearrangements of the ret-oncogene (so called ret/PTC-rearrangements) are strongly correlated with the human PTC, further genes are supposed to be essentially involved in the thyroid tumourigenesis. The genetic mechanisms of human FTC remain widely unknown. In this study, a mouse model of radiation-induced thyroid tumours was used to perform high resolution genome-wide array-CGH (comparative genomic hybridization) analysis, with the aim to analyze in a comprehensive way genetic changes and to gain insight into the carcinogenetic mechanisms.

Material and methods: The murine tumours have been induced with an injection of radioiodine (I131) into iodine-deficient fed mother mice. The DNA was isolated from the formalin-fixed paraffin-embedded (FFPE) thyroid tissue derived from 21 different mice (25 samples): two normal thyroid glands, 13 goiters, two follicular thyroid adenomas (FTA), and eight FTC. The analysis was done using a 1-Megabase BAC (bacterial artificial chromosome) array-CGH.

Results: The tumours showed a broad spectrum of chromosomal alterations. In 46 % of the hyperplasias a variety of different small chromosomal gains and losses (between 0.5 and 119 Mb) was observed. Regional polyploidies on the chromosomes 4 and 5 (about 20 Mb) were demonstrated in an FTA. In contrast, the FTCs exhibited frequent partial or complete losses of the chromosomes 4 (p16) (88 %), 9 (50 %), and 14 (Rb1) (38 %).

Discussion: The extent of genetic aberrations correlates well with the histological phenotype of the lesion: Hyperplasias showed only inconspicuous and infrequent gains and losses, which could represent a genetic instability, predisposing to malignant transformation. The polyploid chromosomal changes of the FTA may be a hint for the location of oncogenes. Interestingly, aneuploidies could be demonstrated to be characteristics of the murine FTC, harboring similarity to the human counterpart. In conclusion, aneuploidy is supposed to play a crucial role in thyroid tumour initiation and/or progression in mice. The tumorsuppressor genes p16 (chromosome 4) and Rb1 (chromosome 14) are putative candidates.

Chronic nephropathy in guinea pigs, pathological classification and correlation with changes in haematological and urine parameters.

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Introduction: Chronic nephropathy is a common, often incidental, finding at post mortem examination of guinea pigs in both the experimental and pet population. These lesions seem to occur more often in older animals. However, not much is known of the pathogenesis or the impact the lesions have on the individual. Only few studies have described and characterised the lesions in detail. To our knowledge no other studies have examined, if a correlation between the macroscopic and histological findings and changes in blood and urine parameters exists. In the current study, kidneys from adult guinea pigs were examined and classified into four categories according to macroscopic severity. Correlation between severity and age, histological changes and selected blood and urine parameters were made.

Material and methods: Kidneys of 19 adult guinea pigs were classified into four groups according to severity of macroscopical changes. Prior to euthanasia urine samples and blood samples were taken on the anaesthetized animal. At post mortem examination, samples for histological evaluation were taken. The main histological changes (interstitial fibrosis, infiltration with lymphocytes, tubular and glomerular changes, degree of mineralization and presence of hyaline casts in tubules) were graded semi-quantitatively. Blood samples were examined for blood urea nitrogen (BUN) and creatinine. Specific gravity and pH were measured on urine samples and examination for blood, glucose, bilirubin, ketones and protein was done.

Results: Of the examined kidneys 74 % showed lesions and 43% of these were severe. Lesions were often bilateral. The histological evaluation showed a good agreement between the macroscopic grading and the grading of the different histological changes. No significant correlation between BUN or creatinin and the severity of kidney lesions was found. Urine specific gravity varied between 1.004 and 1.048 g/ml and pH measurements were 8.0 or 8.5 with no significant difference between animals or the groups based on lesion severity. All urine samples were negative for glucose and bilirubin and only in one animal trace levels of ketones were found. Protein content varied without significant correlation to the severity of kidney lesions. Blood was found in many samples but with no significant correlation to the degree of kidney lesions. Also no correlation was seen between the severity of lesions and the age.

Discussion: Macroscopic lesions can be used to classify chronic nephropathy into four categories with a good agreement between macroscopical lesions and microscopical lesions. The number of animals with kidney lesions was high, but no correlation was found between increasing age and severity. Correlations between the degree of lesions in the kidneys and the haematological and urine parameters were not significant. Only the level of BUN showed a slight tendency to increase with severity of lesions. The frequent blood content in the urine may be related to the sampling method. These findings might indicate that kidneys in the guinea pig have a large capacity to compensate even severely pathological changes.

Presence of missense mutations in p53 gene of selected canine skin tumours.

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Introduction: The aim of the study was the histological and immunohistochemical diagnostics of selected canine skin tumours with detailed epidemiological characterization and molecular analysis of canine p53 gene.

Material and methods: Studies were performed on 135 canine skin tumours using conventional histological methods and a range of commercially available antibodies to recognise the morphology and histogenesis of these tumours. Moreover, for detection of point mutations in exons 5-8 of the p53 gene PCR amplification and temperature gradient gel electrophoresis (TGGE) were used.

Results: Mixed breeds, Boxer and German shepherd were the races most predisposed to develop skin tumours. A significant age predilection was observed in dogs below the first year of their life and in 11-years-old dogs. The most common sites of the body for neoplasia development, in decreasing order, were head, legs and pelvis. The most prevalent skin tumours origin was mesenchymal with 55.6%, while tumours of epithelial origin occurred in 40.0% and those of melanocytic origin in 4.4% of all tumours. The most frequent groups of skin neoplasms were tumours with adnexal differentiation, histiocytomas and mast cell tumours.

During molecular analysis of exons 5-8 of p53 gene, only two cases of point mutation were detected: in well differentiated squamous cell carcinoma and apocrine gland adenocarcoma. Both cases revealed missense mutations in exon 8. A mutation CGC>CAC (arginine>histidine) was detected in codon 282 in the apocrine gland adenocarcinoma and a mutation GAG>TAG (glutamic acid>stop codon) in codon 294 in the well differentiated squamous cell carcinoma.

Discussion: The study showed that the TGGE method can be very useful in an efficient screening of large amounts of tumour DNA samples for mutations and it can be an alternative to laborious and expensive sequencing of the entire p53 gene. TGGE also allows to differentiate homozygous alleles from heterozygous ones.
The type of mutation of exon 8 we found in the case of canine apocrine gland adenoma was not described before based on the available literature.

Perianal (hepatoid) gland hyperplasia due to acariasis of perianal region.

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Introduction: In September 1995, a 3-year-old cross breed male dog with erythematous and erythematosquamous papules which showed itching and particularly affected the face, abdomen, legs, buttocks, and also perianal area was referred to a veterinary clinic. Many of the symptoms of mite infestation are due to an allergic reaction towards the parasites. Infestations may be more severe in debilitated or immunosuppressed hosts. Inflammatory cytokines and growth factors can induce hepatoid gland hyperplasia. In this case of infestation with sarcoptic manges, acute purulent eosinophilic inflammation occurred. The incubation period for S. scabiei var. canis in dogs is 10 days to 8 weeks. Pigs experimentally infected with S. scabiei var. suis become symptomatic in 2 to 11 weeks. Most of the symptoms of sarcoptic mange are caused by allergic reactions towards the parasite, and the incubation period is generally longer the first time an animal is infested by the parasite.

Material and methods: Formalin-fixed, paraffin-embedded sections from selected blocks were cut in 5.0 μm sections. After drying and incubation in a 60°C convection for 30 to 60 min, slides were deparaffinized in xylene, hydrated through a series of graded alcohol to distilled water and stained with H&E.

Results: The lesions were found on the ventral chest and abdomen. Other common locations are the ears, periorbital region, elbows, legs and perianal area. The typical lesion is an intensely pruritic papular rash with thick yellowish crusts. Scratching and rubbing led to a variety of lesions, including erythema, ulcers, bleeding and haemorrhagic crusts due to secondary bacterial infections. Emaciation and peripheral lymphadenopathy can be seen.
The ulcerative hyperplasic perianal region was removed by surgery. Histological examination revealed a slightly acanthotic epidermis, perianal pustules, severe purulent cellulitis, syringitis and hepatoid gland adenitis. Hyperplasia of hepatoid perianal glands also occurred. A mite of scabies could be seen in a burrow within the epidermal cornified layer. In the superficial dermis, there was a perivascular inflammatory infiltrate of mixed cell type with polymorphonuclear cells, mainly neutrophils, eosinophils and also mononuclears such as lymphocytes, plasma cells and macrophages. Tracking cellulitis, fistulated discharging abscesses and deep suppurative wounds which were subject to secondary cutaneous infection were seen.

Discussion: In classic scabies, the lesions are few and roughly symmetrical. These are frequently eczematous and occur in the elbows and the axillary folds. Other typical locations are female mammary glands, the area around the umbilicus, penis and the lower portion of the buttocks. However, we are reporting a special form of scabies resulting in atypical locations and an unusual extension of the disease to the perianal region. Thus, it can masquerade as psoriasiform dermatosis, contact dermatitis, bullous pemphigoid, malignant lymphoma, and systemic or cutaneous lupus erythematosus, among other entities.

Paraneoplastic dermatitis in one cat.

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Introduction: In addition to direct effects, tumours may cause a variety of systemic clinical signs termed paraneoplastic syndromes. Dermatological manifestations of systemic diseases are starting to be documented in the cat. Héripet (1999) enumerated the following paraneoplastic dermatological diseases: Pancreatic paraneoplastic alopecia, superficial necrolytic dermatitis, acquired cutaneous hyperfragility syndrome, paraneoplastic exfoliative dermatitis, paraneoplastic pruritus and degenerative mucinous lymphocytic mural foliculitis.

Material and methods: Short-hair domestic cat, spayed female, 10 years of age. Euthanasia by intravenously administered T 61 injection, usual autopsy examination. Histology: Formaldehyde-fixed and paraffin-embedded tissue, staining methods: H&E, trichrome, modified van Gieson, Berlin's blue, PAS reaction, silver impregnation after Gömöri. Immunohistochemistry: Binding of antibodies for identification antigens CD 3, CD 79αcy, Ki 67, chromogranin A, epithelial membrane antigen, neuron-specific enolase, p 53 protein, S 100 protein, and synaptophysin. All the antibodies came from DakoCytomation, Glostrup, Denmark and their binding was identified by Universal DakoCytomation LSAB 2 System, alkaline phosphatase and fast red substrate-chromogen system (DakoCytomation, Glostrup, Denmark).

Results: Clinical examination: Foci of ulcerative dermatitis were disseminated on almost all parts of the body. Bullous formations developed in the back region and at the base of the tail. Due to progressive deterioration of general health state the cat was euthanatized six weeks after the onset of clinical symptoms. Gross pathology: No lesions were found in the oral cavity and cervical organs. A solid, lobulated tumour of yellowish-gray colour, 4.5 x 3 x 3 cm of size, was located on the omentum and was connected with the pancreas. Numerous, small, slightly light-coloured foci were visible in the pancreas. Moderate venostasis was observed in the lung, liver and renal medulla. Remaining internal organs were free of pathological lesions. Histopathology: Skin: Segments of irregular hyperplasia of the epidermis with akanthosis, hyperkeratosis both ortho- and parakeratotic types, represent typical lesions. Vacuolation of some cells in the spinal layer or segments of oedema and necrosis of epithelium in the stratum spinosum and the stratum granulosum were often observed. Disseminated foci of mixed inflammatory cell infiltrates were frequently present in the corium. Tumour: Differentiated adenocarcinoma of the exocrine pancreas was diagnosed by means of histology and immunohistochemistry, in the tumorous formation located on the omentum and in the pancreas. In the interstitium of neoplastic and normal pancreatic tissues, were numerous strips or foci of lymphocytes. Immunohistochemical examination of the lymphocyte subsets did not lead to reliable results. Liver: Dilatation of sinusoids, compression of the hepatocytes in central and middle zones of the lobules and haemosiderin deposits in cytoplasm of the hepatocytes and Kupffer cells was diagnosed.

Discussion: This case report demonstrates paraneoplastic dermatitis of superficial necrolytic dermatitis nature caused by differentiated adenocarcinoma of the exocrine pancreas. Contribution of the liver alteration to the development of the cutaneous lesions cannot be excluded.

Papillomavirus-associated squamous cell carcinomas in two Iberian lynxes (Lynx pardinus).

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Introduction: The Iberian lynx is the most endangered wild felid in the world. There are less than 200 animals left divided in two separate isolated populations, one in the Doñana National Park area and the other in Sierra Morena (Spain). The remaining populations show signs of inbreeding. Some studies have reported the presence of tuberculosis and parasitic infections. A generalized immune depletion and membranous glomerulonephritis have also been indicated in this species.

Material and methods: We report the presence of squamous cell carcinomas in the skin of two Iberian lynxes (male and female, of 11 and 14 years of age, respectively). In one case the tumour was multicentric (face, ears and forelimb), in the other one it was located only in the ear. Tissue samples of the tumours were obtained at necropsy and routinely processed for H&E and immunohistochemistry for the detection of papillomavirus.

Results: The histology revealed squamous cell carcinoma in the two animals. The tumours arose from an in situ squamous cell carcinoma similar to what is seen in the feline Bowenoid in situ squamous cell carcinoma (BISC). The presence of papillomavirus inclusion bodies was detected with immunohistochemistry in both tumours.

Discussion: Papillomaviruses have been reported in many wild felids. A relation between the presence of this virus and BISC/squamous cell carcinomas has been noted in the cat and in the snow leopard (Uncia uncia). It is also known that papillomavirus induces lesions in immunodepressed cats and humans. This is the first report of papillomavirus-associated squamous cell carcinomas in the Iberian lynx. The detection of papillomavirus indicates, that they are viral induced as occurs in other species, including wild felids and the domestic cat.

Accumulation of PrP deposits in the pregnant uterus of ewes naturally infected with scrapie.

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Introduction: Ovine placenta is an important source of contamination with prions (PrP), a causative agent of scrapie in sheep. It has been shown, that maternal infection of the lamb is determined by the genotype of the foetus and not the maternal one, however, not many data are available about the presence and distribution of prions in the pregnant and non-pregnant uterus in relation to the age of the sheep and the number of lambings.

Material and methods: Uteruses were collected from 36 sheep genetically susceptible for scrapie (VRQ/ARQ genotype) from a flock naturally infected with classical scrapie. Only sheep that reacted positive for scrapie in rapid post-mortem test were included in the study. Sheep were Suffolk-cross with domestic breeds. Birth data were not available and age was determined by the status of teeth ranging from approximately 18 months up to more than 4 years. The pregnant uterus horn was opened and placenta and embryo exposed to fixation by immersion into 4 % buffered formaldehyde prior to paraffin embedding. PrP reactivity and localisation were detected with immunohistochemistry.

Results: 30 sheep were determined as pregnant during necropsy and 6 as non-pregnant. All pregnant sheep were in the early pregnancy. 18 months old sheep were evaluated to be pregnant for the first time. Presence of PrP immunoreactivity was evident unevenly distributed within placental trophoblasts in all age groups and also within the uterine glandular epithelium. Different levels of immunoreactivity were found in 30.6 % of uteruses, 25 % of placentas. 50 % of the sheep in the age group of 18, 24 and >48 months revealed PrP immunoreactivity in the uterus and / or placenta, but only 23.1% at the age of 48 months.

Discussion: Despite some previously published evidence that the uterine wall is not an important site of PrP accumulation, our results showed PrP reactivity in some cases within the endometrial epithelium and endometrial glands. Presence of PrP immunoreactivity was evident in all age groups, suggesting that PrP starts to accumulate in the reproductive tract independently of the reproductive status and that the number of lambings probably does not significantly affect the amount of infectivity in the ovine reproductive organs of the sheep with VRQ/ARQ genotype.

Infectious canine hepatitis - two cases of canine adenovirus (CAV-1) infection in Germany.

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Introduction: Infectious canine hepatitis (IHC) is a systemic disease of mainly young dogs, foxes, other canidae and ursidae, caused by canine adenovirus type 1 (CAV-1). CAV-1 replicates in vascular endothelial cells and hepatocytes resulting, in oedema, diffuse haemorrhages and hepatic necrosis. Widespread vaccination has greatly reduced the frequency of the disease and IHC has not been reported in Germany for many years. Two cases of IHC which occurred in September 2006 and in February 2007 are described in the following.

Material and methods: Case 1 was a 4-months-old male mongrel dog which had been imported from Tenerife shortly before the onset of the disease and had probably never been vaccinated. The clinical symptoms were vomiting, anorexia, apathy, abdominal pain and blood counts leading to the suspicion of IHC. The dog was euthanized due to poor prognosis. Case 2 was a female Collie at 10 weeks of age that had developed fever and died within 24 hours. The puppy was born in Germany and had not yet been vaccinated.

Results: The necropsy findings in case 1 included oedema and diffuse haemorrhages in subcutaneous tissues, lymph nodes and lungs, ascites and intestinal petechiae. The liver was swollen, friable and showed an irregular yellowish and bright red mottling. The attachments of the gall bladder were oedematous. The main histological findings besides oedema and haemorrhages were severe liver necrosis with numerous intranuclear inclusion bodies in vital hepatocytes and single inclusion bodies in renal glomerula. Electron microscopically, the intranuclear inclusions were identified to be of adenoviral origin.
In case 2, similar gross pathological and histological changes were observed and adenoviral inclusions were confirmed by electron microscopy.

Discussion: One of the two dogs described above came from Tenerife and had probably already been infected before it was transferred to Germany. However, the second dog had never been outside Germany. It has to be considered that IHC might reemerge in areas where it has not been reported within the last years. The trade with puppies from other countries and the tourism with dogs should be kept in mind as possible dangers of spreading IHC to susceptable dogs in areas thought to be free of IHC.

Pathologic characteristics of penile fibropapilloma in young bulls.

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Introduction: Fibropapilloma of the penis is caused by the bovine papillomavirus. The virus gains entrance into the skin through wounds, causes neoplastic growth of fibroblasts and forms up tumours in the distal penis portion. This study describes gross and histopathological lesions of this tumour in two young bulls.

Material and methods: Two 12- and 18-month-old bulls were presented with a history of gradually enlarging masses on the glans of the penis, which showed ulceration, haemorrhage and secondary infection. The masses were multiple, varied size from 0.5 to 5 cm in diameter, were well circumscribed, non pigmented and attached to the glans by a relatively broad pedicles. Tissue samples were fixed in 10% buffered formaldehyde, embedded in paraffin, sectioned at about 5 μm, stained with H&E and studied microscopically.

Results: Histopathologically, the masses proved to be papillomatous features with irregular hyperplasia and epidermal rete ridges. Abundant connective tissue was covered by a thickened hyperplastic stratified squamous epithelium and elongated epidermal-dermal interdigitations penetrated deeply into the connective tissue. In the epidermis, orthokeratotic hyperkeratosis, many koilocytes with variably sized keratohyalin granules, and intranuclear inclusion bodies could be observed. Gross and histopathological findings of the lesions indicated fibropapilloma. Recurrence occurred in one case two months after surgical treatment.

Discussion: This tumour is caused by a venerally transmitted virus. Transmission frequently occurs as a consequence of homosexual activity among bulls. Although this tumour is benign, potentially adverse consequences include secondary infections, or adhesions between the preputial layers. Large tumours can lead to either paraphimosis or phimosis.

Validation of tissue microarrays for immunohistochemical analyses of canine malignant lymphomas.

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Introduction: In most validation studies of tissue microarrays, a fixed number of cores with a given diameter is analyzed to determine the degree of accuracy by which the microarray represents the whole section. In the present study, a statistical model is described which predicts this property for various combinations of 2 core sizes (0.6 mm and 1.2 mm) and different core numbers.

Material and methods: The model was based on artificial arrays generated from Ki67 and active caspase-3 immunostains of 40 canine malignant lymphoma samples. Positivity was scored on a continuous scale and a large number of cells was analyzed with the help of semi-automated cell counting. Two physical tissue arrays were assembled based on the model computations and using materials from the 40 cases. They consisted of two 1.2 mm and four 0.6 mm cores, respectively, and they were tested for their suitability to immunophenotype the tumours.

Results: The model predicts the maximum width of the 95%-confidence interval within which the true mean of each tumour is contained. Based on Ki67 data this is expected to be 1.119, 0.268, 0.162, 0.122, 0.101, 0.088, 0.079 for 2, 3, 4, 5, 6, 7, and 8 0.6 mm cores, respectively and 0.328, 0.079, 0.047, 0.036, 0.030, 0.026, 0.023 for the corresponding numbers of 1.2 mm cores. Despite considerable differences in range and distribution of Ki67 and active caspase-3 positivity values, the model predictions were almost identical for both markers. Comparison of 0.6 mm and 1.2 mm cores indicated that the use of small cores necessitates inclusion of a larger number of samples but requires counting of a markedly smaller number of cells.
Both physical arrays proved to accurately determine the immunophenotype of the lymphomas (Cohen's k(0.6mm)=0.79; k(1.2mm)=0.91).

Discussion: The study provides a basis for the use of tissue microarrays in future high-throughput immunohistochemical investigations of selected markers in canine lymphomas. The statistical model presented assists to determine an optimal tissue microarray design depending on a desired accuracy.

Generation and pathologic analysis of novel kidney disease models derived from the Munich ENU mouse mutagenesis project.

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Introduction: One characteristic of renal disease is the failure of adequate urinary excretion of metabolism products like urea, which was used as parameter to screen for novel kidney disease models in the Munich ENU mouse mutagenesis project.

Material and methods: Randomly mutagenized C3H inbred mice were generated by using ethylnitrosourea (ENU). Clinical blood chemistry analysis was carried out on over 15,000 G1 offspring and 500 G3 pedigrees to screen for dominant and recessive mutations leading to an increased plasma urea level. After generation of mutant lines, further analysis included a screen for additional clinical chemistry and morphological alterations.

Results: After identification of 44 animals consistently exhibiting increased plasma urea concentrations, transmission analysis of the altered phenotype of 23 mice to the subsequent generations were done and led to the generation of five mutant lines. Other clinical chemistry alterations in the mutants of all five lines were significantly decreased urinary urea levels and increased plasma creatinine levels, whereas decreased urinary creatinine levels were only observed in some of the mutant lines. Morphological kidney findings in the five mutant lines ranged from no macroscopical and light microscopical kidney alterations to decreased kidney-to-body weight ratio, dilatation of the renal pelvis and severe glomerular lesions, respectively. To identify the causative mutation, a special breeding protocol and linkage analysis are currently being performed.

Discussion: Thus, use of the plasma urea level as parameter in the high-throughput screen of randomly mutagenized mice resulted in the successful establishment of several nephropathy mouse strains. These strains might be valuable tools for molecular studies of mechanisms of renal function or might represent interesting models for human kidney diseases.

Evaluation of apoptosis after Ciprofloxacin inducement in rat testis.

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Introduction: Ciprofloxacin is a synthetic antibacterial agent belonging to the family of fluoroquinolones. It affects a very broad spectrum of microbial pathogens, especially Gram-negative bacteria, what has been approved in more than 100 countries world-wide. The aim of this study was to see apoptotic effects of ciprofloxacin after injection in rat testis.

Material and methods: Twenty male wistar rat were selected and randomly divided into two groups; control (n=10) and test (n=10) group. The test group received 12.5mg/kg (PO) ciprofloxacin daily for sixty days; the control group just received placebo. On the sixtieth day, the testis tissue of rats in both groups was removed and prepared for light microscopy. The Terminal Uridine Nick End Labeling (TUNEL) technique was used to identify apoptosis.

Results: Light microscopic studies of testis tissue slices of the test group showed apoptotic bodies in (15.11±3.523 %) and (7.3±0.762 %) in the control group. The rate was significantly increased in the experimental group when compared with the control group ($P<0.01$).

Discussion: Since our study showed, that ciprofloxacin had side effect such as an increased rate of apoptotic bodies in testis cells in rat, we suggested that using ciprofloxacin in humans can induce many histopathogical disorders and may be decrease the rate of fertility.

Pathomorphological investigations on wild birds in the period between October 2005 and February 2006 in Austria.

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Introduction: There are only few publications about comprehensive pathomorphological studies on wild birds. The past epidemic of High Pathogenic Avian Influenza (HPAI) in 2005/2006 entailed throughout Europe the entry of unusually many wild birds to diagnostic institutes. The Austrian Reference Laboratory for HPAI has examined about 5000 wild birds for the present of HPAI since autumn 2005. Based on the lab capacities, a contingent of these animals and samples were processed for pathoanatomical investigations within the scope of a doctoral thesis for histopathological investigation. The aim of this presentation is to give an overview about the detected organic and systemic diseases regarding the frequency, and the geographical and seasonal distribution.

Material and methods: Between October 2005 and February 2006, prior to the detection of the first case of HPAI in Austria, around 400 dead birds (369 wild birds) were investigated pathomorphologically to clarify the cause of death. Following necropsy and organ sampling, all investigated birds were tested negative for H5N1 by PCR. Furthermore, histopathololgy on formalin-fixed and paraffin-embedded tissue samples stained with H&E and special stainings was performed, and afterwards statistical analysed.

Results: Organic changes: Inflammatory changes: hepatitis (219), enteritis (205), pneumonia (107), tracheitis (39), nephritis (20), epi- and endocarditis (7), serositis (7), pancreatitis (5), splenitis (4), encephalitis (4) and meningitis (2);
Degeneration: hepatic fatty degeneration (57), tubulonephrosis (5);
Intra- and extracellular deposition: hepatic siderosis (114), pulmonary anthracosis (58), amyloidosis (14), renal tubular concrements (4).

Results Aetiology: Trauma (154), starvation (40), bacterial infection (81), unspecific bacteria (79), tuberculosis (2), parasitic infestation (22), suspicion of ethylene-glycol poisoning (2).

Discussion: The highest mortality rate was due to trauma and starvation most likely based on the very cold autumn and winter and the frequent change of location in this period. Pulmonary anthracosis seems to be an indicator for environmental influence. Many authors found lead poisoning as an important cause of death in swans. However, in the present investigation no histological changes, typical for lead poisoning were noticed. Infectious diseases (including zoonoses) also played an important role in the cause of mortality in wild birds which needs to be considered in epidemiological studies.

Canine Gastrointestinal Stromal Tumours (GIST).

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Introduction: In dogs, non-lymphoid mesenchymal tumours arising from the wall of the gastrointestinal tract are commonly referred to as "gastrointestinal stromal tumours". However, neoplasms with smooth muscle, neural, and fibroblast differentiation have all been included in this definition. In humans, the term "gastrointestinal stromal tumour (GIST)" is strictly applied to mesenchymal tumours of the gastrointestinal tract that most likely originate from the interstitial cells of Cajal. All GISTs express KIT, a receptor tyrosine kinase and activating mutations of c-kit have been identified in the juxtamembrane domain, most commonly exon 11. The purpose of this study was to characterize the microscopic and immunohistochemical features of canine GISTs in order to more accurately diagnose and prognosticate these neoplasms.

Material and methods: Forty five canine non-lymphoid intestinal mesenchymal tumours (NIMT) that had been previously diagnosed as leiomyosarcomas, fibrosarcomas, spindle cell sarcoma and anaplastic sarcoma were included in the study. All neoplasms were reviewed microscopically and immunohistochemistry (IHC) for KIT, vimentin, desmin, smooth muscle actin (SMA), S-100, and PGP9.5 was performed on all neoplasms. DNA was extracted from all neoplasms positive for KIT by IHC and exon 11 of the juxtamembrane domain and exon 17 of the kinase domain of c-kit were sequenced in search of mutations.

Results: Based on the microscopic and immunohistochemical characteristics 18 (40%) neoplasms were identified as GISTs. Morphologically, these neoplasms could be further classified into a spindeloid, pleomorphic, epitheloid and schwannoma pattern. In addition to KIT, all GISTs were positive for vimentin and 14 (78%) were positive for S-100 and 6 (33%) for SMA. Seven (39%) GISTs had an activating mutation in exon 11 of c-kit. Fifteen (33%) of the NIMTs were re-classified as leiomyosarcomas. Five (33%) leiomyosarcomas were well differentiated microscopically and expressed strong staining for SMA, but only weak staining for vimentin. In addition, 2 other neoplastic entities were identified in this study. Four neoplasms that only expressed vimentin and S-100 and had stellate to spindle shaped cells, arranged in short interwoven bundles, were thought to be schwannomas. Two neoplasms that only expressed vimentin and PGP9.5 and were composed of loosely arranged spindle cells in long interwoven bundles were thought to originate from the myenteric plexus.

Discussion: Canine NIMTs should be classified using both microscopic and immunohistochemical features to more accurately diagnose and prognosticate these neoplasms. A diagnosis of a GIST has to be based on positive expression of KIT and such neoplasms should be screened for activating mutations. In contrast to human GISTs, canine GISTs occurred mainly in the small intestine (67%). As described with human GISTs, they metastasized more commonly (5/18) than leiomyosarcomas with spread to liver, lymph nodes and omentum. Further studies are necessary to better characterize a potentially different biological behavior and therapeutical response of poorly versus well differentiated leiomyosarcomas as well as schwannomas and myentiric plexus neoplasms.

Diagnosis of feline intestinal lymphoma on surgical biopsy samples.

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Introduction: The differentiation of primary intestinal lymphoma from inflammatory bowel disease (IBD) in live cats is currently based on clinical signs in conjunction with the morphologic evaluation of intestinal biopsies. Both diseases are common and represent clinically as chronic diarrhea, vomiting, and weight loss. Outcome and therapy depend on the correct diagnosis. However it is extremely difficult to determine the cause of intestinal disease based on morphology alone. The analysis of lymphoid infiltrates is complicated by the fact that small lymphocytes represent the main cell type in intestinal T-cell lymphomas as well as in inflammatory reactions. Especially cases of intestinal lymphomas in which neoplastic cells do not extend into the muscularis pose a challenge to the surgical pathologist. Unfortunately endoscopic biopsies containing only mucosa and submucosa are most commonly submitted by most veterinary practitioners, since they are relatively easy to collect and minimally invasive. The purpose of this study was to develop a diagnostic algorithm for surgical intestinal biopsies from cats with a history of chronic diarrhea based on a step wise testing strategy using morphologic assessment followed by immunophenotyping (IPT) and finally PCR to determine clonality of the lymphoid cells to differentiate IBD from intestinal lymphoma.

Material and methods: In a retrospective study we analyzed 64 formalin fixed, paraffin embedded intestinal biopsies from 64 cats with a history of chronic diarrhea and a previous diagnosis of intestinal lymphoma or IBD. Each section was scored based on the location and density and the morphology of the lymphocytic cell infiltrates and their epithelial involvement. Sections were read blind and a diagnosis of IBD versus lymphoma was made. IPT (CD79a and CD3) was performed on all sections. Sections were than read blind a second time combining H&E and IPT. Following extraction of DNA from shavings of paraffin section of each of the surgical biopsies, rearrangement of T cell receptor gamma (TCRG) was determined by multiplex PCR of the CDR3 region. Clonality of suspected B-cell lymphomas was determined by analyzing CDR3 of the immunoglobulin heavy chain variable region (IGH V) with multiplex PCR.

Results: Using morphology only, 38 cases were determined to be T cell lymphomas, 7 cases to be B cell lymphomas and 19 cases were determined to be IBD. Following immunophenotyping 5 cases morphologically identified as IBD were re-classified as T cell lymphomas, 2 B-cell lymphomas were re-classified as T cell lymphomas, 1 T-cell lymphoma was re-classified as a B-cell lymphoma and 1 T-cell lymphoma was determined to be IBD. Following PCR, an additional 5 cases previously identified as IBD by H&E and IPT were re-classified as T-cell lymphomas.

Discussion: Combining H&E with IPT greatly enhanced the diagnostic accuracy. In particular intra-epithelial clusters and plaques of T-cells were found to be highly predictive of T-cell lymphoma. PCR to determine clonality further improved the diagnostic accuracy. Since clonality is not synonymous with malignancy, it is important to recognize that histopathology, IPT and PCR for clonality should always be used in conjunction with each other.

Construction of a stable transfected permanently secreting BHK Tet-On cell line carrying the single-chain canine IL-12 for application in the tumour immunotherapy in dogs.

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Introduction: Besides being the most important tumour patient in veterinary medicine, the dog is a promising model for tumour immunotherapy in man. As one of the approaches in tumour immunotherapy, the cytokine stimulation of Natural Killer (NK) and cytotoxic T cells represents a promising way to specifically fight the tumour cells. Among the cytokines showing potent anti-tumour activities the heterodimeric interleukin-12 (IL-12) plays a significant role. However, in contrast to mice and human, detailed studies on this area in dogs are lacking. In order to provide reliable sources for future investigations on the field of tumour immunotherapy in dogs, we (1) constructed a single-chain canine IL-12 sequence, (2) stably transfected it into a baby hamster kidney (BHK)-Tet-On cell line, which would serve as a constant and inducible (Doxycycline) protein source and (3) demonstrated the biological activities of the constructed protein.

Material and methods: Both sequences coding for the canine IL-12, p35 and p40, were amplified by PCR, ligated as a single chain in a pTRE/luciferase vector and afterwards stably transfected into the BHK-Tet-On cell line. Using canine interferon-gamma (IFN-gamma) ELISA and cytotoxicity test (Rose Bengal Assay), the bioactivity of the IL-12 containing supernatants was investigated.

Results: Canine IL-2 blasts incubated with the supernatant from the transfected clone showed significantly increased IFN-gamma production, which was completely blocked by an anti-canine IL-12 neutralizing antibody. The supernatant also showed increased cytolytic activity of the IL-2 blasts against the canine thyroid adenocarcinoma (CTAC) target cells at 50:1 ratio.

Discussion: The transfected clone produces the single-chain canine IL-12 in the supernatant, which shows the full bioactivity like the commercially available IL-12. Perspectively, this cell clone can be used for further investigation of the IL-12 induced anti-tumour effects of canine lymphocytes, especially NK cells. Moreover, the nucleotide sequence of the single-chain IL-12 could be used in gene delivery studies in dogs.

Histological and ultrastructural changes of frontal cerebral brain cortex under the influence of sodium hypochlorite solution, applied to T-2 toxicosis of piglets.

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Introduction: T-2 toxin is a low-molecular composition, the development of special protective measures against which is practically very labour-intensive, as far as providing conjugation with protein and high-molecular substances is a matter of some difficulty. We can accelerate neutralization processes by applying suitable detoxicants and antidotes. Thorough research on the influence of sodium hypochlorite (SHC) solutions as detoxicants has recently occupied a very important place among pharmacological studies. However, in neuromorphological aspect these questions are still waiting for their solving.

Material and methods: The experiment was carried out on 2.5-month-old spiglets aged which weighed 18 - 20 kg. The first (screening) group was fed and watered on good quality, with adequate fodder. Animals of the second and the third (experimental) groups were fed on mixed fodder contaminated with T-2 toxin (content of T-2 was 61 mg per 1 kg of fodder) for 20 days. Starting on the 10th day the animals of the third group were given (SHC) solution with a concentration of 200 mg/l instead of water. In groups of four, the animals on the 10th day from groups I and II and on the 20th day - from groups I, II, III were sacrificed and dissections were conducted, pieces of frontal cortex of the cerebrum were sampled for histological, histochemical and electron microscopic analysis.

Results: According to the results of the histo- and ultrastructural research conducted on the frontal cerebral brain cortex it was determined that on the 10th day of toxicosis the phase of destructive changes of neural elements progressed. The energy resources were not sufficient to provide a normal exchange, not only of functional, but also structural proteins and this in turn led to emersion of vacuoles, membrane insertions, myelin-like bodies and dissociation of structural proteins of cytoplasm. This was accompanied by a gross violation of structural organisation of the most mitochondria and their partial fragmentation. This histologically manifested itself in hydropic degeneration of neurons, oedema of oligodendrocytes, clasmatodendrosis and emersion of ameboid forms of astrocytes.
On the 20th day the phase of destructive change prevailed in the remaining structures of the cerebrum, which was accompanied by the mitochondria swelling and renovation of their cristas, granular reticulum cisterns proliferation in the perinuclear zone, nucleolus enlargement, nuclear chromatin re-distribution, and nuclear capsule rugosity. In such state a cell compensates the tissue insufficiency at the expense of spare capacities.

Discussion: One of the reasons which predetermined morphologic reconstruction and functional activity of neurons may be the active providing brain tissue with oxygen, as far as sodium hypochlorite displays biological influence at the expense of atomic oxygen liberation. Displayed under the influence of SHC solution nuclear and cytoplasm volume hypertrophy, activation of apparatus of energetic, aleuronic synthesis in the frontal cerebrum neurocytes, are, in our opinion, the evidence of sodium hypochlorite atomic oxygen influence on the intracellular and biosynthetic processes of brain tissue.

PRDC (Porcine Respiratory Disease Complex) in Korea: Prevalence, pathologic aetiology and microscopic lesions.

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Introduction: A retrospective study of natural cases of Porcine Respiratory Disease Complex (PRDC), recorded from 2005 to 2006, was performed to determine its prevalence in Korea. Porcine Respiratory Disease Complex (PRDC) of nursery and grow-finish pigs is characterized by cough, fever, lethargy, loss of appetite, laboured breathing and possibly death. Combinations of viral and bacterial agents are generally involved in the aetiology of Porcine Respiratory Disease Complex. The most common pathogens reported are porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), porcine circovirus-2 (PCV-2), Mycoplasma hyopneumoniae (Myco), Actinobacillus pleuropneumoniae (APP), Pasteurella multocida (Pm) and Haemophilus parasuis (HPS).
The purpose of this study was to investigate common aetiological agents and characteristic microscopic lesions associated with Porcine Respiratory Disease Complex (PRDC) in Korea.

Materials and Methods: Diagnostic data from 481 respiratory cases in pigs received at NVRQS between 2005 and 2006 were analyzed retrospectively. The samples were tested for the presence of respiratory pathogens by routine methods such as PCR. Tissue specimens were fixed in 10% neutral buffered formaldehyde for 24-48 h and embedded in paraffin wax according to the standard laboratory procedure. For detection on paraffin embedded tissue of PRRSV, PCV-2 and SIV, avidin-biotin-complex-peroxidase staining procedure was used. The cases with respiratory problems were up to 22 weeks old.

Results: Of the 481 cases of PRDC examined, 348 (72.3%) samples showed co-infections. Of the 348 co-infection cases, 175 contained two agents, 112 contained three agents, 48 contained four agents, 11 contained five agents and two contained six agents. Of the co-infection cases containing two agents, the combination of PRRSV/PCV-2 was found to be the most common and occurred in 78 (22.4%) of the samples. The combination of PCV-2/HPS occurred in 29 (8.3%) samples. Of the three agent co-infection cases the combination of PRRSV/PCV-2/HPS most commonly occurred in 39 (11.0%) samples, with PRRSV/PCV-2/Pm occurring in 20 (5.7%) cases.
The present results show that PRRSV and PCV-2 are common pathogens involved in PRDC in Korea. The microscopic lesions of PRRSV include alveolar septa thickening and the occurrence of degenerated PMNs in alveolar and bronchial lumina. The lesions in PCV-2 infected cases are alveolar septa thickening, peribronchial and perivascular cuffing and fibroplasia. SIV infected cases showed bronchiolitis (degeneration and necrosis of epithelial cells).
Bacterial infections resulted in different histopathological lesions: Pm caused bronchopneumonia and macrophages were found in the interstitial space of the lung. APP led to zonal necrosis and infiltration of oat cells. HPS was characterized by pleuritis.

Discussion: Microscopic observations are important when conducting a complete diagnostic investigation. Using such methods, one or more pathogens can be identified.

Comparison of different techniques for the diagnosis of porcine proliferative enteropathy.

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Introduction: Lawsonia intracellularis is an obligate intracellular bacterium causing porcine proliferative enteropathy (PPE), a transmissible enteric disease which is common among growing and finishing pigs throughout the world. The bacterium affects the aboral small intestine, in particular the ileum. Various methods for the diagnosis of PPE have been described, including PCR as well as different staining techniques of histological slides. In many laboratories the infection ist still diagnosed by evaluating gross and histological lesions in the ileum and (by demonstrating the bacteria) by Warthin-Starry silver staining. This technique, however, is not specific, little sensitive and has limitations when applied to necrotic or autolyzed samples. Therefore, the objectives of the present study were to compare different diagnostic techniques and to find suitable and reliable tools for future routine diagnostics.

Material and methods: 204 pigs with a history of diarrhoea and / or retarded growth were used for sample collection. At necropsy, mucosal scrapings were taken from the ileum and faecal samples were collected from the large intestine. The aboral part of the ileum was fixed in 7 % neutral buffered formaldehyde and embedded in paraffin wax. The following diagnostic techniques were performed: H&E staining; Warthin-Starry silver staining; immunohistochemistry (IHC); in situ hybridization (ISH); PCR from mucosal scrapings, faecal samples and paraffin wax-embedded tissue. All diagnostic techniques were compared with the results from PCR from mucosal scrapings (gold standard).

Results: 32 pigs were found to be infected with L. intracellularis based on a positive PCR result of mucosal scrapings. Nine ileal samples showed gross lesions consistent with the typical changes of chronic PPE, whereas 13 H&E-stained sections showed typical histological lesions. By Warthin-Starry staining intracellular bacteria were demonstrated in only 11 samples. Using IHC, L. intracellularis antigen was detected in the different layers of 22 ileal samples. In most cases signals were demonstrated within epithelial cells but also within the cytoplasm of mononuclear cells in the lamina propria and submucosa. ISH detected ribosomal RNA of L. intracellularis in 16 cases, mostly within epithelial cells. 29 pigs were positive by PCR from faeces, while PCR from paraffin-embedded tissue gave only 13 positive results.

Discussion: Warthin-Starry is a not specific staining technique which only detects severe infections and is sometimes difficult to evaluate. From all histological staining techniques, which have the advantage to localize the bacteria within lesions, IHC was the most sensitive. This technique shows signals within different layers of the intestine, which allows to estimate the state of infection. Alternatively, ISH might be used with similar advantages in laboratories without access to (non-commercially available) antibodies. The sensitivity of PCR from paraffin-embedded tissue was not satisfactory. IHC and ISH are also considered useful for retrospective studies. For ante mortem diagnosis, PCR from faeces proved to be an efficient diagnostic tool, but intermittent shedding could cause false negative results.

Wildlife Disease Monitoring at the National Zoological Gardens, South Africa.

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Introduction: Since the National Zoological Gardens (NZG) was incorporated into the National Research Foundation in 2004, it has developed a research and education program in line with its vision of advancing awareness, knowledge, and innovation in the conservation of Africa's biodiversity for the benefit of society. This ambitious program is intended to make significant research contributions to facilitiate sound scientific decisions for wildlife management (1) and is a timely development in the face of recent introduction of Classical Swine Fever to the country and the pending extension of the global Avian Influenza epidemic to southern Africa. Economic growth is at least partly dependent on ecotourism and therefore on biodiversity. Compared to domestic animals and animals kept in zoos, little is known about disease in free-ranging African wildlife.

Material and methods: The Zoological Pathology and Research Program entails disease monitoring in captive and free-ranging non-domestic animals and a disease database which, with stored necropsy samples, is available for research and training purposes. Systematic necropsy examinations and storage of disease information facilitates the identification of disease trends and generates prospective research projects. Samples are stored at BiobankSA making retrospective studies feasible.

Results: Expected benefits include early identification of emerging animal and human diseases, rational guidelines to translocation, quarantine and testing protocols, a central information centre for wildlife managers, veterinarians and pathologists, and relevant research projects focussed on animal welfare, health and biodiversity.

Discussion: Zoos are well placed to act as early warning systems as they house a variety of different species with differing susceptibilities, are staffed by veterinarians skilled in detection of new diseases, trap pest animals that may act as vectors, often take in sick or dead animals from surrounding areas, and evaluate imported animals that may be carrying diseases (2). Zoos are also ideally placed to educate and train school children, veterinarians, laboratory technicians, pathologists, curators, game rangers and tour guides, etc. The NZG views this program as central to the country's need to safeguard animal and human welfare for the future.

1.Building a Future for Wildlife, The World Zoo and Aquarium Conservation Strategy, World Association of Zoos and Aquariums, Berne, Switzerland, 2005
2. T McNamara West Nile Virus - Lessons learned. C L Davis Foundation, Pathology Symposium, University of Dublin, Ireland, Sept 8-20, 2003

Mycobacterium xenopi mesenteric lymph node adenitis in an adult Ruffed Lemur (Variegata variegata).

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Introduction: Mycobacterial infections are extremely rare in Lemurs (1, 2, 3). This to the authors knowledge is the first report of a Mycobacterium xenopi infection in a Lemur.

Material and methods: A 10 year old male captive Ruffed Lemur (Varecia variegata) that died suddenly was subjected to detailed necropsy examination. Lymph node tissue was cultured and subsequently tested with a 5'-16S rDNA PCR-sequencing assay that identifies and speciates Mycobacterium spp.

Results: Death was found to be due to severe necrogranulomatous haemorrhagic mesenteric lymph node adenitis and haemoperitoneum. Rare acid-fast organisms were found in necrogranulomatous inflammatory foci in the lymph node and scattered throughout the mesentery. Culture and PCR analysis identified Mycobacterium xenopi in lymph node tissue.

Discussion: Mycobacterium xenopi causes disease in mainly immune compromised humans (4), and occasionally in domestic cats (5, 6). Here, we report the first case of M. xenopi infection in a primate. The origin of the infection is unknown, but a human source cannot be ruled out. Epidemiological and management implications of M. xenopi infections are briefly discussed.

1. R E Junge 2003 Prosimians in Zoo and Wild Animal Medicine, 5th Ed. Eds M E Fowler and R E Miller, Saunders, p337
2. K Steiger et al, 2003 Uncommon mycobacterial infections in domestic and zoo animals. Deuts. Tier. Wochen. 110: 382
3. P Wilson et al. 1984 P. Mycobacterium bovis infection in primates in Dublin Zoo. Laboratory Animals 18: 383
4. E Platia & K Vosti 1983 Mycobacterium xenopi pulmonary disease. West J Med 138:102
5. P MacWilliams et al 1998 Lymphadenitis and peritonitis caused by Mycobacterium xenopi in a cat. Vet. Clin. Pathol. 27:50
6. A Tomasovic et al 1976 Mycobacterium xenopi in a skin lesion of a cat. Aust. Vet. J 52:103

KIT immunoreactivity of canine melanocytic tumours: a preliminary study.

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Introduction: C-kit gene product, CD117-KIT, is a tyrosine kinase receptor known to be important in the development of several cell types, including melanocytes. It is also involved in the pathogenesis of some tumours, including GIST and mast cell proliferative diseases in men and animals. In these tumours, KIT immunoreactivity is useful for diagnostic and prognostic purposes and identification of specific c-kit mutations is becoming a promising substrate for novel therapeutic approaches.
The role of CD117 in the development of human melanocytic tumours is still debated: former studies demonstrated a loss of KIT immunoreactivity with tumour progression, while recent ones identified several mutations of c-kit in distinct anatomical subtypes of melanomas, associated with increased KIT immunoreactivity.
Melanocytic tumours are frequent in dogs and the prognosis is based on the classical histologic hallmarks of malignancy and proliferative activity. A further prognostic feature is the anatomical site, with oral, nailbed and mucocutaneous tumours being more aggressive than cutaneous or ocular ones; the reason of these differences are still unclear. Little is known about the role of KIT in the development of normal canine melanocytes and its involvement in melanocytic neoplasia has not been demonstrated to date.
Aim of this study is to evaluate KIT immunoreactivity in canine melanocytic tumours from different anatomical sites.

Material and methods: 26 canine melanocytic tumours (8 cutaneous, 9 oral, 3 palpebral, 1 ocular and 2 metastatic) were selected from the archives and re-evaluated. Only tumours with at least some degree of melanin in neoplastic cells were selected. Formalin-fixed paraffin-embedded tumours were immunostained with polyclonal rabbit anti-human CD117 antibody. The percentage of immunoreactive cells (none, rare <10%, mild 10-50%, diffuse >50%), the intensity (weak, moderate, strong) and the pattern of staining of neoplastic cells were recordered.

Results: KIT immunoreactivity was detected in a majority (21/26: 81%) of melanocytic tumours. In detail 6/8 of cutaneous, 7/9 of oral, 2/3 of digital and all the few palpebral, ocular and metastatic tumours showed various degree of immunostaining. The immunoreactivity was rare in 4, mild in 10, and diffuse in 7 cases, with intensity ranging from weak and focal to strong and diffuse. Several staining patterns were identified, namely weak cytoplasmic with membrane accentuation, diffuse cytoplasmic and cytoplasmic granular with stippling. Mixed staining patterns were often present in different areas of the same tumours.

Discussion: The role of KIT both in human and canine melanocytic tumours needs to be further investigated. Even if based on a small number of cases from retrospective archival material, not related to follow up data, these results may be, in our opinion, a starting point for a prospective evaluation of KIT immunoreactivity, protein and mRNA expression in a larger series of canine melanocytic tumours. These data may be useful for subclassification of the tumours based on anatomical sites, biologic behaviour and, finally, prognosis.

BSE agent identification in sheep by analysing brain distribution of disease associated prion protein (PrPd) - Comparison with natural scrapie cases.

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Introduction: Possible transmission of the bovine spongiform encephalopathy (BSE) agent to small ruminants has been considered for several years. To date, only one case of BSE infection has been confirmed in a French goat using both analysis of enzymatic cleavage sites of disease associated prion protein (PrPd) and mice transmission studies. These methods are time consuming and hard to elaborate.

Material and methods: In the present study, qualitative and semi-quantitative analyses of the distribution of PrPd were performed in the brain of two sheep (A136 R154 Q171 homozygous prion genotype) clinically affected with BSE, using immunohistochemistry and PET-blot methods. For comparison, similar analyses were performed in 10 sheep, clinically affected with natural scrapie bearing identical or different genotypes.

Results: In the brain of the two BSE affected sheep, a particular PrPd distribution was demonstrated: a strong PrPd accumumation in basal nuclei, a dense fine granular accumulation in the piriform cortex and in hippocampal commissure, a dense linear accumulation in the substantia nigra and a weak extracellular fine deposition near of Purkinje cells in the cerebellum were identified. In the brain of all scrapie affected sheep different sites and types of PrPd accumulation were observed.

Discussion: Despite a limited number of cases, this preliminary study indicates that this approach could offer a complementary and more rapid method for identification of BSE agent in clinically affected sheep.

Widespread meningoencephalitis in a monkey (Macaca sylvanus) naturally infected with tick-borne encephalitis virus (TBEV).

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Introduction: Tick-born ecephalitis (TBE), a zoonotic disease caused by TBEV, a flavivirus, is reported predominantly in humans and seldomly in dogs and horses. TBEV uses rodents as maintenance and amplifying host and is transmitted by Ixodes ricinus in Central Europe. The incidence of TBE in Germany has increased enormously during recent years. Experimental infection of macaques with TBEV has been described, but natural infection has not been reported previously.

Clinical signs: Incoordination, paresis of the hind legs and intermittent opisthotonus were observed in a 15-month-old female barbary macaque (Macaca sylvanus) monkey from a group of about 200 kept in a large outdoor enclosure of a monkey park located in a TBE-risk area in Southern Germany. Four days after the onset of clinical signs the animal became comatous and was euthanized.

Macroscopic and microscopic findings, immunohistochemistry: No gross lesions were seen at necropsy. Histopathologic examination revealed moderate perivascular inflammatory cuffs and mild diffuse infiltration of brain parenchyma and meninges with mononuclear cells in almost all brain areas. Single neuronophagias were observed. Immunohistochemistry using a rabbit polyclonal hyperimmune serum revealed TBE-viral antigen mainly in Purkinje cells of the cerebellar cortex and to a lesser extent in pyramidal neurons of the temporal cortex.

Virological investigation: The presence of TBEV was confirmed in cerebrum, cerebellum and brain stem by nested RT-PCR and after virus isolation from cell cultures. Sequence data indicate that the isolated TBEV is closely related to Kumlinge disease virus, a TBEV strain that is closely related to strain Neudoerfl, the prototype strain of the European TBEV subtype.

Discussion: We report a fatal case of naturally acquired TBE in a barbary macaque. Lesions and antigen distribution were comparable to that observed in fatal human TBEV infections with a short clinical course.

Granulomatous pneumonia caused by Mycobacterium genavense in a dwarf rabbit (Oryctolagus cuniculus).

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Introduction: The organism Mycobacterium genavense has been identified as a new member of MOTT (mycobacterium other than tuberculosis) in 1992 (taxonomic identification in 1993) and has been diagnosed in HIV-positive patients as the causative agent of disseminated mycobacteriosis since then. Up to now infections in animals have been described in many birds, one dog, one cat, two ferrets and one monkey.

Material and methods: In the present case a juvenile dwarf rabbit was assigned for autopsy to the Institute of Veterinary Pathology, LMU-Munich with the preliminary report of dyspnea and suspected ascites.

Results: The main macroscopic finding was a watery red thoracal effusion and some light-coloured striated foci in the lung; furthermore there were multifocal scars in the cortex of the kidney. The histological examination of the lung showed a severe granulomatous pneumonia with detection of some acid-fast bacilli; in the kidney an interstitial chronic lymphoplasmacellular nephritis with interstitial fibrosis was diagnosed. A multifocal granulomatous and partly necrotising encephalitis was found in the brain. In the lung Mycobacterium genavense was verified by PCR and gene sequencing.

Discussion: This is the first report of Mycobacterium genavense infection in a rabbit known to the Institute of Veterinary Pathology. Whether or not the suspected encephalitozoonosis (nephritis, encephalitis) is a precurser for the observed Mycobacterium genavense infection remains speculative. In the presented case the method of infection also remains unclear. Lastly, a potential zoonosis risk should be kept in mind.

Borna disease virus transmission: From the virus-inoculated rat pups to the nursing mother.

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Introduction: The possibility of secondary infection of Borna disease (BD) from the virus-inoculated rat pups to the nursing mother was examined for the two variants of Borna disease virus (BDV).

Material and methods: Thirteen BDV-inoculated mother rats nursing pups were used. Within 24 hours after birth, the pups were inoculated with 2 x 10^3 FFU of CRNP5 (mouse-adapted BDV variant) or CRP3 (rat-adapted BDV variant) intracranially. Mother rats nursed their pups until the pups developed severe BD or weaned at postnatal day 28. Subsequently the mother rats were observed for about 8 weeks after giving birth or showing clinical symptoms and developing severe BD. After observation, the mother rats were killed and histological studies, virus titration of the brain samples, and antibody titration of the sera were performed. The expression of the antigens of BDV was also examined immunohistochemically by using two primary antibodies, monoclonal mouse anti BDV p24 protein (HP062) and anti BDV p40 protein (HN123) antibody (kindly donated by Dr. Ikuta, Osaka University, Japan).

Results: One out of 13 mother rats developed neurological signs, suspected of indicating BD. This rat nursed pups infected with CRNP5 which died with neurological signs after the 19th day post-infection. Subsequently, the mother rat developed neurological signs one month after the pups had died. On the third day after the onset of the clinical disease, the general condition of the mother deteriorated and the rat was killed and autopsied. Histopathologically, the rat had a nonsuppurative meningoencephalitis. The lesions were distributed throughout the brain to various degrees, including the brainstem, but were more severe in the frontal part of the cerebral hemisphere, the rhinencephalon, and the hippocampus. In the hippocampus there were perivascular cuffings and neuronal degenerations with diffuse glyosis, especially in the hippocampal CA3 region. The regular cell layer arrangements, perpendicular to the long axis of the pyramidal neurons, had been lost in places. The neurons of the granular layers of the dentate gyrus had also degenerated and the neuronal cell arrangements were loosened. The positive reaction against BDV protein antibodies was distributed throughout the central nervous system, including the diencephalon, the brainstem, the cerebellum and the trigeminal ganglion, but was more intense in the rhinencephalon and the hippocampus. Cytologically, the positive reaction was observed mainly in the nucleus of the neurons. The perikaryon of some of the neurons was also positive and less intensely positive in neuropils. In the brain sample, the virus titer was 1.2x10^7 TCID50/g. On the other hand, twelve mother rats without clinical symptoms had no histopathological changes and no positive reactions against BDV antibodies immunohistochemically in the brain, and BDV was not recovered from the brain. However, the anti-BDV antibody assay revealed 3 rats out of twelve had positive titers for BDV.

Discussion: Infection of a nursing mother rat by BVD from infected newborns led to a fatal disease. Histopathological examination of the mother rat showed a nonsuppurative meningoencephalitis, particularly in the olfactory system and the hippocampus. Furthermore, the positive reaction against BDV antibodies was more intense in the same areas. The above mentioned findings strongly suggest that a mother rat was infected by its pups via the olfactory route, but that its incidence was low.

Gross and histopathological lesions of coccidiosis in sheep and goats.

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Introduction: Small ruminant coccidiosis is a contagious protozoal disease that especially occurs in kids and lambs, with a worldwide distribution. In this study, gross and histopathological lesions of coccidiosis in sheep and goats are described.

Material and methods: Between 1998 and 2005, a total number of 21 small ruminants, including 6 goats (1 male and 5 female) and 15 sheep (11 male and 4 female) with a varying age from 20 days to 4 years (mostly less than 3 months old), were studied. At first, each animal was subjected to postmortem examination.
Gross lesions were noted carefully and wet smears from intestinal mucosa were taken. For histopathological diagnosis, appropriate specimens of small and large intestines and mesenteric lymph nodes were fixed in 10% buffered formaldehyde, embedded in paraffin, sectioned at about 5 µm, stained with H&E, and studied microscopically.

Results: Gross lesions were seen mostly in jejunum and ileum of affected animals and included small whitish foci as well as nodules in intestinal mucosa. These nodules were pinpoint to proliferative and polyp-like. The most important histopathological lesions in either sex and different age groups included papillary hyperplasia of the epithelium, the presence of developmental stages of Eimeria in the lamina propria and the glands in crypts of Lieberkühn, along with eosinophilic enteritis. The affected villi were greatly distended and disorganized due to the developmental stages of Eimeria and inflammatory reaction, which mainly consisted of eosinophils and lymphocytes. Several of the crypts of the Lieberkühn were disorganized or obliterated due to the growth of giant schizonts. The giant schizonts were easily discernible in histological sections. They were seen mainly in the crypts of Lieberkühn. The mature schizonts contained numerous minute merozoites arranged in whorls arising from the margin of protoplasmic spheres in the middle of the schizont.

Discussion: Coccidia are obligate intracellular pathogens and exquisitely host- and tissue-specific protozoa. Because of diminished epithelial turnover in young animals, they are most susceptible to the disease.This study showed that most of the lesions in sheep and goats affected to coccidiosis were nodular and polypoid. The term pseudoadenomatous has been used to describe these polypoid lesions and the oocyst patches or plaques. The plaques and polyps may be the result of mitogenic stimuli from progamonts. The immature stages in crypt epithelium, which appear to divide by binary fission in synchrony with the infected host cell, would thus supply a continuous stream of infected cells.

Laser capture microdissection: its application to the study of the pathogenesis of pseudorabies virus infection in mink.

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Introduction: Pseudorabies virus (PRV) is an alphaherpesvirus that causes a neurological disease in many wild and domestic animals, although only the adult pig is considered its natural host. The neuropathology elicited by PRV in the nervous system is quite consistent regardless of the host, with the remarkable exception of mink, in which it is characterized by a widespread vasculopathy, rather than by an encephalitis. Thus, the objective of this study was to gain a better understanding of the pathogenesis of PRV infection in mink, focusing on a possible vascular basis of the disease in this species.

Material and methods: Nervous system of mink, natural or experimentally infected with a field PRV strain, was used. Paraffin-embedded tissue samples were stained with H&E or phosphotungstic acid haematoxylin methods. Furthermore, we used immunohistochemistry (polyclonal rabbit antiserum against PRV antigens) and laser capture microdissection (LCM) followed by polymerase chain reaction, to investigate the relationship between the presence of PRV and the occurrence of the vascular lesions in the mink brain.

Results: The inflammatory reaction in the nervous system was minimal or even absent and the main lesions consisted of haemorrhages and ischaemia associated with a systemic vasculopathy. Both haemorrhagic and ischaemic infarcts were found associated with fibrinoid degeneration of the capillary walls and loss of the endothelial lining. We separately captured vessels by LCM from the brain stem (where PRV-infected cells were numerous) and from the cerebral cortex (where no PRV-infected cells were detected by immunohistochemistry) and extracted the total DNA. PCR performed on LCM samples with PRV gE-specific primers demonstrated the presence of virus DNA in vessels from both regions.

Discussion: The results obtained indicate that mink exhibit a PRV-specific immunodeficiency, since they do not appear to mount a normal immune response against the virus. In addition, we demonstrated that the virus is present in damaged cerebral vessels, suggesting that PRV endotheliotropism is connected with the vasculopathy characteristic for the disease in mink.

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Morphologic features of physiological to pathological luteal tissue in the cow.

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Introduction: Different luteal structures can be present in the bovine ovary. Corpus luteum (CL) is a transient endocrine gland which displays different morphological features in relation to the phase of oestrus cycle. Cavitary CL (cCL) differentiates for the presence of a central cavity, which origin and significance have not been fully established. cCL has to be distinguished from luteal cyst (LC), a frequent pathological condition of the bovine ovary. The aim of this paper is to evaluate, if gross examination is able to distinguish among physiological and pathological luteal structures.

Material and methods: From 483 slaughtered beef cows, 305 luteal structures were collected and grossly analyzed. Compact CLs were distinguished in 4 morphological stages and correlated to blood progesterone (P4) levels. CLs in stage I (0-4 days of oestrus cycle) were 0.5-1.5 cm in diameter, bloody in colour and had a prominent and haemorrhagic papilla. CLs in stage II (5-10 days) were characterized by larger size and a double colour of the luteal tissue at section, red next to the apex and orange in the remainder. CLs in stage III (11-17 days) were 1.6-2.5 cm in diameter and uniformly orange. CLs in stage IV (18-21 days) were under 1.5 cm in diameter with colour ranging from deep orange to yellow lemon and had an invaginating papilla. We considered cCLs those having a central cavity over 0.5 cm in diameter, filled with yellowish fluid and an evident ovulation papilla. LCs were distinguished from cCLs mainly for the absence of the papilla.

Results: 209/305 (69%) compact CLs were identified and grouped as follows: 9% in stage I, 24% in stage II, 47% in stage III and 20% in stage IV. P4 levels were 1-2 ng/ml in stage I and IV and 7-9 ng/ml in stage II and III. A double ovulation was recorded in 7% of these cases. 58/305 (19%) cCLs were found and easily identifiable by size (frequently over 3 cm in diameter), reduced consistency, marked tendency to protrude from the ovarian profile and often for an abnormally small ovulation papilla. 6/305 (2%) persistent CLs were strongly suspected for the presence in uterus of abundant purulent exudate, or remnants of died embryos or foetuses. 32/305 (10%) LCs were characterized by large size due to an inner cavity over 2.5 cm in diameter, soft consistency, spherical appearance and thin to thick wall, partially or totally luteinized. 88% of these cysts were single, 18% were associated to ovarobursal adhesions, 31% to functional CLs.

Discussion: Gross appearance of luteal structures allows to estimate with good accuracy the phase of oestrus cycle and to differentiate between cCLs and LCs, which are often clinically, ultrasonographically and histologically indistinguishable. cCL has to be regarded as a non-pathological condition and probably arises from an abnormal closing of the ovulation site. LCs are pathological structures resulting from spontaneous or induced luteinization of follicular cysts. A mechanical anovulation was suspected when ovarobursal adhesions were present. The association of LCs and CLs could be related to the resolving of the condition, spontaneously or after specific treatment.

Macrostomia in a calf: pathological and radiological observations.

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Introduction: Facial clefts are developmental disorders caused by failure of the fusion of facial processes such as the frontonasal, maxillary, and mandibular processes, in an early foetal period. They are assumed to have various phenotypes in relation to the disordered site and foetal organs such those originating by the branchial arch. The defects appear in the lateral or median site of the rostral face as cleft lip, jaw, and palate. In humans, midline facial anomalies accompanied with defects in the intermaxillary segment are frequently combined with severe brain anomalies, such as holoprosencephaly and cyclopia, representing the most severe form of hypotelorism. The association of hypertelorism and various forms of median clefts are called median cleft face syndrome or frontal dysplasia. Facial clefts may occur in several animal species and have been only occasionally reported in cattle. Aim of the paper is to describe the pathological and radiological findings of a case of facial cleft in a calf.

Material and methods: A live female crossbred calf was delivered after a normal pregnancy from a 7-years-old Friesian cow. The calf died spontaneously after 7 days because of an inability to stand and suckle. It was frozen and sent to the Unit of Veterinary Pathology of the University of Messina for necropsy, which was performed after slow defreezing at 0-4°C temperature. During the dissection, the head was removed and radiologically evaluated. Samples of tissues and organs were collected and routinely processed.

Results: Externally the calf showed macrostomia due to a large unilateral transverse cleft involving the right oral commissure and the right ear concha, displacement of the right auricular pinna with atretic auricular external meatus, thin and shortened upper and lower right lips. Radiological examination of the head revealed asymmetry of the mandible horizontal ramuses with hypoplasia of the right one, which was also luxated and rotated, and a lack of the temporomandibular joint. Petrous temporal bones and tympanic bullae appeared asymmetric and differently conformed. Lateral displacement of premolars and cleft of hard palate were observed, too. After jaw removing, most of the radiological features were grossly evident. Examination of the central nervous system revealed only cerebellar hypoplasia.
Gross examination of internal organs revealed abnormalities only at genital level. A bilateral ovarian agenesia was detected together with a juvenile uterus which, histologically, showed only scant endometrial glands and remnants of Wolffian ducts. Furthermore, no organized mammary tissue was found. The remaining body organs were grossly unremarkable.

Discussion: Based on pathological and radiological findings, the malformation was classified as a particular case of cleft lip, jaw and palate associated with genital abnormalities. On the basis of macroscopical changes observed in the presented case, the authors discuss a possible comparison with similar human syndromes.

Agnathia in a lamb: gross and radiological observations.

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Introduction: Agnathia (dysgnathia or agnathia-otocephaly - AO) is a lethal malformation complex of the first and second branchial arche, characterized by absence of the mandible, microstomia, aplasia or hypoplasia of the tongue and low-set or medially fused ears toward the midline. The facial anomalies show considerable variability, particularly in the external appearance of the ear. In some cases, the ears approach one another anteriorly and are occasionally fused in the position of the absent mandible, thus the terms “agnathia”, “otocephaly” and “synotia”, often used in literature as “dysgnathia” as synonym. Embryologically, AO without brain anomalies is believed to result from a defect of the ventral portion of the first an second branchial arch and may be caused by defective neural crest migration or proliferation in the anterior portion of the embryonic disc, or by a mesodermal deficiency in the arch itself.
The condition has been described in humans and it may occur spontaneously in several animal species, including mice, sheep, lambs, guinea pigs, and dogs. Aim of this paper is to describe the gross and radiological findings of the condition observed in a lamb foetus.

Material and methods: A male Comisana lamb was collected dead from the uterus of a 3.5-years-old Comisana sheep at the end of pregnancy during slaughtering. The lamb was sent frozen to the Unit of Veterinary Pathology of the University of Messina, where necropsy was performed after slow defreezing at 0-4°C temperature. Prior to the dissection, the skull and the proximal districts of the alimentary and respiratory apparatuses were radiologically evaluated using two orthogonal radiographs with and without contrast medium.

Results: Externally the lamb showed astomia, proboscis, lack of mandible, bilateral hypoplasia of the maxilla and slightly prominent fluctuant pharyngeal diverticulum. The pinnae of the ears were fused in the ventral midline (synotia) with a common athresic external auditory meatus, also confirmed by the contrastographic x-ray examination. The ocular orbits were displaced ventromedially with nearly fused eyes resembling synophtalmia. Direct radiographic evaluation, consisting of two radiographs of the skull obtained orthogonal to each other, revealed the absence of the mandible, hyoid bones, timpanic bullae and hypoplasia of the maxilla. Contrastographic x-ray examination and the following dissection confirmed the presence of a laryngopharyngeal diverticulum and cranial dilation of the oesophagus. The remaining body organs were grossly unremarkable.

Discussion: Based on gross findings, the malformation was classified as agnathia and associated to the type 4 malformation of the first branchial arch in sheep or “ateloprosopia”, as proposed by Dennis and Leipold (1972).
Authors discuss the possible pathogenic mechanisms of this condition, infrequently reported in veterinary practice, pointing out the importance of the knowledge of animal malformations in breeding management.

Congenital hypotrichosis in Valle del Belice sheep: gross and histopathological study of skin lesions.

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Introduction: Congenital alopecia consists in a partial or complete amount of hair loss apparent at birth or that develops during the neonatal period. The condition may be regional or multifocal, but it is usually generalized. The ectodermal defect may involve hair follicles but it may also involve teeth, claws or hooves, sweat and lacrimal glands. Congenital hypotrichosis is commonly reported in cattle, frequently in swine and rarely in sheep, goats, horses, dogs and wild animals.
The hairless (hr) gene is often responsible for this disorder in men, mice and rats. Recent experimental data support the genetic control of the ovine hypotrichosis as a Mendelian recessive trait in Valle del Belice sheep, an ovine-breed biotype reared in Sicily for milk production.

Material and methods: Between September 2004 and November 2005, two distinct Sicilian farms rearing Valle del Belice sheep were monitored for congenital hypotrichosis. The reared animals consisted in 140 and 250 subjects, respectively, and the defect was present in both the farms ranging in the order of 2-3% of animals. Both farms showed a consistent number normal lambs. The anamnesis did show no particularity and previous skin lesions, hitching or dental anomalies were not recorded. Only few subjects presented onychogriphosis.
For the present study, 4 Valle del Belice lambs of different age and sex were evaluated. Two of them spontaneously died and underwent necropsy (only skin lesions are discussed), while skin biopsies were performed in the remaining lambs.

Results: The four subjects showed similar gross skin features characterised by: large areas of alopecia, thin and smooth skin which allowed to see the underlying vascularisation, and moderately severe traumatic lesions. Histopathological findings, similar in all cases, were characterised by: variations in skin thickness, lack or markedly reduction of hair follicles, hair erector muscles and sweat glands, cystic dilation of some keratin filled follicles, and prominent sebaceous glands.

Discussion: The observed findings confirmed the diagnosis of hypotrichosis. Histopathological findings overlapped those observed in animals with congenital hypotrichosis, as described in literature. Grossly, the distribution of alopecic areas proved to be similar to those observed in Dorset sheep, whereas the distribution of alopecic and normal skin areas in Valle del Belice and Dorset sheep showed to be opposite to those observed in Guernsey, Jersey, Holstein, and Hereford bovines. Furthermore, the constant findings of hyperplastic/hypertrophic sebaceous glands proves particularly interesting if compared to the different forms of animal hypotrichosis, being the same only just found in one case of congenital hypotrichosis in a horse.

Imperfect myelination does not hinder electrical conduction in the optic nerve of the 2-50 transgenic mouse.

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Introduction: The 2-50 transgenic mouse suffers a severe, early loss of oligodendrocyte precursor cells with a resulting delay in onset of central nervous system myelination and is dysmyelinated as an adult. Some internodes have thin myelin, or remain unmyelinated, some have thicker than normal sheaths, others are of normal thickness but poor compaction. Although pups display a shaking phenotype for a short period during the period of peak myelination, the adult animals are apparently normal. We performed extracellular electrophysiological recording of optic nerves to compare neurotransmission in hemizygous transgenic animals and their non transgenic siblings.

Material and methods: 2-50 transgenic mice of either sex were screened for the presence of the transgene using PCR. They were deeply anaesthetised, decapitated, and the optic nerves were dissected for recording. Animals aged 29 and 120 days were used. Nerves were placed in a bath of circulating oxygenated, artificial CSF at room temperature and allowed to equilibrate for 60 minutes. Each end of one nerve was drawn into a suction electrode. Voltage and duration response curves were recorded for each nerve. Voltage response was measured using increments of the threshold voltage at 0.01s stimulus duration. Duration response was measured similarly as increasing stimulus pulse length at the threshold voltage for 0.01s. Maximal stimulation was assumed when no appreciable change in amplitude was detectable for three increases of voltage or duration. Compound action potentials were analysed using peak fitting software and statistical analysis was performed to compare 1. conduction speed 2. the contribution of the first and second conduction groups to the compound action potential and 3. the likelihood of a third conduction group being detectable.

Results: No difference referrable to the transgene status could be detected although the age of the animal was found to affect the results.

Discussion: The CNS myelin of the 2-50 tg mouse has been shown by electron microscopy and protein isoform analysis to be abnormal in physical and chemical structure and myelin sheaths on individual optic nerve fibres are incomplete, but function of the optic nerve is not obviously impeded.

The whole nerve recording technique obviously measures only axons that do transmit an action potential; it is possible that the transgenic animals have a significant difference in the number of axons firing, if these axons are distributed across the conduction groups and not clustered in one, or that ephaptic coupling occurs and stimulates axons that would otherwise fail to transmit. However, the successful axons transmit in a close to normal fashion, suggesting that the thickness, completeness and quality of the myelin is adequate for physiological function, although not necessarily “normal”. This supports the mathematical models which predict that, while extensive failure of myelination will lead to conduction block, some degree of variation is tolerated well.

Cytological features of upper respiratory pathways in acute influenza infection in mice - experimental model.

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Introduction: Murine experimental models are often used to elucidate some of the pathogenetical issues in influeza for testing different vaccine formulas. It is known that local immunity provided by NALT (nasal associated lymphoid tissue) plays an important role in influenza infection of the upper respiratory pathways and its spreading in lungs. This investigation proposes an experimental murine model of influenza infection and searches the way of cytological expression of lesions in nasal and tracheal mucosae.

Material and methods: Four strains of mice between 6 to 8-weeks-old have been used: Balb/c, TCR-HA, Ins-HA and dTg. A/PR8/34 influenza virus strain from allantoic liquid was intranasally instilated, using 268.8 HAU/mouse. The animals were euthanized 48 hours after the infection. Influenza infection was confirmed for each mouse using a quick test for virus identification. Scrapings of nasal and tracheal mucosae were May-Grünwald-Giemsa stained in two animals for each strain.

Results: Different mucosal reaction occurred in each strain and also between tracheal and nasal mucosa of the same mouse. Nasal scraping presented: Balb/c - degenerated epithelial cells, inconstant loss of cilia and condensed nuclei, numerous nuclei without cytoplasm; Ins-HA - rare active and degenerated neutrophils, degenerated epithelial cells, inconstant loss of cilia and rare multinucleated epithelial cells; TCR-HA - rare active neutrophils, normal features in almost all epithelial cells; dTg-numerous active neutrophils, rare lymphocytes, mixed population of normal and degenerated epithelial cells.
Tracheal scrapings presented: Balb/c - rare active neutrophils with foamy cytoplasm, degenerated epithelial cells; Ins-HA - rare active neutrophils, normal and degenerated epithelial cells, multinucleated epithelial cells; TCR-HA - numerous neutrophils and macrophages with foamy cytoplasm, rare lymphocytes, degenerated and normal epithelial cells with condensed nuclei; dTg - numerous active neutrophils and macrophages, rare lymphocytes, degenerated and normal ciliated epithelial cells, rare binucleated cells.

Discussion: Balb/c and Ins-HA (ideal mice strains for study in influenza infections) presented a lack of acute inflammatory reaction in investigated mucosae. Important cytological features occurred in epithelial cells. Epithelial syncytial cells were observed in Ins-HA, being a feature observed in other respiratory viral disease. In mice strains resistant to PR8 influenza virus strain (TCR-HA and dTg) a strong inflammatory reaction as catarrhal rhinitis and tracheitis occurred (active neutrophils and macrophages, lymphocytes). Considering the disease pathogenesis, we explain why tracheal inflammatory reaction is stronger than nasal mucosa response.

Histopathological and serological study on aborted foetuses caused by Bovine Viral Diarrhea in dairy herds of Tehran.

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Introduction: Bovine Viral Diarrhea (BVD) infection is one of the main causes of bovine abortion in Iran. In this survey, BVDV was detected in 56 cases of aborted foetuses from dairy herds of Tehran submitted from Summer 2005 to Spring 2007 on the basis of histopathological findings and monoclonal capture enzyme-linked immunosorbant assay (ELISA) test for antigen detection.

Material and methods: To detect the cause of abortion, 150 aborted foetuses were submitted for necropsy, microscopical examination and ELISA test. Tissue samples were obtained from the brain (cerebellum), heart, liver, spleen, kidney and lung and were fixed in 10% neutral buffered formaldehyde, routinely embedded in paraffin and stained with H&E. To detect antigens by capture ELISA test, blood samples obtained from the heart of foetuses were examined by IDEXX laboratories BVDV Ag/Serum-plus kit.

Results: Gross findings during necropsy were non-specific, but in 3 aborted foetuses cerebellar hypoplasia was seen. Microscopically, the cerebellar lesions were necrosis in external granular layer, focal haemorrhage in the cortex followed by focal cavitations of the white mater and atrophy. Leptomenigitis characterized by accumulation of lymphocytes was seen. In addition, interstitial pneumonia, interstitial nephritis, periportal hepatitis and focal necrosis in the spleen were observed. Fifty-one of the 56 BVDV infected fetuses (based on histopathological findings) were found to be positive in the ELISA. Normalized OD values (ODv = ODs - ODn) for these 51 positive samples ranged from 0.56 to 1.16, with a mean of 0.85 and standard deviation of 0.15.

Discussion: BVD viruses are classified in the virus family Flaviviridae and are members of the genus pestivirus. BVDV is a RNA virus that has two genotypes: type 1 and 2 and each genotype beein divided into 2 biotypes: cytopathic (CP) and non-cytopatic (NCP).
The effects of BVDV depend on the stage of gestation when a heifer or cow is infected. The BVD virus is capable of passing easily from the uterus of an infected cow to the foetus, which is particularly vulnerable to the BVD virus during the first 6 months of pregnancy. BVDV can cause abortions, stillbirths, resorption and mummification of the foetuses. If the foetus survives, calves are often born persistently infected (PI) or with various congenital defects. The type of lesions in CNS (i.e. cerebellar hypoplasia) depends on the age of the foetus and the stage of CNS development at the time of infection. All of the lesions are due to vascular impairment, resulting from vasculitis.

In this study, the cause of abortion in 56 out of 150 cases (37.4 %) was detected by gross findings, microscopical examinations and BVDV antigen detection by monoclonal Ag/Serum capture ELISA in 51 cases (91.1 %).
ELISA for the detection of BVDV antigen in the blood of PI cattle has been compared and is highly sensitive, specific and considered valuable in eradication programs when monitoring large numbers of animals. It is an effective way to monitor herds for persistently infected animals. Moreover, there is no need for sample preparation, saving time and effort.

Immunohistochemical expression and genetic mutations of p53 in feline mammary carcinoma.

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Introduction: In normal cells p53 controls several biological outcomes (growth arrest, apoptosis, DNA repair etc). In human cancer overexpression and p53 mutations, particularly of the missense type, are frequently observed (about 40%). Although, only partial correlation exists among immunohistochemistry (IHC)-based methods and sequencing analyses. Only little is known about the correlation of p53 mutation and overexpression in feline neoplasms, but probably they might occur with a different frequency then in humans. The aim of the study is to investigate the p53 expression with a panel of different antibodies and to evaluate the presence of mutations in p53 DNA Binding Domain (DBD) in a group of feline mammary carcinomas (FMC).

Material and methods: Feline mammary samples from the CEROVEC Animal Tumour Registry of 26 cats submitted for histological examination were classified according to the WHO and tumours (23) were graded (G) according to the Nottingham Grading System. IHC was performed with CM-1, Pab 240, Pab 1620, Pab 246, BP 53-12 and DO-1 according to manufacturer's instructions. The DBD coding region (exons 4-8) was sequenced; the human residues numeration was used to identify feline p53 aminoacids. DNA from formalin-fixed and/or paraffin-embedded tissue was extracted and amplified. PCR products were checked on agarose gel, purified (GenElute PCR Clean-up kit - SIGMA), sequenced three times on both strands (Big Dye Terminator cycle sequencing) and analysed (ABI Prism 310 G.A.-Applied Biosystems) according to manufacturer's protocol. Sequencing data were analysed (SeqScape v2.5 Applied Biosystems) and aligned with the consensus sequence (Genebank ID26608).

Results: Lesions were non neoplastic (3) or carcinomas (23). p53 expression was detected with CM-1 antibody: in 9 cribriform (G2=5, G3=4, positive from 2% to 89%) in 6 tubulopapillary (G1=1, 0%, G2=3, 28%, 19%, 0%; G3=2, 85% and 0%); 5 solid (G2=1, 28%, G3=3, 37%, 69%, 0%; G3=1 0%), 1 intraductal (G1, 4%), 1 squamous (G3, 0%), and 1 tubular (G2, 27%). G1 are 50% positive (1/2), G2 are 80% positive (8/10) and G3 are 72% positive (8/11). Morphotypes are variably positive: cribriform 8/9, solid 3/5, tubulopapillary 3/6. Only Pab 240 is able to detect p53 overexpression (7/23), associated with CM-1 (6/7) or not (cribriform). In non-neoplastic lesions p53 is undetectable. Three single point mutations were collected: CGA196TGA, (nonsense, cribriform), ATC195TTC (missense, tubulopapillary) and TTC270TTY (silent, healthy); the silent polymorphism in codon 163 was confirmed, with other polymorphisms and some mutations in non-coding regions.

Discussion: Only two clones, Pab 240 and CM-1, are able to detect feline p53 overexpression: 74% CM-1, much higher respect those reported in literature for mammary carcinomas and 30% Pab 240, with a cribriform positive for Pab 240 but negative for CM-1. Two mutations are lower than expected compared with human carcinomas. ATC195TTC is strongly positive to both antibodies while CGA196TGA is negative, as expected, for Pab240 and 32.2% for CM-1. Further evaluations on the feline p53 are needed to assess its role in feline carcinogenesis.

Mutations in E-box motifs overlapping with glucocorticoid response elements (Egre) affect disease induction by the T-cell lymphomagenic gammaretrovirus SL3-3.

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Introduction: The gammaretrovirus SL3-3 is a potent ecotropic virus that strictly induces T-cell lymphomas in mice, after a latency period of 2 to 4 month, depending on the mouse strain. In this study, the disease pattern of SL3-3 mutated in the basic-helix-loop-helix (bHLH) factor binding sites (E-box motifs) was investigated. These binding sites are glucocorticoid response elements overlapping E-boxes (Egre) that can be found trice in the enhancer sequence of SL3-3. BHLH factors play an important role in the development of lymphoid cells and in this study it could be shown that mutations in their binding sites cause a shift in disease pattern.

Material and methods: Paraformaldehyde and formalin fixed, paraffin embedded tissues from lymph nodes, spleen and liver from 23 mice infected with mutated SL3-3 (mutation in the E-boxes overlapping glucocorticoid response elements [mEgre]) were histologically analysed. Five µm thick sections were cut and stained with H&E or with chloroacetate esterase (CAE). Immunohistochemistry (CD3, B220, TdT, myeloperoxidase) was performed when indicated. The classification of the lymphoid and nonlymphoid haematopoietic neoplasms was performed according to the Bethesda proposals.

Results: E-box mutants induced, with a significantly extended latency period, multiple haematopoietic malignancies, including myeloid leukaemia, B-cell and T-cell lymphomas. Fourteen of 23 SL3-3 mEgre injected mice were diagnosed as pre-T-cell lymphoblastic lymphomas. Four SL3-3 mEgre injected mice showed a myeloid leukaemia (with or without maturation). Plasmacytoma was found in two SL3-3 mEgre infected mice and three mice were diagnosed as mixed tumours (B-cell and T-cell origin).

Discussion: In this study, it could be shown that bHLH binding sites play an important role in T-cell lymphoma induction by SL3-3, although the disruption of T-cell lymphomagenesis was incomplete. Mutations of this E-box enhancer sites allow the strictly T-cell lymphomagenic SL3-3 virus to induce different haematopoietic malignancies including T-cell lymphoma, myeloid leukaemia, plasmacytoma and mixed B- and T-cell lymphomas. This finding strongly supports the central role of these E-box motifs in SL3-3 for T-cell lymphomagenesis. The phenotypic heterogeneity suggests that SL3-3 mEgre either is able to infect and transform several cell types or can target a haematopoietic progenitor.

An outbreak of Pasteurella multocida septicaemia in lambs.

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Introduction: Pasteurella multocida is an important veterinary and opportunistic human pathogen and exists as a commensal in the upper respiratory tract of many livestock, poultry and domestic pet species. Infection in humans is often associated with an animal bite, scratch, or lick, but infection without animal contact may occur. This study describes the clinical, bacteriological and pathological aspects of an outbreak of Pasteurella multocida septicaemia in lambs.

Material and methods: Six dead lambs from a total of 120 affected animals were presented for postmortem examination. Systematical postmortem examinations were performed. Tissue specimens were taken for bacteriology and histopathology. Tissue specimens were fixed in 10 % buffered neutral formaldehyde, 6 μm tissue sections were stained with H&E and examined histologically. Differential tests were done for bacterial isolation.

Results: The clinical course was short, with the first sign often being sudden death. Affected animals that were found alive were febrile, anorectic, showed recumbency, and death within 24 hours. At necropsy, generalized petechiation on serosal surfaces, marked ecchymoses in the surface of the lungs and enlargement of the lymph nodes were observed. Specimens from the liver, lung, kidney and blood were sent to the diagnostical laboratory for bacterial detection. On bacterial isolation a small, gram-negative coccobacillus with bipolar staining was identified and based on its biochemical characteristics, confirmed as Pasteurella multocida biotype A. Histopathological examination revealed severe hyperaemia with multifocal haemorrhages in the lung, liver and the kidneys.

Discussion: Human diseases caused by Pasteurella multocida include respiratory infections, sepsis and meningitis. Certain serological types are the aetiologic agents of severe pasteurellosis, such as fowl cholera in domestic and wild birds and bovine haemorrhagic septicaemia. The young lambs are highly susceptible to biotype A infections, which progress rapidly to a fatal septicaemia. The organism is also a primary pathogen in goat kids. This disease probably does not warrant a separate classification but is kept separate because some outbreaks are manifest only with septicaemia in lambs. The authors believe that this is the first record of septicaemic pasteurellosis in lambs in Iran.

Cutaneous lymphoma with unusual morphology in a dog.

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Introduction: Among the cutaneous tumours of haemotopietic cell origin in dogs, histiocytic, plasmacytic and lymphocytic tumours are common along with mastocytoma. Morphological features of these tumour cells are characterized by round nuclei especially in the lymphomas, although some atypical tumour cells reveal anisokaryosis or multinucleation. We encountered a solitary cutaneous lymphoma with unusual morphological feature of tumour cells in a dog and examined histopathologically and immunohistochemically.

Material and methods: A 7-year-old neutered chihuahua dog showed slowly growing solitary cutaneous mass in the thoracic ventral area. The surgically resected mass was fixed in 10% phosphate buffered neutral formaldehyde solution (pH 7.4) and sent to our laboratory for histopathological examination. No recurrence has been observed for more than 6 months after surgery in the dog.

Results: The tumour mass was well demarcated and consisted of closely packed round or polygonal cells. Tumour cells infiltrated from just beneath the epidermis to the surface of dermal muscle and subcutaneous adipose tissue and partially infiltrated into epidermal layers or outer root sheath of the hair follicle (epitheliotrophic). Tumour mass was predominantly composed of individualized round cells with relatively abundant eosinophilic cytoplasm and irregular-shaped elongated nuclei. Some tumour cells had eccentric elongated nuclei displaced to cytoplasmic rim. Nuclei of other tumour cells were horseshoe or doughnut-shaped. The morphological feature of these cells was similar to those of cutaneous plasmacytoma rather than lymphoma. Cells with round nuclei similar to those of lymphocytes aggregated only in a part of the mass. Immunohistochemically, almost all tumour cells with elongated nuclei were positive for CD3 and were negative for CD20, CD79, kappa light chain, and lambda light chain. Metachromasia was also negative in all tumour cells by toluidine blue stain. Small numbers of CD20 and CD79 positive cells mingled between the tumour cells. However, these cells were intermingled normal B-lymphocytes.

Discussion: From the results of immunohistochemical examination, the present case was diagnosed as a cutaneous T-cell lymphoma despite of unusual morphplogical feature of the tumour cells. Cutaneous T-cell neoplasma classifies as cutaneous epitheliotrophic lymphoma (mycosis fungoides type and pagetoid reticulosis type) and cutaneous nonepitheliotrophic lymphoma (nonepitheliotropic lymphoma, vasotropic and vasoinvasive nonepitheliotropic lymphoma and cutaneous intravascular lymphoma). Among these subtypes of T-cell lymphoma, the present case might be a cutaneous epitheliotropic lymphoma: mycosis fungoides type. However, the clinical and morphological feature were quite different from ordinal cases.

High frequency of spontaneous rhabdomyosarcomas in older MDX mice with X-linked muscular dystrophy.

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Introduction: Mdx mice (X-linked muscular dystrophy) lack dystrophin and show a progressive muscle pathology which usually remains clinically silent. However in older individuals, we found a high incidence of subcutaneous and muscular tumours and checked the histological nature of these neoplasms.

Material and methods: Necropsy records and histopathology from 36 mdx and 36 control mice born in 2005 and killed in 2007 (age range, 16-26 months).

Results: The spontaneous incidence of neoplasms in mdx (16/36) and control mice (17/36) was similar. Males and females were equally affected. Out of 17 tumours in control mice, 16 were malignant lymphomas (mostly mesentery and Peyer's patches) and 1 a histiocytic sarcoma. The 16 tumours in mdx mice were 7 malignant lymphomas (including 6 mesenteric) and 9 rhabdomyosarcomas. The rhabdomyosarcomas were of the spindle cell type, they were located mostly on the thigh (4/9) and shoulder (3/9). The rhabdomyosarcomas in mdx mice were characterized by their high mitotic activity, high local invasion, but usually absence of distant metastases (except one case with pulmonary metastases).

Discussion: Rhabdomyosarcomas are very unusual tumours in mice, including C57BL/10ScSn mice (the genetic background of mdx mice). The common occurrence of this tumour type in mdx mice suggests a link with the muscular dystrophy, either due to a chronic stimulation of muscle regeneration, or due to a role for dystrophin in controlling myoblast replication.

Pathogenesis of the experimental infection of Highly Pathogenic Avian Influenza H5N1 A/turkey/Turkey/05 (Asian lineage) in Pekin ducks.

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Introduction: Highly pathogenic avian influenza (HPAI) is caused by infection with influenza A viruses of the family Orthomyxoviridae, with devastating consequences for poultry and zoonotic potential. While wild birds and waterfowls act as reservoirs for influenza viruses, it has been generally considered that HPAI did not cause clinical disease or deaths in ducks or wild aquatic birds. However, recently emerged HPAI H5N1 has shown an increased lethality for wild bird populations. This study investigates the pathological changes and virus dissemination of HPAI H5N1 A/turkey/Turkey/05 over time in Pekin ducks.

Material and methods: Fifteen 4-week-old Pekin ducks (Anas platyrhychos) were inoculated via intranasal and intraoral route with 104 EID50 HPAI H5N1 A/turkey/Turkey/05. Three randomly selected ducks were euthanized and necropsied on days one to five post infection. Tissue samples from multiple organs, including the respiratory, alimentary, integumentary, endocrine and nervous system, were collected, fixed in buffered formalin and routinely processed for histopathology. Immunohistochemical detection of Influenza A nucleoprotein on tissue sections was used to determine viral distribution.

Results: Infected animals displayed clinical signs from 4 dpi, including depression, reluctance to feed, in-coordination and torticollis, showing rapid deterioration by day 5. On post mortem examination, airsacculitis was observed from day 3 and myocardial linear necrosis from day 4. Histological changes were observed from day 2, comprising multifocal pancreatic necrosis, acute rhinitis and mild interstitial pneumonia, airsacculitis, myositis and non-suppurative encephalitis. Myocarditis with multifocal necrosis of myocardiocytes and necrosis of the adrenal cortex was observed from 4 dpi. Viral antigen was detected in a small number of cells in lung, nasal cavity and spleen on day 1, with a widespread organic distribution by day 2. The organs where immunolabelled cells were more numerous and consistently present throughout the infection were lung, air sac, heart, pancreas and brain. Immunolabelling of breast and thigh muscle myocytes and epidermis of the feather follicle wall and epidermal collar was observed from 2 dpi. No immunolabelled enterocytes were observed throughout the infection.

Discussion: Infection of Pekin ducks with HPAI H5N1 A/turkey/Turkey/05 virus results in systemic viral spread with a lethal outcome. This broader tissue tropism and severe encephalitic and myocardial changes will account for the increased pathogenicity of this isolate in ducks. Early spread of the virus to meat and feathers may play a role in the transmission of the disease.

Ashworthius sidemi in European bison (Bison bonasus) - pathological changes in the abomasum and duodenum.

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Introduction: Ashwortiosis is caused by bloodsucking nematodes from genus Ashwortius, family Trichostrongylidae, which are located in the abomasum of ruminants. Since the 50th of 20th century, helminth fauna of European bison in the Polish part of Bialowieza Primeval Forest has been regularly and exactly investigated and above mentioned parasites have never been observed. In 1997 in the Bieszczady Moutains, Demiaszkiewicz and Lachowicz, in the Belarusian part of Bialowieza Forest Kochko (personal communication 2000) found for the first time single specimes of A. sidemi in bison. It was the reason to take up helminthological necropsies of bison culled in the Polish part of Bialowieza Forest. From 2005 to 2006 the anatomo- and histopathological examination were performed, too.

Material and methods: 34 European male and female bisons, aged 4 months to 26 years were examined post mortem to define pathological lesions. The abomasal and duodenal walls were collected for histopathological examination. Specimens were stained with H&E. The abomasum and duodenum were completely examined for worms using the sedimentation method.

Results: All examined bison were infected with A. sidemi. The invasion intensity was from 632 to 14,890 individuals. Medium intensity of invasion was 4,907. The histopathological examination of the abomasal and duodenal wall showed infiltration of inflammatory cells with eosinophils, enlargement of lymphoid follicles and infiltration of lymphoid cells in mucosa and submucosa. In some cases small necrotic foci, focal destruction of epithelium and parasitic nodules were observed.

Discussion: Nematodes of the genus Ashwortius are treated as dangerous parasites which locate in the abomasum of ruminants in Africa and Eurasia. A. sidemi is considered a parasite initially typical for Asiatic deer, especially Cervus nippon, with which it was introduced to many countries of the former USSR and Europe. These worms feed on the mucosa, causing bleeding, or suck blood actively. The pathomorphological changes observed in all infected bison show a reaction against A. sidemi.

The urothelial carcinoma due to Capillaria plica infection of urinary bladder in a dog - case report.

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Introduction: The Capillaria plica infection in dogs causes haematuria, dysuria, and/or a catarrhal cystitis, but the chronic infection may result in urine bladder cancer.

Material and methods: A female dog, 18 months old, mixed breed, kept in a household with a garden. Periodical haematuria has been reported for 8 months. Urine laboratory tests, the USG, diagnostic laparotomy (cystotomy), H&E slides of the bladder's wall, and parasitological examination of the urine were performed.

Results: The H&E slides of the urinary bladder's wall showed: papillary urothelial (transitional cells) carcinoma of a low-grade. Moderate infiltration of bladder tissue by inflammatory cells and a haemorrhage were noticed as well. Parasites were found within the epithelium and numerous eggs of Capillaria plica were present in the urine.

Discussion: The lesions were surgically removed from the wall and deworming was performed with several medicines. The haematuria stopped and the dog's general condition has improved. It is very likely that haematuria was due to the urothelial carcinoma which would have been caused by a long-term irritation with C. plica.

The deworming has not been successful and single morphologically changed eggs have been found in the urine. To prevent reinfection, the final host should not be allowed to have contact with the domestic composting unit in the garden, which is the natural place of the earthworms - the intermediate host of C. plica.

Insulin-induced hypoglycaemic peripheral neuropathy in spontaneous diabetic WBN/Kob rats.

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Introduction: Intensive insulin therapy has often brought an increased incidence of hypoglycaemia. These patients sometimes developed hypoglycaemic neuropathy. However, it still remains unclear how much of hypoglycaemic conditions induce the morphological and clinical changes of peripheral neuropathy. Spontaneously diabetic WBN/Kob rats develop diabetic peripheral motor neuropathy characterized by primary segmental demyelination and secondary axonal degeneration. We, therefore, examined the short-term effects of hypoglycaemia on neuropathic changes in WBN/Kob rats.

Material and methods: Seventy five-week-old spontaneous diabetic WBN/kob rats were given insulin implants (bovine insulin) for 40 days. These rats were divided into 3 groups by blood glucose levels as follows; normoglycaemic / slightly hyperglycaemic (60-250 mg/dl, N group), hypoglycaemic / slightly hyperglycaemic (35-200 mg/dl, H group), non-treated spontaneously diabetic (350-420 mg/dl, D group). Sciatic-tibial motor nerves were subjected for measurement of conduction velocity and qualitative and quantitative histomorphological analysis.

Results: The conduction velocity of N group was higher than that of the D and H groups. Morphological analysis of sciatic-tibial motor nerves of the H group showed severe changes such as nerve fiber loss, myelin distention, demyelination, axonal degeneration and endoneurial fibrosis. These changes tended to occur in large-sized myelinated fibers. In N and D groups, only mild changes such as slight myelin distention, demyelination and endoneurial fibrosis were seen. The degree and distribution of these degenerated nerve fibers in the H group were significantly higher than in the N and D groups.

Discussion: These results suggest that hypoglycemia with levels of less than 60 mg/dl induced by treatment with insulin implants induces severe peripheral neuropathy in diabetic WBN/kob rats.

Evolution of T-lymphocytes and cytokine expression in the ileum of calves inoculated with the Bovine Viral Diarrhoea Virus.

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Introduction: The objective of this work was to evaluate the quantitative changes and cytokine expression by T-lymphocyte populations in the ileum of calves during Bovine Viral Diarrhoea (BVD), as well as their implication in the pathogenesis of B-lymphocyte apoptosis.

Material and methods: Ten colostrum-deprived Friesian calves of 6-8 weeks old were used. Two animals were used as uninfected controls, while the remaining calves received an intranasal inoculation of noncytopathic BVD virus genotype-1 strain 7443. Animals were slaughtered in groups of two at 3, 6, 9 and 14 days post-inoculation (dpi). Samples of ileum were fixed in 10% buffered formaldehyde, Bouin's solution and zinc salts fixative and routinely processed for structural and immunohistochemical studies. The avidin-biotin-complex-peroxidase method in combination with different antigen unmasking techniques were used for immunolabeling B-lymphocytes (CD79), T-lymphocytes (CD3), T-lymphocyte subpopulations ($CD4^+$, $CD8^+$, and gamma/delta) and cells, mainly T-lymphocytes, expressing cytokines (IFN-gamma and IL-4). To determine the proliferation of lymphocytes, Ki67 antibody was used. Positive cells against CD4, CD8, gamma/delta, IFN-gamma and IL-4 were counted and tested for significance ($P< 0.05$) by Mann-Whitney´s U-test. A semiquantitative counting for CD79, CD3 and Ki67 immunolabelled cells were performed.

Results: Despite lymphoid depletion observed mainly in the follicles of Peyer´s patches, activated T-lymphocytes ($CD3^+$) showed an increase in the follicles, interfollicular areas and lamina propria of ileum from 3 dpi, peaking at 6-9 dpi. Inside the follicles, $CD4^+$ T-lymphocytes displayed a significant increase from 3 dpi, peaking at 6 dpi, while the increase of gamma/delta and CD8+ T-lymphocytes was lower and observed from 6 and 9 dpi, respectively, until the end of the experiment. However, in the final stage of the disease, the number of $CD4^+$ T-lymphocytes located in the lamina propria and interfollicular areas was lower than $CD8^+$ and gamma-delta T-lymphocytes, which showed a significant increase at 14 dpi. At the beginning of BVD, an increase in the number of cells immunolabeled against IL-4 was observed, peaking at 3 dpi in the different studied areas of ileum and declining afterwards. However, cells expressing IFN-gamma peaked at 14 dpi in the lamina propia and interfollicular areas, but not inside the follicles. The number of B-lymphocytes (CD79+) and Ki67 positive-cells was increased from 3 dpi, peaking at 6 dpi.

Discussion: The increased expression of IL-4 by T-lymphocytes next to the small number of T-lymphocytes immunolabeled against IFN-gamma at the initial stage of BVD could be related with alterations in the Th1/Th2 immune response. Moreover, the existence of T-lymphocytes expressing IL-4 from 3 dpi widespread in lymphoid follicles, could induce an abnormal proliferation and blastic transformation of B-lymphocytes ($CD79^+$), which were increased next to Ki67 positive-cells from 3 dpi. These changes could be involved in the massive apoptosis of lymphocytes. The role played by the antigen-presenting cells as dendritic cells in this pathogenic mechanism should be evaluated.

Characterization of E-cadherin glycans in canine mammary carcinoma.

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Introduction: E-Cadherin is a transmembrane protein that mediates the homotypic cell-cell adhesion in a calcium-dependent manner, playing pivotal roles in cell differentiation, transformation and invasion. E-Cadherin can be post-translationally modified by phosphorylation, O-glycosylation and N-glycosylation. Little is known about carbohydrate chain structures of this glycoprotein and their role in the stability of adherens-junctions. The importance of the patterns of E-cadherin glycosylation in the process of malignancy is also not clearly understood. Many studies have demonstrated that malignant transformation of a number of human cell types correlates with changes of cell surface N-linked oligosaccharides, but so far nothing is known in canine cancers. Aim of the study: Characterization of the pattern of E-cadherin glycosylation and study of the carbohydrate profile of E-cadherin in canine mammary carcinoma.

Material and methods: Canine mammary carcinoma cell line (CMTU) was used for immunofluorescence labeling of carbohydrate antigens. Immunoprecipitation studies of E-cadherin followed by Western-blot analyses were performed using lectins (Sambucus nigra agglutinin, SNA; Maackia amurensis agglutinin, MAA; Phaseolus vulgaris agglutinin, PHA-L) and specific monoclonal antibodies for the detection of carbohydrate antigens.
Characterization of glycosylation was carried out by SDS-PAGE electrophoresis of the immunoprecipitated E-cadherin and in-gel deglycosylation using PNGase F for glycan release. N-glycans were separated by Matrix-Assisted Laser Desorption/Ionization mass spectrometry (MALDI MS) and their structures identified by the computer matching of the resulting masses with those derived from a sequence database.

Results: Evaluation of expression of carbohydrate antigens in the CMTU cell line showed expression of Sialyl Lewis x, Lewis a and Lewis x. These carbohydrate antigens were not observed on the 120 KDa range which correspond to the molecular weight of E-cadherin.
The results also revealed that E-cadherin from canine mammary carcinoma carries oligosaccharide chains with a-2,3 and a-2,6 linked sialic acid. E-Cadherin also posses GlcNAcb-1,6 branched complex type glycans.

Discussion: These results reveal the overall glycosylation pattern of E-cadherin from canine mammary carcinoma cell line and suggest that the complex type N-glycans at the surface of E-cadherin could be implicated in the neoplastic transformation of canine mammary carcinoma. Future studies will determine how the glycans structures affect canine E-cadherin adhesive function and tumour progression.
We made use of a rapid and sensitive method for the profiling and sequencing of glycoprotein-associated N-linked oligosaccharides from protein gels, which constitute a modern method in veterinary pathology.

Implication of cell type-specific regulation of Borna disease virus transcription efficiency in neurons and astrocytes of experimentally infected Lewis rats.

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Introduction: Borna disease virus (BDV) causes a severe neurological disorder of the central nervous system (CNS) which is characterized by a non cytolytic virus propagation and persistence in the CNS. The underlying mechanisms of the viral persistence are yet incompletely understood, but might be achieved by various strategies, for example direct modification of the viral genome, control of transcription and regulation of viral protein expression. The latter was initially shown by a restricted glycoprotein (BDV-GP) expression in certain brain areas. To investigate further strategies of viral replication and transcription, the expression of BDV-specific transcripts was analyzed in experimentally infected rat brains in different cell types.

Material and methods: BDV-transcripts specific for the nucleoprotein (+ssBDV-N), glycoprotein (+ssBDV-GP) and intron I-specific +ssRNA (+ssBDV-Intron I) were quantified either in neurons and astrocytes at 14, 24, 42, and 90 days post infection (dpi) using laser microdissection and RT-PCR.

Results: A general higher expression of BDV-N transcripts was only seen in neurons. The single cell analyses revealed a predominantly temporal regulation of BDV-specific transcripts in neurons. The expression maximum of BDV-N and BDV-Intron I +ssRNA was detected at 24 dpi, while highest levels of BDV-GP +ssRNA were found at 42 dpi. In astrocytes, BDV-N- and BDV-GP-specific transcripts varied not significantly between all time points investigated. Only BDV-Intron I +ssRNA increased significantly from 14 to 24 dpi in astrocytes.

Discussion: In conclusion, this data indicate cell type-specific regulatory mechanisms of BDV transcription.

A murine model of porcine Pasteurella multocida pneumonia.

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Introduction: Pasteurella multocida is a major cause of porcine bronchopneumonia and associated with the Porcine Respiratory Disease Complex (PRDC). In addition, systemic spread of P. multocida may occur. The pathogenesis related to both initial lung infection and subsequent dissemination is unknown. To investigate the initial phase and later progress of infection a murine model of P. multocida pneumonia was developed. The aim of the present study was to examine if the model corresponds to natural infection in pigs and whether this model could distinguish between different patterns of lesions and influence on systemic infection caused by P. multocida strains isolated from different types of porcine pneumonia.

Material and methods: Twenty female BALB/c-J mice (Taconic, Denmark), approx. 20 g, were separated into four groups of five. Three groups were infected with one of three strains of P. multocida isolated from clinical cases of necrotizing, suppurative and non-suppurative chronic porcine pneumonia, respectively. Mice were transiently anaesthetized using isoflurane and infected with 10^4 cfu/ml of P. multocida in 10 µl broth applied through the nostrils. A control group received sterile broth only. All mice were sacrificed upon clinical signs of infection. Specimens of lungs and the spleen were collected for bacteriological and histopathological examination.

Results: All mice showed clinical signs of pneumonia 24 h p.i. Differences between the strains of P. multocida were not reflected in the viable counts. Histopathology showed acute bronchopneumonia in all animals. However, the pattern of distribution of lesions varied from local to diffuse. The cellular infiltration consisted mainly of neutrophils and was located around bronchioles and arteries. To a variable extent bacteria were present in the lung tissue from mice in all infected groups. Lung lesions were mainly characterized by deposition of fibrinous material in alveoles and bronchioles, perivascular oedema, suppuration and necrosis. In the spleens, the red pulp was drained of erythrocytes and multiple microabscesses were evident. A non-significant difference in the quantity of lesions was seen according to the strain of P. multocida used.

Discussion: This study shows that the aerogenous murine model is suitable for induction of pneumonic lesions by P. multocida of porcine origin. Patterns corresponding to the differences in the porcine pneumonias were not present 24 h p.i., but similarity between the porcine and murine inflammatory response was evident.

Association of necrotizing enteritis in piglets with in situ beta-toxin secretion by Clostridium perfringens Type C.

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Introduction: Clostridium perfringens is the putative cause of important enteric diseases in veterinary and human medicine. Different toxinotypes of C. perfringens mediate their pathogenic effect via a large array of secreted exotoxins. Which of these toxins are responsible for development of intestinal tract lesions is, however, largely unknown. Necrotizing enteritis in neonatal piglets is an important disease in veterinary medicine caused by Clostridium perfringens Type C and potentially Type A strains. The characteristic lesion is a segmental haemorrhagic necrosis of the small intestine. Currently applied prophylactic programs have been inefficient in reducing the incidence of disease outbreaks in Switzerland. Two toxins, beta and beta2, are hypothesized to be the key factors in the development of lesions. The goal of our project was to associate necrotizing enteritis with the presence of toxigenic C. perfringens as well as in situ beta- and beta2-toxin secretion.

Material and methods: 199 animals were necropsied and evaluated by routine pathological and bacteriological analysis of the intestinal tract, including the genotyping of isolated C. perfringens. Additional direct multiplex real-time PCR analyses for the detection of C. perfringens toxin genes were performed on intestinal tissue samples. In vitro and in situ toxin secretion was evaluated using western blot analyses of culture supernatants and intestinal samples as well as immunofluorescence studies on intestinal sections. In addition to post mortem analyses, 1400 swab samples of live piglets were analysed bacteriologically to determine the prevalence of C. perfringens Type A and Type C strains in piglets in Switzerland.

Results: Bacteriological investigations of the intestinal tract showed a significant association of necrotizing enteritis with beta-toxigenic C. perfringens Type C. Whereas Type C strains were almost exclusively detected in pig herds affected by necrotizing enteritis, the prevalence of beta2 toxigenic Type A strains in clinically healthy piglets was more than 95 %. All isolated C. perfingens Type C strains secreted beta-, and beta2-toxin in culture. Immunofluorescence revealed binding of beta-toxin to endothelial cells of mucosal vessels, specifically in areas affected by necrotizing lesions. Beta 2-toxin could not be demonstrated in intestinal sections.

Discussion: Our results demonstrate that necrotizing enteritis in piglets is associated with beta-toxin secretion of C. perfringens Type C strains. Additionally, they suggest that beta-toxin binding to endothelial cells leads to vascular damage and subsequent ischaemic necrosis of the small intestine. In contrast to Type C strains, beta2-toxigenic C. perfingens Type A strains are part of the normal intestinal flora of piglets and do not play a predominant role in the development of necrotizing enteritis in piglets.

Rapid reversal of epithelial invasion in a mouse model of microbially-induced carcinoma.

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Introduction: By infecting 129/SvEv recombinase-activating gene-2 (Rag2)-deficient mice, which lack functional lymphocytes, with Helicobacter hepaticus (Hh), we showed that innate immune response is sufficient to induce colitis-associated colon cancer. Adoptive transfer of regulatory T-lymphocytes (TR) derived from 129/SvEv wild type donors (WT TR) prior to infection, inhibited inflammation and cancer. However, adoptive transfer of TR cells obtained from IL10-deficient donors (IL10-/-TR) failed to protect and instead exacerbated a malignant epithelial phenotype such that 100% of recipient Rag2-/- mice rapidly developed mucinous colonic tumours that invaded the peritoneal cavity. In this study, we assessed the effect of the adoptive transfer of WT TR in Rag2-/- recipients of IL10-/-TR with established large, infiltrative colonic tumours.

Material and methods: Hh-infected Rag2-/- transferred with IL10-/-TR remained untreated or were further treated with a) different doses of WT TR obtained from both Hh-infected and non-infected donors b) cytokine-neutralizing antibodies (anti-TNFα, anti-IL6) c) IL10-Ig fusion protein. Samples collected from mice upon necropsy were analyzed by histopathology, immunohistochemistry, flow cytometry, ELISA and real-time PCR.

Results: Hh-infected Rag2-/- transferred with IL10-/-TR mice developed large, infiltrative mucinous colonic tumours. IL10-/-TR cells expressed Foxp3 and localized in colonic tumours. Mice with colonic tumours had elevated IL6 protein levels in serum. Significant elevations of IL6 but not TNF-α gene expression were evident in colonic tissue. K-ras and the epithelial oncogene Pim1 but not Bcl3 or c-Myc and the Tgfβ pathway members Tgfβ1 TgfβRII and Smad4 but not TgfβRI were also significantly over-expressed. Each one of the three different methodologies used in this study to treat mice with established tumours led to the regression of the tumours, abolished malignancy and normalized inflammatory cytokine and oncogene expression levels.

Discussion: We have demonstrated that invasive colonic carcinomas are rapidly reversible through IL10-mediated restoration of epithelial homoeostasis. Adoptive transfer of IL10-competent regulatory cells not only reversed intestinal pathology in this mouse model, but also improved activity and overall body condition of recipient mice, perhaps providing insights into links with systemic health. The finding that microbially-triggered colitis induces universal upregulation of IL6 highlights possible roles for systemic inflammatory responses throughout the body. The ability of competent TR cells to normalize epithelial signaling and restore epithelial homoeostasis substantiates links between host immunity, epithelial homoeostasis and malignancy. Because dysregulation of IL-6 is a frequent feature of invasive malignancies, it will be important to consider immune-mediated effects among the steps initiating or modulating cancer and associated neoplastic invasion in humans.

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Virus distribution in the organs of Crocidura leucodon naturally and persistently infected with Bornavirus.

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Introduction: The precise pathogenesis and epidemiology of natural Borna Disease Virus (BDV) infections are unknown; however, several unique epidemiological features point towards the existence of BDV reservoir populations other than the final hosts. Here we report two cases of naturally and persistently infected bicolored white-toothed shrews, Crocidura leucodon, which were caught in an endemic area in Switzerland as candidates for Borna virus reservoir.

Material and methods: Here we present the detailed viral antigen distribution in several organs of the shrews by immunohistochemistry, confirmed by RT-PCR technique (TaqMan®).

Results: Borna Disease Virus was detected in the brain, liver, myocardium, kidney, lungs, and skin, mainly in neurons and nerve tissue of several organs, but also in parenchymal (e.g. hepatocytes, Leydig cells), and epithelial cells, i.e. bronchial epithelial cells, transitional epithelial cells of the urethra, as well as in salivary gland, sebaceous glands and epidermis.

Discussion: Crocidura leucodon appears to be a vector species for BDV, having high amounts of virus in almost every organ, without any evidence of disease or tissue pathology. The way of acquisition and distribution of the infection is presently unknown, but subject to further research plans.

Aberrant expression of major histocompatibility class II molecules by tubular epithelial cells during Leptospira pomona-associated tubulointerstitial nephritis.

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Introduction: Leptospirosis is an important disease in pigs and interstitial nephritis is the most frequent lesion in infected swine. Although pathogenetical mechanisms leading to nephritis are still poorly understood, the antigenic stimulus induced by the persistent colonization of renal tubules by leptospires might play an important role. Antigen presentation by cells expressing major histocompatibility class II molecules (MHCII) on their surface induces the epitope-specific immune response against bacteria. In inflammatory conditions, cytokines and antigenic stimuli can evoke MHCII-overexpression by professional APC, including histiocytes and lymphoid cells, as well as MHCII-aberrant expression by non-professional APC such as tubular epithelial cells.

Material and methods: At slaughter a kidney was collected from each of 20 8-months-old pigs within a group with a high prevalence of interstitial nephritis (white spotted kidney) and from each of 5 8-months-old pigs within a group without renal lesions. Kidneys were tested by PCR for the detection of Leptospira spp. The kidneys were also submitted to microscopic and immunohistochemical (IHC) examination, the latter using a mouse monoclonal antibody raised against α-chain of human MHCII and a rabbit polyclonal antibody raised against Leptospira interrogans serovar pomona. Double antigen IHC-staining on the same tissue sections was also performed using the same primary antibodies.

Results: Kidneys from pigs of the control group were histologically normal and negative for leptospiral infection by PCR and IHC. On the contrary, 4 histological patterns of interstitial nephritis were observed in the 20 pigs in the group with a high prevalence of white spotted kidney: The 4 cases with a perivascular lymphocytic pattern were negative for Leptospira-IHC staining and PCR. In these cases mild MHCII-aberrant expression by tubular cells was observed. The 6 cases with lymphocytic interstitial pattern and the 8 cases with lympho-histiocytic interstitial pattern were positive at PCR and/or IHC for Leptospira. In these cases variable amount of Leptospira antigen was detected both in the tubular lumen and within the epithelial cells. Furthermore, prominent MHCII-aberrant expression by intralesional regenerating tubular epithelium was correlated with the severity of inflammation. The 2 cases with subacute lympho-histiocytic and granulocytic interstitial nephritis were positive at PCR and IHC for Leptospira with abundant antigen in tubular lumen, cytoplasm of tubular cells and neutrophils. The MHCII-aberrant expression by tubular epithelial cells within foci of interstitial inflammation was moderate in both cases. No colocalization between Leptospira antigen and MHCII-aberrant expression in tubular structures was observed in any cases by double antigen IHC.

Discussion: Our results indicate that MHCII is not primary involved in the triggering of the inflammatory response since tubular epithelium engulfed or lined by Leptospira antigen does not express MHCII. It can be supposed that regenerating MHCII-positive tubules, associated with the severe interstitial inflammation, acquires a new phenotypic profile under the influence of the cytokinic milieu. Such regenerating tubules are probably more “alert” from the immunological point of view reducing the possibility of a de novo Leptospiral colonization. Indeed, leptospires were never detected in MHC II positive tubules.

Stroke - the development of a large animal model with sheep for a better scientific and therapeutic understanding.

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Introduction: Stroke is the third frequent cause of death in human beings. Until recently, the animal model of choice for clinical, pathomorphological and therapeutic investigations had mainly been the rodent. In order to create an economically and ethically justifiable animal model with a better comparability to the human anatomy, the sheep was established as a large animal model for stroke.

Material and methods: Via transcranial surgery the medial cerebral artery (MCA) was occluded using a high frequency bipolar forceps. 30 Merino rams were randomly assigned to 4 groups: proximal (n=10) and distal (n=5) MCA occlusion (MCAO), sham operation (MCA was touched but not occluded, n=5) and controls (no operation, n=10). Neurological examinations were performed before and after surgery until day 42 after MCAO (day of killing). Further effects of MCAO were recorded using magnetic resonance imaging (MRI), magnetic resonance angiography (MRA), as well as PET examinations. The animals were euthanized and the brain was thoroughly fixed in formalin (perfusion and immersion), routinely embedded in paraplast and sections were stained with H&E and pikrosirius red. Representative slides were investigated immunohistochemically (glial fibrillary acidic protein (GFAP)).

Results: Proximal MCAO results in a large cortical lesion and clear neurological dysfunctions, whereas distal MCAO produces a smaller cortical lesion and reduced functional deficits. Macroscopically, the infarct extension deeply depends on the localisation of occlusion with the proximal MCAO showing the widest infarction area. Nevertheless, comparing the macroscopical area of infarction to the area with histomorphological changes, the latter always is a little more extensive. Histomorphologically, the ischaemic necrosis resembles changes described for stroke in several animal models and in humans with fat granule cells presenting the majority of visible cells in the necrotic areas. At the border to still viable neuropil (penumbra) fibrosis and granulation tissue can be detected and the neuropil shows decent astrogliosis (GFAP) as well as hypoxic cell changes of the neurons.

Discussion: The sheep model serves as an adequate large animal model in order to investigate changes caused by stroke to be transferred to human medicine. The perspective of this study is to investigate the efficiency of autologous bone marrow and umbilical cord blood cell therapy on clinical/functional and pathomorphological parameters in this new large animal model.

Apoptosis involvement in hepatitis of naturally porcine circovirus type 2 (PCV2) infected pigs.

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Introduction: Porcine circovirus type 2 (PCV2) belongs to the genus Circovirus of the Circoviridae family and is the causative agent of postweaning multisystemic wasting syndrome (PMWS). PCV2 ORF3 gene encodes the ORF3-protein, which is apparently involved in viral induced-apoptosis in vitro. PCV2 induced hepatitis is characterized by cellular alterations such as hepatocyte cytoplasmatic swelling and vacuolation, karyomegaly and single hepatocyte apoptosis/necrosis with formation of Councilman bodies. Investigations using the TUNEL assay have resulted in conflicting results in respect to this phenomena´s occurrence in PCV2 induced hepatitis.

Material and methods: Apoptosis in formalin fixed liver tissue was assessed with cleaved caspase-3 (CCasp3) immunohistochemical detection. Five control livers and 31 hepatitis cases from pigs infected with PCV2. Quantification of serum PCV2 load was assessed by RT-PCR in 28 hepatitis cases. Apoptotic rates were measured using a CCasp3 semi-quantitative scoring approach. Double immunolabelling for detecting CCasp3 and PCV2 genome was done in a severe hepatitis cases to potentially assess the correlation between PCV2 infected cells and apoptosis.

Results: CCasp3 labelled figures rate was minimal in normal liver. Negative and mild PCV2 infected livers also displayed low rates of apoptosis. In contrast, livers with higher amounts of PCV2 showed higher apoptotic indices; apoptotic hepatocytes and pre-apoptotic hepatocytes were frequently labelled with the anti-CCasp3 antibody. Overall, apoptotic rates increased significantly with the severity of hepatitis and with the intensity of PCV2 infection of the liver. Double-labelling showed that hepatocytes could be found both stained for CCasp3 (cytoplasm) and PCV2 DNA (nucleus), only cytoplasmatic stained for CCasp3 or only PCV2 DNA labelled, both nuclear and cytoplasmatic or simply nuclear. PCV2 genome amounts in the liver were positively correlated with PCV2 viraemia load.

Discussion: It was concluded that (1) caspase-3 is indeed activated in PCV2 associated hepatitis, (2) PCV2 is an apoptosis-inducer virus in hepatocytes under natural infection conditions, and (3) cleaved caspase-3 immunohistochemistry method offers an alternative to routine histological apoptosis assessment and its quantification in pig liver tissue.

Genetic and pharmacological tools suggest that uterine Interstitial Cajal-like cells induce KIT dependent myometrial contractions in the mouse.

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Introduction: In the gastrointestinal tract, Interstitial Cells of Cajal (ICC) have a pacemaker activity: they generate slow waves that trigger and coordinate gut smooth muscles contractions. Their post-natal development, survival and activity depends on the receptor tyrosine kinase KIT, encoded by the Kit gene. Based on KIT expression, ICC have been described in other muscular tissues such as the urethra and urinary bladder.
Mouse uterine myometrium also shows spontaneous contractions. However, the identity of the cells responsible for these contractions is still unknown and the presence of ICC in the uterus is a source of controversy.

Material and methods: We investigated the presence of KIT-positive, functional uterine ICC in adult nullipare and ovariectomised 129 S2/SvPa female mice. To detect Kit uterine expression, we used either wild-type or heterozygous mice for the KitW-lacZ allele, which allows easy labelling of gut ICC.
Ex vivo myometrial spontaneous activity was recorded. To test for a potential KIT dependence of this activity, we used both genetic and pharmalocogical approaches, respectively with Kit mutant mice and Imatinib mesylate (Glivec ND), a potent KIT inhibitor drug (an inhibitor of KIT tyrosine kinase signalling and a therapeutic agent used in cancerology against tumours whose proliferation is KIT dependent).

Results: We demonstrated the presence of KIT-expressing cells between uterine muscular layers. The uterine myometrium of 129 S2/SvPa Kit+/+ mice exhibited both longitudinal and circular spontaneous organized contractions. Absence of one Kit copy did not induce a haploinsuffuciency phenotype. In the homozygous hypomorphic KitW-v/W-v mutant mice, a dramatic reduction of longitudinal myometrial contractions was observed. Furthermore, Imatinib abolished spontaneous circular and longitudinal myometrial contractile activity in a dose-dependent manner.

Discussion: This experiment showed Kit-positive ICC-like cells in the murine myometrium. Future experiments have to be done: transmission electron microscopy might confirm the ultrastructural ICC identity, and recordings of their electric slow waves might be KIT-dependent.
With both genetic and pharmacological approaches, we obtained a KIT-dependent inhibition of longitudinal and circular uterine contractions in mice. This result could lead to new treatments against myometrial hypercontractility in order to enhance embryonic implantation. Furthermore, as gastrointestinal stromal tumours (GIST) are distinguished from leiomyosarcomas based on KIT expression, uterine stromal tumours could be diagnosed by the same means. This distinction could lead to treatment of uterine stromal tumours with inhibitors of tyrosine kinases, which have proven to be very efficient for GIST.

Unusual case report of Marek's disease in Tehran.

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Introduction: Marek's disease is a Herpes virus infection of chickens and rarely turkeys in close association with chickens, seen worldwide.

Material and methods: This is a case report of a broiler chicken. It was referred to the Department of pathology, Tehran University. A small tumour mass (lemon-like) was on its head with 3 cm in diameter. It was suspected to be a brain or skull tumour, but no clinical signs were seen. The chicken was euthanized. After a midsagital incision on the head, the mass was seen under the skin and upon the skull, so its origin was not bone or brain. For histological examination, tissue samples were chosen from the tumour mass and other organs such as the lung, kidney, liver and brain. Samples were fixed in 10% buffer formaldehyde. Thin paraffin sections were stained with H&E.

Results: Grossly, the tumour mass was circumscribed with a thin capsule. Cut surface of the neoplasm was yellow to gray. Histopathology revealed a diffuse infiltration with a pleomorphic population of lymphomatous cells. Infrequently, large, darkly stained so called Marek's disease cells were present amongst the infiltrate and it confirmed the diagnosis Marek disease.

Discussion: Only a small percentage of infected chickens develop clinical MD. Diagnoses, therefore, must be based on disease specific criteria such as pathology, epidemiology and possibly tumour specific antigens. It must be differentiated from leukosis. Cytologically, lymphoid leukosis tumours are generally composed of a homogeneous population of lymphoblasts. In contrast, the growths of MD contain lymphoid cells that vary in size and maturity from lymphoblasts to small lymphocytes and plasma cells may also be present.

Histological signs of testicular dysgenesis in 8 dogs: counterpart of human Testicular dysgenesis syndrome?

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Introduction: Human male reproductive disorders increased during the last fifty years, demonstrating a higher prevalence of cryptorchism and hypospadia, decreasing semen quality and increase incidence of testicular cancer. All these changes are symptoms of a common entity called testicular dysgenesis syndrome (TDS). Environmental chemicals, such as pesticides and phthalates, acting as hormone disrupters and interfering with male sexual differentiation, have been advocated in the pathogenesis of TDS. TDS has been reported in humans, fish, reptiles, birds and in wildlife mammals and was reproduced in laboratory animals by phthalates administration. This study describes the occurrence of morphological findings suggestive of TDS in canine species.

Material and methods: In this study testes from 38 dogs (3 to 16 years old) where checked for the histological features considered typical of human TDS: dysgenetic tubules with thick and hyaline basal membranes, Sertoli cell only tubules (SCO tubules), and Leydig cell hyperplasia. Moreover, immunohistochemistry for placental alkaline phosphatase (PLAP) was employed to recognize tubules lined by gonocytes, named carcinoma in situ (CIS) and described in human pathology as seminoma precursor lesions.

Results: In 8/38 dogs (21%), ranging in age from 8 to 16 years, lesions consistent with TDS features were detected. These features were present singly or in combination and displayed different degrees of severity. Moreover, in all the 8 cases testicular tumours were present and frequently both of the testes were affected. Seminomas (SEM) were present in all cases and coexisted with PLAP-positive CIS-like tubules. Leydig cell tumours were detected in 2/8 dogs.

Discussion: The occurrence of histological features described in humans and wildlife animals as suggestive of TDS were observed in the 21% of canine testes examined in this study. Moreover, in all the cases examined, SEM and CIS-like structures coexisted, suggesting that in canine species, as in man, CIS may represent a possible precursor lesions for testicular germ cell cancer. Seminiferous tubules characterized by a thick and hyaline basal membrane have been frequently described as aged-related changes in the testes of men and animals and frequently they have been associated to an increase in interstitial fibrosis. Although, the animals examined presented lesions similar to the human TDS were aged, no signs of fibrosis were present in adjunct to the TDS lesions. In human, the most severe cases of TDS present a combination of clinical signs but, in less severe cases, only the microscopic dysgenetic features may be present. Further investigations on a wider number of dogs, including young animals, are required to corroborate the findings of this study and to evaluate the incidence of TDS and its consequences on reproductive functions in canine species. Moreover, further studies are also required to ascertain the impact of environmental factors on the pathogenesis of male reproductive disorders in the dog.

A real-time PCR assay for the detection of Lawsonia intracellularis in porcine intestinal mucosa samples.

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Introduction: Lawsonia intracellularis is an obligate intracellular bacterium causing porcine proliferative enteropathy (PPE), a transmissible enteric disease which is common among growing and finishing pigs throughout the world. The bacterium affects the aboral small intestine, in particular the ileum. Data of Warthin-Starry silver impregnation and immunohistological examination point to a correlation between the number of bacteria present and the severity of lesions and disease. The aim of this study was to establish a quantitative detection method for Lawsonia intracellularis in tissue samples. The results were linked to those of a previous study which used conventional PCR, IHC, ISH and Warthin-Starry silver staining.

Material and methods: In the years 2005 and 2006 204 pigs with a history of diarrhoea and / or retarded growth were submitted for necropsy to the Institute of Pathology and Forensic Veterinary Medicine of the University of Veterinary Medicine, Vienna. Mucosal samples were scraped from the ileal wall, tissue samples were taken from the ileum for histologic examination, and samples of colonic contents were collected. 51 of these animals showed positive or questionable results for Lawsonia in a previous study with IHC, ISH, Warthin-Starry silver staining of histological sections and / or conventional PCR of tissues and faeces. A TaqMan real-time PCR protocol was established for specific detection of the 16S ribosomal RNA of Lawsonia intracellularis. The probe complementary to the genome was labelled with FAM at the 5' end and TAMRA at the 3' end. Plasmids containing a correlating 61 bp insert from L. intracellularis served as positive control. 10 animals which had been tested negative in the previous study were taken as negative controls. Results from the real-time PCR were compared to those previously obtained.

Results: The established real-time PCR assay yielded clear and constant signals. The achieved Ct values and a serial dilution of the positive control plasmids allowed a quantification of the genetic copies extracted from the tissue samples.

Discussion: Real-time PCR, although more expensive, has great advantages to the more time consuming conventional PCR. The additional fluorescent probe needs to hybridize with the produced amplicons which increases the specificity of the assay. Furthermore, the detected signal can be used to quantify the amount of target gene in the sample. This may be especially useful for distinguishing between acute infections harbouring large amounts of bacteria from resolving infections in which the bacteria are markedly reduced in number and sometimes even undetectable by morphological labeling techniques.

In-situ hybridization as useful tool for the detection of gastrointestinal protozoa in snakes.

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Introduction: Inflammation of the digestive tract in snakes often is associated with protozoa. Because of the special climatic conditions that reptiles require, these animals usually are kept in confined enclosures. Especially parasites with a direct life cycle profit from a lack of hygiene and therefore may lead to repeated autoinfections and spread quickly between terraria. If the parasitic burden amounts to high numbers of protozoa and / or the snake is challenged with immunosuppressive factors, parasites may lead to serious illness. A specific diagnosis is important for the correct choice of preventive steps and therapeutic measures.

Material and methods: During the years 1996 to 2005 a total number of 208 snakes were submitted for necropsy at the Institute of Pathology and Forensic Veterinary Medicine of the University of Veterinary Medicine Vienna. Paraffin embedded tissue sections from the digestive tract of these animals were reinvestigated histologically with H&E and PAS staining. Protocols for chromogenic in-situ hybridization were established detecting sequences from the 18S ribosomal RNA of Cryptosporidium spp., Entamoeba spp., Entamoeba invadens and Monocercomonas spp. Samples from inner organs were investigated additionally in those snakes which were positive for Entamoeba spp. or Monocercomonas spp.

Results: Seven snakes had positive results for Cryptosporidium spp. in stomach or small intestine. 13 animals were positive with the general Entamoeba probe in the colon but only nine of them were also positive with the Entamoeba invadens probe. In six of these nine animals E. invadens was found spreading into extraintestinal organs. In 34 snakes Monocercomonas spp. could be detected in stomach, small and / or large intestine.

Discussion: The detection of protozoa in standard histological sections can be difficult. They are easily overlooked especially if present in only small numbers and if the infected tissue does not show extensive and typical inflammatory reaction. Small organisms like Monocercomonas spp. show very little inner structure and blend in with surrounding structures and cells. Special stains like PAS may help to recognize organisms but are not specific. With in-situ hybridization also similar looking protozoa can be differentiated. The possibility of their localisation inside tissue sections and of the evaluation of surrounding inflammatory reaction helps in distinguishing pathogenic protozoa from pseudoparasites. The latter, being parasites of the prey, are apathogenic for the snake but are passed through its digestive tract and thus may lead to false positive diagnoses.

First report of circoviral infection in pigs in Republic of Macedonia.

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Introduction: Porcine circovirus type 2 (PCV2) is nowadays considered the causative agent of post-weaning multisystemic wasting syndrome (PMWS), an emerging disease affecting nursery and growing pigs.

Material and methods: Tissues for this research were collected at necropsy of debilitated 5 to 12-week-old piglets, exhibiting multiple clinical signs and multisystemic lesions. Formalin-fixed, paraffin-embedded tissues (lymph nodes, lungs, intestine, spleen, pancreas, kidneys and liver) from 30 pigs originated from 4 different pig farms in Republic of Macedonia, were investigated by immunohistochemistry (IHC) for the presence of circoviral antigen using F217 2C6-H9-A2 monoclonal antibody and EnVision Kit (Dako ChemMate, Denmark). Also, histological sections of all selected cases were stained with H&E technique and reviewed histologically to verify the presence of histopathological changes.

Results: Commonly PCV2 affected pigs were those of 2 to 3.5 months of age. The symptoms include wasting as a major sign, but also unthriftness, pallor of the skin, respiratory distress, diarrhea and occasionally icterus. Gross lesions in affected piglets include slight increase in the size of the lymph nodes, lymphadenopathy, hepatitis and nephritis. Moreover, those pigs had a moderate to severe diffuse interstitial pneumonia with intense interstitial oedema, multifocal haemorrhages and disseminated intravascular coagulation.
Histological lesions were distinctive and consisted of depletion and occasional necrosis of lymphocytes in the cortex and paracortex of lymph nodes. There were often numerous multinucleated giant cells scattered in the same areas. The lungs showed variable hyperplasia of bronchus-associated lymphoid tissue. In some cases it was possible to see large histiocytic and multinucleate giant cells in the thickened interalveolar walls. Lympho-histiocytic inflammatory infiltrates were observed in kidneys, pancreas, intestine and myocardium.
Circoviral antigen was present in large, round or dendritic cells, in remnants of follicles and less frequently, in giant cells and fixed sinusoidal mononuclear phagocytes of lymph nodes. Also, the antigen was present in scattered large cells within the hyperplastic bronchus-associated lymphoid tissue and lymphoid follicles in the palatine tonsils. Sporadically, the virus was detect in the cytoplasm of renal and respiratory epithelium, vascular endothelium, hepatocytes and enterocytes.

Discussion: The present study indicates that PCV2 can be a predisposing factor for development of PMWS lesions. The observed pathomorphological lesions in affected pigs, typical of PMWS, correlated with the detection of PCV2 antigen. PCV-antigens were regularly detected in cells of monocyte/macrophage lineage of multiple organs. A strong correlation has been observed between the amount of PCV2 nucleic acid and the severity of microscopic lesions of lymphoid tissue.

Cerebellar cortical atrophy in a Belgian Blue cow associated with lesions described in human Norman-Jaeken disease.

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Introduction: Cerebellar cortical atrophy is characterized by selective degeneration of Purkinje cells of cerebellar cortex. It can be divided in two major groups. The first group is called “abiotrophies”. They have been described in most of the domestic animals species and in a few rodents and primates. The other group is called “hypoplasia”, which indicates that the cerebellum has failed to develop to its full potential. This can result from in utero viral infections. The abiotrophic process is more viewed as affecting the organ after it has developed its full cellular complement. Differentiation from the effects of in utero infection with bovine virus diarrhea is important in cattle. This is not difficult in those abiotrophic disorders in which calves are not affected until several months of age. However, in some cases, calves are affected at or within a week of birth. In humans, cerebellar cortical atrophy is mainly described as primary degeneration of the granular layer, also known as Norman's type.

Material and methods: In April 2007, a Belgian Blue cow, twenty-one months and originating from 105-unit herd (mixed and meat types) was presented for slaughter with neurological symptoms, including ataxia, tendency to fall, myoclonia, hypersalivation and hyperexcitation. The owner declared that the animal already had similar problems since birth. Subsequently, the veterinary inspector included the animal in the routine protocol for ruminants that are suspected of transmissible spongiform encephalopathy. Samples of the brain stem, the cerebrum and cerebellum were taken for further examination for BSE, rabies and BVD. Large samples of the three major brain parts mentioned were fixed in a 4 % phosphate-buffered formaldehyde solution, processed routinely, paraffin-embedded, and sectioned at 5-µm thickness. Sections were stained with H&E.

Results: The BSE and rabies tests were negative. The histopathological lesions were mainly concentrated in the cerebellum with focal loss of granular cells in different stages (varying from limited loss, over remnant part of the layer to total absence of the granular cells) and Purkinje cells that were frequently arranged in clumps or dislocated into the molecular layer.

Discussion: Our study reports for the first time on cerebellar cortical atrophy in the Belgian Blue breed. Additionally, in contrast to all other cases described in cattle, it concerns an adult animal. The histopathological lesions found were more compatible to the human counterpart, also called the Norman type of cerebellar ataxia (or recently Norman-Jaeken type). Finally, this report also indicates that cerebellar cortical atrophy should be added to the list of differential diagnosis of bovine spongiform encephalopathy.

Transmissible Spongiform Encephalopathies: Ultrastructural findings and immunohistochemical study of the intestine in an experimental murine model of Scrapie.

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Introduction: Despite the importance of the knowledge of the pathogenesis of Transmissible Spongiform Encephalopathies (TSE) and their relevance in public health, there are only a few studies concerning the early steps of the infection at the entry sites. The aim of our study was to evaluate the possible role of cytokines in the intestine and to describe the ultrastructural changes that could appear in these locations after an experimental inoculation with a TSE agent.

Material and methods: 84 C57BL mice were orally inoculated with the scrapie RML strain, while 30 animals with the same characteristics were maintained without inoculation and used as negative controls. Animals were slaughtered in batches of six from 15 to 300 days postinoculation (dpi). Samples from intestine were fixed in 10% buffered formaldehyde and zinc solution and embedded in paraffin-wax. H&E and avidin-biotin-complex-peroxidase techniques were used for morphological and immunohistochemical studies. Samples from the same locations were fixed in 2.5% glutaraldehyde, postfixed in 2% osmium tetroxide and embedded in Epon 812 for transmission electron microscopy.

Results: Animals showed typical symptoms of the disease from 270 dpi, consisting in stagger, arched back and erected tail and hair. However, no gross or microscopical lesions were found in the necropsies or in the structural study. The immunohistochemical study carried out, using the Rb486 antibody, showed the presence of PrPd in tingible body macrophages and in dendritic cells of Peyer's patches from 60 dpi onwards. The number of macrophages stained against MAC-3 antibody peaked at 60 dpi, while TNF-alpha expression was decreased at this time. No significant changes were found in IL-1alpha, IL-6, IL-10 and IFN-gamma expression.

Discussion: Despite the increased number of macrophages observed at dates where PrPd deposits were detected for the first time, these cell populations showed the lowest TNF-alpha expression at this timepoint. This non-activation signal, in addition to the low expression of IL-1alpha and IL-6 observed, could be due to a low production of IFN-gamma by T-lymphocytes but not to a high expression of IL-10. Furthermore, prions could play a role in the recruitment with no secretory activation of macrophages in these locations, mechanism by which the agent could replicate and spread through the organism. On the other hand, ultrastructural study will contribute to the knowledge of the pathogenesis of prionic diseases, evaluating subcellular changes that could appear in the gut-associated lymphoid cells and intestinal cells due to the presence of the agent.

Thymic neuroendocrine carcinoma in a dog: a case report.

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Introduction: A 9-year-old, 26-kg intact female Labrador Retriever was referred after an unsuccessful chemotherapy for a presumed mediastinal lymphoma. Physical examination revealed a severe stomatitis. Thoracic radiographs disclosed a large mediastinal mass. Multidetector computed tomography (MDCT) showed a well defined, inhomogeneous mass, surrounded by thymic tissue with normal CT characteristics. The regional lymph nodes were normal.

Material and methods: Punch biopsies of the oral cavity lesions were obtained. Samples were obtained from CT-guided surgical debulking of the mass and processed for histopathological examination.

Results: Specimens of the mass showed a mixed lymphoid population. Oral cavity biopsies revealed an erosive severe stomatitis suggestive of erythema multiforme (EM). Histological findings of the mass consisted of sheets of polygonal cells separated by delicate fibrovascular stroma. Neoplastic cells possessed lightly eosinophilic finely granular cytoplasm, centrally placed nucleus with fine chromatin and one nucleolus. Mitotic count was 1-2/HPF. Areas of coagulative necrosis and aggregates of lymphocytes were seen. Differential diagnoses included: paraganglioma, metastatic neuroendocrine carcinoma, thymic neuroendocrine carcinoma, thymoma, large cell lymphoma, mast cell tumour. Immunohistochemistry revealed positivity for cytokeratin AE1/AE3, chromogranin A, synaptophysin with accessory CD3 positive lymphocytes and vimentin positive connective stroma. Based on histological and immunohistochemical findings a diagnosis of thymic neuroendocrine carcinoma was performed. At the moment of the writing the dog is on good general conditions, in absence of any sign of recurrence.

Discussion: In human medicine neuroendocrine carcinomas of the thymus are rare and encompass two entities: thymic neuroendocrine carcinoma (TNC) and small cell neuroendocrine carcinoma (SCNC). The vast majority of TNCs and all reported SCNCs were presented with signs of thoracic structural displacement. TNCs have an indolent course with occasional recurrence, rare metastatic spread and are surgically resectable. Fairly 30% of case are associated with Cushing's syndrome and up to 25% of TNCs can occur as a component of MEN-1. Differential diagnoses comprise: metastatic neuroendocrine carcinoma, thymoma, large cell lymphoma, mast cell tumour, paraganglioma and germ cell tumour. Immunohistochemical markers are helpful in the diagnostic workout. TNCs stain for cytokeratins, chromogranin, synaptophysin. They often stain for hormones such as ACTH, somatostatin, serotonin, and B-endorphin. The present case illustrates a case of TNC and EM of the oral cavity. It clearly shows that immunohistochemistry is necessary to investigate mediastinal masses and that cytology alone is unreliable, because of the lymphoid component of any mediastinal structure, misleading toward a lymphomatous proliferation. EM is commonly diagnosed as paraneoplastic condition in a variety of neoplasms of dogs. Although never reported before, an association between EM and the TNC object of this case presentation it is likely. To the best of the authors' knowledge this is the first case of TNC described in a dog.

P-Glycoprotein immunodetection as a prognostic marker of canine cutaneous mast cell tumour.

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Introduction: One of the most frustrating aspects of canine mast cell tumour (cMCT) treatment with chemotherapy agents is the development of multidrug resistance (MDR) due to increased levels of Permeability-glycoprotein (Pgp). The aim of this study was to describe Pgp expression and to verify the relationship between histological grade and survival time.

Material and methods: Surgical samples of 42 cases of cMCT were evaluated according to WHO diagnostic criteria. Immunohistochemical staining was performed using mouse monoclonal antibody against human Pgp (C494). Pgp-positive cells were evaluated semiquantitatively counting 10 HPF areas. The percentage of neoplastic cells was scored on a scale of 0 to 3. The correlation between Pgp expression and the MCT grade was investigated using the nonparametric Spearman correlation coefficient and a survival analysis approach was used on a study period of 12 months. All the analyses were performed with the software SPSS 12.0 (SPSS Inc., Chicago).

Results: The 42 cases of cMCT were allocated into grade I (24), grade II (9) and grade III (9). Pgp positive cells were found in 32/42 (76.2%) MCTs with 15/24 (62.5%) grade I, 8/9 (88.9%) grade II, and 9/9 (100%) grade III. Statistical analysis performed showed: grade I vs III ($U=4.50$; $P<0.001$) and grade II vs III ($U = 0.0$; $P<0.001$) and a positive correlation between Pgp expression and histologic grade of canine cutaneous mast cell tumour (Spearman $Rho = 0.704$; $P< 0.001$). Survival analysis showed that a higher positivity score for Pgp is associated with a shorter overall survival time (Wilcoxon (Gehan) statistic = 29.340; $P<0.001$).

Discussion: Overexpression of Pgp is associated with resistance to different classes of anticancer compounds. This study describes Pgp expression in cMCT with preliminary follow up data. MDR gene products like Pgp are highly conserved between human and canine tissue. Accordingly, cross-reacting anti-Pgp antibodies can be used for the immunohistological detection of Pgp expression in canine tissues. It was also reported that Pgp is expressed in spontaneous canine lymphomas and that the level of expression is correlated with the chemotherapeutic response. In a paper describing Pgp and MRP expression in cMCT, the authors found higher levels of expression in well differentiated neoplasms. In this investigation, immunohistochemical detection revealed a higher percentage of Pgp positive cells in MCTs with a more malignant phenotype (grade III) with Pgp immunostaining predominantly distributed along the cell membrane.
A positive relation exists between Pgp expression and histological grade. Moreover, in agreement with literature data that deals with overall survival time and MCT grade, survival analysis showed that patients with an elevated percentage of Pgp positive cells (overexpressing Pgp) exhibited a shortened overall survival time. Follow up data and preliminary survival analysis revealed that high grade and an elevated percentage of Pgp positive cells/HPF are correlated with a poor prognosis and a shorter survival time. On the basis of this study, we can consider Pgp expression as an indicator of worse prognosis, but a more extensive follow up study after chemotherapy protocol would be extremely useful in suggesting a routine immunohistochemical evaluation for Pgp as a MDR marker.

Evaluation of Imatinib effect in an ex vivo culture system of skin samples from dog with grade II mastocytoma.

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Introduction: Mast cell neoplasms are the most common cutaneous tumours in dogs, metastasizing to local lymph nodes, liver, spleen, and bone marrow. Canine MCT are difficult to control by local treatment and these tumours are resistant to systemic chemotherapy as well. Canine mast cells express the c-KIT proto-oncogene that encodes the type III receptor tyrosine kinase KIT (CD117), an extracellular ligand binding domain. Mutations in the c-KIT proto-oncogene have been implicated in the progression of canine cutaneous MCTs. In this tumour, the most important mutations observed are deletions and internal tandem duplication (ITD) found in the juxtamembrane domain of c-KIT. These ITD c-KIT mutations are significantly associated with aberrant KIT protein (CD117) localization in MCTs. Recently, different studies indicated 3 patterns of KIT protein localization in canine MCTs (peri-membrane, cytoplasmatic stippling to focal, and diffuse cytoplasmatic KIT localization). Well-differentiated tumours weakly express KIT and poorly differentiated tumours have a high expression of KIT (3). The selective tyrosine kinase inhibitor imatinib mesylate (STI-571 or Gleevec®) has recently been licensed. However, the ability of this drug to inhibit signal transduction and induce apoptosis in canine MCTs is not investigated. Our goal was to examine the ability of STI-571 to induce apoptosis in an ex vivo culture system.

Material and methods: 20 to 3 cm width - punch biopsies, taken from a canine voluminous grade II mast cell tumour, were cultivated for 24 to 110 h in vitro with normal RPMI 1640 with l-glutamine, 10% FCS, 1% penicillin-streptomycin-fungizone medium (control) or the same medium containing a concentrations of 15.8 mg (corresponding to 10 mg/kg) of STI-571. Cultured biopsies were stopped daily, fixed, paraffin embedded, and stained with Ki67 (MCTs proliferation), Bcl-2 (MCTs anti-apoptotic activity), and CD117 (KIT accumulation in MCTs). Apoptosis was assessed by TUNEL method.

Results: Biopsies treated with STI-571 showed at 48, 72, and 96 h a progressive and significant increase in TUNEL$^+$ MCTs compared to untreated ones. A reduction in Ki67 and BCL-2 expression was observed. CD117 positivity was unaltered throughout the entire experiment in control samples for intensity and pattern of distribution. In treated samples, a high reduction of expression, associated with a strong cytoplasmatic-perinuclear intensity stain was observed in scattered remained cells.

Discussion: This study demonstrates that the tyrosine kinase inhibitor STI-571 induces caspase-dependent apoptosis in canine MCTs. Induction of apoptosis is delayed and only occurs after several hours of drug exposure, suggesting the involvement of complex intermediate pathways. Survived CD117-strong stained cells may represent a STI-571-resistent/poor differentiated cells, probably involved in tumour recurrence.

Intestinal immune response in sheep with different pathological forms of paratuberculosis.

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Introduction: Mycobacterium avium subsp. paratuberculosis (MAP) is the causative agent of paratuberculosis, a chronic granulomatous enteritis of wild and domestic ruminants. Characterization of the T-cell subsets in intestinal lesions in sheep with paratuberculosis may contribute to understand the pathogenesis of this disease.

Material and methods: Immunohistochemistry was performed on 8 normal sheep and 14 naturally infected and clinically diseased sheep (8 with lepromatous and 6 with tuberculoid forms). The phenotype and distribution of lymphocytes has been determinated in the normal sheep intestinal mucosa and in MAP infected sheep. Immunoperoxidase labelling was carried out on paraffin and frozen sections of ileum using monoclonal antibodies against ovine CD4, CD8 and regulatory ($CD4^+CD25^+$) T cells.

Results: In all three sample groups, cells appeared to be non-randomly distributed throughout the lamina propria. Higher densities of lymphocytes were present in villus than in crypt areas. $CD8^+$ cells were located principally around the epithelial basement membrane, whereas $CD4^+$ cells were localized towards the central villus area of the lamina propria. Sheep with lepromatous-multibacillary form showed a reduced number of $CD4^+$ T cells, compared to animals affected by tuberculoid-paucibacillary form. The quantity of $CD25^+$ T cells in the ileum had a similar trend and the difference was not significant. Additionally, RT-PCR demonstrated higher levels of mRNA for TNF-α, Il-1β, IFN-γ, MCP-1, IL-12, IL-10 and TGF-β in infected animals than in control sheep. In ileal tissues of sheep with tuberculoid-paucibacillary form IFN-γ, MCP-1 and IL-12 mRNA was upregulated while in sheep with lepromatous-multibacillary form TNF-α, Il-1β, TGF-β, and IL-10 mRNA overexpression was observed.

A subcutaneous thoracic mass on a Sibirian tiger (Panthera tigris altaica).

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Introduction: The present case report describes a malignant peripheral nerve sheath tumour in a Siberian Tiger (Panthera tigris altaica). Until now, only one single case of a benigne peripheral nerve sheath tumour in a large felid has been reported.

Material and methods: Tissue samples from the skin and the internal organs were processed for H&E staining. Immunohistochemistry was performed using the antibodies for CD3, CD79a, desmin, factor VIII related antigen (von Willebrand factor), glial fibrillary acidic protein (GFAP), lysozyme, MAC 387, melan A, neuron specific enolase (NSE), pan cytokeratin (Lu5), smooth muscle actin, S100 protein, and vimentin.

Results: Three cutaneous, ulcerating masses were located dorsally and ventrally of the last rib. In addition, multiple greyish, firm, lobulated masses were found in the lung and mediastinum. Histologically, the well demarcated, invasively growing masses consisted of highly pleomorphic cells arranged in bundles or sheets separated by a fine fibrovascular and collagenous stroma. The cells were partly spindle-shaped and partly plump fusiform to epithelioid with an abundant eosinophilic homogeneous cytoplasm. In some areas, several multinucleated giant cells were visible. The metastases showed less extracellular matrix and were less differentiated. Rarely, the neoplasm showed differentiation to a myxoid pattern with abundant extracellular matrix consisting of mucopolysaccharide with embedded stellate cells. The neoplastic cells were labelled with antibodies specific for vimentin, S100, GFAP and NSE. The remaining antibodies tested were negative.

Discussion: The histomorphological appearance and the relatively rapid clinical course were initially suggestive of an anaplastic sarcoma with giant cells (previously classified as malignant fibrous histiocytoma), rather than a malignant peripheral nerve sheath tumour (MPNST). However, the positive signals for neural and mesenchymal markers (S100 protein, GFAP, NSE, and vimentin) indicated that we were dealing with a malignant peripheral nerve sheath tumour of the skin with metastases in the mediastinum and lungs. A mass with similar histological appearance and immunohistochemical results has been described in a domestic cat. Malignant PNSTs may vary significantly in morphology, requiring the application of a panel of appropriate immunohistochemical markers to reach a final diagnosis for tumours that lack clear morphological characteristics. In conclusion, we propose MPNST as a differential diagnosis for an anaplastic sarcoma with giant cells.

Evidence of Parachlamydia in bovine abortion - a potential emerging zoonotic risk?

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Introduction: Waddlia chondrophila type strain WSU 86-1044 was implicated as an abortigenic agent in 1986 when it was detected in the lung, liver, and other tissues of an aborted bovine fetus in the USA. A growing body of evidence supports the role of Parachlamydia and Simkania as agents of pneumonia in humans. The significance of these recently discovered Chlamydia-like organisms in ruminant abortion, as well as their potential zoonotic risk to humans, remains largely unexplored. Hence, we aimed to investigate the incidence of Chlamydia-like organisms infections in abortions in cattle from Switzerland.

Material and methods: During the breeding seasons of 2003 - 2004, formalin-fixed and paraffin-embedded placenta specimens (n = 235) from late-term abortions in cattle were analyzed by histopathology, immunohistochemistry (IHC) using a polyclonal antibody against Parachlamydia and Waddlia, respectively, and polymerase chain reaction (PCR) method targeting the 16S rRNA of the order Chlamydiales, followed by sequencing. Two selected cases were further investigated by transmission electron microscopy (TEM) for the ultrastructural evidence of Chlamydia-like organisms.

Results: In 149 of 235 cases (63.4%), histopathological lesions such as purulent and/or necrotizing placentitis were observed. By 16S rRNA PCR, 43 of 235 cases (18.3%) were detected to be positive and sequencing exhibited 82-100% sequence similarity with Chlamydia-like organisms. From these 43 cases, nine cases revealed high sequence similarity to Parachlamydia (P.) acanthamoebae (91-99%), whereas in the other 34 cases, a definitive identification was not possible. Thus, these isolates were referred to as Chlamydia-like organisms. Placentitis could be found in five of nine cases positive for P. acanthamoebae and in one case (1/9) vasculitis was detectable. By IHC, positive antigen labeling was observed in seven of nine cases for Parachlamydia, but it was negative in all cases for Waddlia. In two cases positive by PCR and IHC for P. acanthamoebae Chlamydia-like structures were observed by TEM. In 28 of 34 cases (82.3%) positive for Chlamydia-like organisms other than P. acanthamoebae, purulent and/or necrotizing placentitis was obvious; four of 28 cases displayed additional vasculitis. By IHC, 23 of 34 cases (67.6%) were positive with the antibody against Parachlamydia, whereas all 34 cases were negative with the antibody against Waddlia.

Discussion: This is the first description of Parachlamydia in the setting of bovine abortion. The organism was not only detected by PCR, but could be demonstrated within the placental lesions by IHC using an antibody specifically directed against Parachlamydia. All cases were negative for Waddlia by PCR or IHC. As Parachlamydia may be involved in bronchitis and pneumonia in humans, caution should be taken when handling bovine abortion material because of the potential zoonotic risk. Overall, the significance of Chlamydia-like isolates other than Waddlia chondrophila remains an open question and needs further investigation.

An ulcerative skin lesion associated with natural Trichinella infection in a cat.

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Introduction: Trichinella spp. are capable of infecting huge varieties of mammalian species, including companion animals. Natural infection occurs by ingestion of raw meat e.g. infected prey. Although experimental and natural Trichinella spp. infections in cats have been reported, clinical manifestations seem to be extremely rare in this species. The present report is the first documented case of clinical trichinellosis in a cat with an ulcerative skin lesion as the main presenting clinical feature.

Material and methods: An 8-year-old, neutered male domestic shorthair cat was presented at a small animal referral practice with an ulcerative skin lesion. The ulcer had appeared two months ago, and it had not been responding to antibiotic therapy with amoxicillin and clavulanic acid (for 2 months) or local treatment with eye drops containing fucidin acid (for 2 months). At the time of presentation a crusting ulcer measuring 7 mm to 4 mm was present at the lower left eyelid. The cutaneous lesion was excised with clear surgical margins under general anesthesia and submitted for histopathology to the referral laboratory in veterinary histopathology (Patovet Finland). Formalin fixed skin biopsy was embedded in paraffin and routinely processed for histopathology. Enzyme-linked immunosorbent assay (ELISA) was used to determine host-produced antibodies against Trichinella.

Results: At histopathology, the epidermis was ulcerated and there was moderate serocellular crusting. The dermis was severely affected by reaction consisting of interlacing bundles of fibroblasts. Numerous lymphocytes, histiocytes, plasma cells, and neutrophilic granulocytes were interspersed among the dense spindle cell population. Moderate to severe perivascular lymphocytic infiltration with few eosinophils was present peripherally within the lesion. In the center of the lesion there was a well preserved coiled nematode larva within an elliptical cyst with collagen capsule. The cat showed anti Trichinella antibodies by ELISA.

Discussion: Trichinella can be identified at the genus level in biopsies based on location (in muscle fiber) and morphology (nurse cell formation, measurements, presence of stichosome in parasite). The presence of cyst capsule and the intensive inflammatory reaction associated with the features of the shape of the cysts suggest that T. nativa may be involved in this case. Epidemiological studies conducted with PCR-based species identification techniques have revealed T. nativa to be the most common Trichinella species in Finnish wild life. When allowed to roam free, most cats are effective hunters, and the owner of this cat had observed that the animal was preying on small mustelidae. The aetiological diagnosis of the present case was initially based on finding a nematode larva identifiable as Trichinella sp. in a skin biopsy and confirmed by detecting Trichinella antibodies in the serum of the cat. The possibility that the presence of the larva in the cutaneous lesion was purely accidental should also be considered. However, unsuccessful treatments with antibiotics and an intralesional location of the larva are suggestive for a role of Trichinella larva in the pathogenesis of this skin lesion.

Changes in lymphocyte subsets in early stages of PRRS virus infection in swine.

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Introduction: Porcine Reproductive and Respiratory Syndrome is a disease in swine caused by a virus belonging to the Arteriviridae family, order Nidovirales. This disease causes important economic losses in pig production. Clinical reports of PRRS have focused primarily on late-term reproductive failure and postweaning mortality losses. The virus primarily replicates in macrophages and is able to spread to the rest of the organs from the lungs.

Material and methods: Twenty eight Landrace x Large White pigs, 5 weeks old, were inoculated by intramuscular route with an European strain of PRRS virus, originated from a Spanish farm which had severe respiratory signs in piglets. Four animals remained as non inoculated animals. Animals were slaughtered painlessly in batches of 4 at 3, 7, 10, 14, 17, 21, and 24 days post inoculation and control animals were slaugthered at the end of the experiment. Blood samples were taken at 0, 3, 7, 10, 14, 17, 21, and 24 dpi and the clinical signs and temperature of animals were monitored daily. Samples from the blood (peripheral blood leukocytes), spleen, retropharyngeal and tracheobronchial lymph nodes were taken and analysed by flow cytometry to detect changes in the lymphocyte subpopulations, using monoclonal antibodies against CD3, CD4, CD8, CD21 and SWC5.

Results: Inoculated animals did not show any significant respiratory or other clinical signs. No significant changes were observed in the total number of leukocytes in inoculated pigs during the experiment. At necropsy, lung lesions consistent with interstitial pneumonia were observed, together with an enlargement of the medial retropharyngeal, tracheobrochial and mediastinal lymph nodes. Most significant changes in lymphocyte subpopulations were an increase in the $CD4^{+}CD8^{+}$ and $CD4^{-}CD8^{high+}$ T lymphocytes and a decrease in the $CD4^{-}CD8^{low+}$ and $CD4^{+}CD8^{-}$ cells.

Discussion: The pathogenesis of immune response to PRRS virus infection is not yet clarified and although vaccines are commonly used, vaccination is only one of several approaches to be considered in designing a control strategy (Prieto & Castro, 2005). A decrease in the $CD4^{+}/CD8^{+}$ ratio has been described by others (Christianson et al., 1993; Shimizu et al., 1996) for infections with American isolates. In this study it has been observed that this decrease is accompanied by an important increase of the number of double positive $CD4^{+}CD8^{+}$ T lymphocytes, a typical memory cell population of pig circulating blood and lymphoid tissues (Zuckermann et al., 1996).

References:
Christianson et al. 1993. Can J Vet Res. 57, 262-268
Shimizu et al., 1996. Vet Immunol Immunopathol. 50, 19-27
Zuckermann et al., 1996. Immunology. 87, 493-499
Prieto & Castro, 2005. Theriogenology. 63, 1-16

Qualitative changes in expression and distribution of immunosuppressive and Th2 cytokines during murine chronic pulmonary tuberculosis.

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Introduction: Control of bacterial growth during chronic infection with Mycobacterium tuberculosis requires Th1 cytokine expression at all times. To avoid collateral damage derived from the inflammatory properties of a continuous expression of Th1 cytokines, the immune system also activates immunosuppressive and Th2 cytokine responses. However, over time the expression of immunosuppressive cytokines (TGFβ and IL-10) and Th2 cytokine IL-4 may override the effect of Th1 cytokines, having a detrimental effect to both, the protective antibacterial response and the development of T cell responses. When this happens, there is a high risk of disease reactivation.

Material and methods: In this study we evaluated qualitative changes in expression and distribution of the immunosuppressive cytokines TGFβ, IL-10 and the Th2 cytokine, IL-4. For this purpose the lungs from C57BL/6 mice infected with a low dose aerosol of Mycobacterium tuberculosis were harvested at 60 and 150 days post challenge and the expression of these cytokines was revealed by immunohistochemistry and in situ hybridization.

Results: Our results showed that after 60 days of the infection, the granulomas appeared as single entities of medium size formed by infected cells surrounded by a lymphocytic cap, with foamy cells located in dilated alveoli. In these granulomas, TGFβ was expressed in cells located in the center of the granuloma and by a few macrophages in the alveoli. Positive expression of TGFβ was associated with early signs of fibrosis in the septae. IL-10 was expressed mainly in macrophages and foamy cells located in the periphery of the granuloma and in a few lymphocytes. IL-4 was expressed highly in the center of the granuloma.

At 150 days post challenge, most of the lung parenchyma was affected by bronchial interstitial pneumonia. The alveolar septae were enlarged with a predominant infiltration by macrophages interspaced with few lymphocytes and neutrophils. At this stage of infection some foamy cells located in dilated alveolae showed early signs of cell death. In areas of the parenchyma showing more advanced stage of infection, there were purulent foci formed by foamy cells and neutrophils. There were also lymphocytes but they appeared more scattered over the lesions than at 60 days post challenge. At this stage, expression of TGFβ was higher than at 60 days post challenge. TGFβ was found mainly in macrophages located within the thickened septae of the alveoli and in fibroblast like-cells showing extensive fibrosis. IL-10 was also expressed highly during this stage of infection, but in contrast to 60 days post challenge, the expression in foamy cells was less intense whereas in perivascular macrophages and lymphocytes this was more intense. Interestingly, many intravascular lymphocytes also expressed IL-10. IL-4 was strongly expressed in foamy cells and in purulent foci.

Discussion: In conclusion, we observed that as the chronic infection with Mycobacterium tuberculosis progresses the expression of the immunosuppressive TGFβ, IL-10 and Th2 cytokines increased and this correlates with worsening of the pathology. The main source of immunosuppressive cytokines is located in macrophages. The expression of IL-4 was always associated with purulent foci in the lungs.

Lack of IL-10 expression during pulmonary chronic infection with M. tuberculosis results in very high levels of expression of the Th1 (IFN-γ and IL-12) and immunosuppressive (TGFβ) cytokines.

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Introduction: It is known that IL-10 down-regulates the expression of Th1 responses. Previous studies indicated that early protective mechanisms appear unaffected by IL-10. However, expression of IL-10 during chronic infection is detrimental to both, the protective antibacterial response and the development of stable mononuclear granulomas.

Material and methods: In this study we further evaluated the role of IL-10 in relation to another immunosuppressive cytokine (TGFβ) during pulmonary infection with Mycobacterium tuberculosis. For this purpose we infected IL-10 gene targeted knockout (IL-10 KO) and C57BL/6 (wild type) mice using a low dose aerosol infection with Mycobacterium tuberculosis. The lungs from infected mice were harvested at 60 days post challenge, and using flow cytometric analysis and immunhistochemistry, we compared T cell populations, Th1 (IL-12 and IFN gamma) and immunosuppressive (TGFβ) cytokines between both groups of mice.

Results: Our results demonstrated that while both groups of mice had similar lung bacterial loads, the lung histopathology differed greatly. Thus, areas of inflammation in the lung parenchyma of IL-10KO mice were more severe than in wild type mice. Furthermore, in the IL-10KO mice there were numerous foci of alveolitis with abundant cell infiltration and the number and size of granulomas were larger than in the wild type mice. When the distribution of T cells and cytokines were compared between both groups of mice, we found that T cells in the IL-10KO mice were interspaced in the parenchyma within the lesions whereas in the wild type mice most T cells were found in clusters of lymphocytes within the granulomatous lesions. In addition, we found that there were significant differences in the expression of Th1 cytokines (IFN gamma and IL-12) and that the IL-10KO mice had significantly higher levels of expression of these cytokines than the wild type mice. Finally, the levels of expression of the immunosuppressive cytokine TGFβ were much higher in the IL-10KO mice than in the wild type mice. Most macrophages within the alveolar septa of the IL-10KO mice expressed high levels of TGFβ.

Discussion: In conclusion our data suggested that IL-10KO mice express high levels of Th1 cytokines and that these mice have higher inflammatory responses than wild type mice because there is no IL-10 to down-regulate the expression of Th1 responses. However, the bacterial load in both groups of mice remained similar because most macrophages in the IL-10KO mice are inhibited by high levels of TGFβ expression.

Antigen-presenting cells: the key of apoptosis during bovine viral diarrhoea?

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Introduction: Antigen-presenting cells as dendritic cells, next to T-lymphocytes, which showed an abnormal expression of IL-4 and IFN-gamma during Bovine Viral Diarrhoea (BVD), are involved in the proliferation and differentiation mechanisms of B-lymphocytes. The aim of this work was to clarify the evolution of dendritic-like cells in the ileum from calves inoculated with the Bovine Viral Diarrhoea Virus (BVDV) and their role in the lymphocyte apoptosis in Peyer´s patches.

Material and methods: Ten colostrum-deprived Friesian calves of 6-8 weeks old were used. Two animals were used as uninfected controls, while the remaining calves received an intranasal inoculation of noncytopathic BVDV genotype-1 strain 7443. Animals were slaughtered in groups of two at 3, 6, 9 and 14 days post-inoculation (dpi). Samples of ileum were fixed in 10% buffered formaldehyde, Bouin's solution, and 2.5% glutaraldehyde and processed for structural, immunohistochemical and ultrastructural studies. The avidin-biotin-complex-peroxidase method in combination with different antigen unmasking techniques were used for immunolabeling infected cells against BVDV (MoAb 15C5 against Gp48) and dendritic cells (S100, alpha-S100).

Results: The number and distribution of the follicular dendritic-like cells immunolabeled against S100 and alpha-S100 antibodies inside the lymphoid follicles of Peyer´s patches did not display significative changes along the experiment. Monocytes-macrophages and lymphocytes were identified by immunohistochemistry as the main target cells for BVDV. In addition, some reticuloepithelial-like cells and follicular dendritic-like cells located in the lymphoid follicles were also immunolabeled against 15C5 monoclonal antibody from 3 dpi. Viral infection of follicular dendritic cells was confirmed by ultrastructural examination. Subcellular structures related to viral infection were membrane-bound inclusions characterized by a floccular, electron-dense content. These subcellular structures contained round or slightly oval particles, roughly 45-55 nm in diameters (core of 25-30 nm) which were tentatively identified as the mature BVD virions. Infected follicular dendritic cells showed an increase in size as well as abundant cytoplasm.

Discussion: The viral infection of the follicular dendritic cells next to the morphological changes showed by these cells, could cause alterations in the antigen-presenting functions during BVD. These changes, could induce alterations in the proliferation, differentiation and maturation processes of B-lymphocytes inside the follicles, which could be involved in B-lymphocyte apoptosis during BVD.

Luminal-like A and B types in canine mammary carcinomas.

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Introduction: A new classification for human breast cancer, initially based on gene expression and now employing immunohistochemistry, exerts 4 distinct entities indicated as luminal-like (types A and B), basal-like, and ERB-B2 positive cancers.

Material and methods: In a series of 39 canine mammary carcinomas antibodies against cytokeratins 19, 14, 5/6, oestrogen receptor (ER), progesteron receptor (PR), vimentin and ERB-B2 were immunohistochemically tested and the results grouped as follows: luminal-like ($CK19^+$, $ER^{+/-}$, $PR^{+/-}$, $CK14^-$, $CK5/6^-$) type A ($ERB\text{-}B2^-$) and type B ($ERB\text{-}B2^+$); basal-like ($CK19^-$, ER^-, PR^-, $CK14^+$, $CK5/6^+$, $ERB\text{-}B2^-$); ERB-B2 ($CK19^-$, ER^-, PR^-, $CK14^-$, $CK5/6^-$, $ERB\text{-}B2^+$). All cases were graded for invasion according to Gilbertson et al. (1983) as: in situ, stromal and vascular infiltrating tumours. Correlation among variables was tested by the Pearson method.

Results: $CK19^+$ cells showed a luminal pattern in all cases furtherly subgrouped into 25 type A and 14 type B. The A or B groups did not substantiate any correlation with the histological type, invasion (in situ: 2 type A and 9 type B; stromal infiltrating carcinomas: 17 type A and 4 type B; intravascular infiltrating carcinomas: 6 type A and 1 type B), and ER status (9 of 25 (36%) type A and 4 of 14 (28%) type B tumours were ER negative). A significantly higher (Pearson $P<0.05$) percentage of PR negative cases was found in the luminal-like B group (10 of 14 (71%) PR negative cases) compared to luminal-like type A neoplasms (11 of 25 (44%) PR negative cases). The proliferation of myoepithelial cells in the complex and mixed types of canine mammary carcinoma failed to reveal a true basal component. Myoepithelial cells were CK14 and CK5/6 positive only when surrounding the epithelial luminal counterpart, whereas the nests of myoepithelial proliferation and the metaplastic component resulted negative to the same antibodies and positive to vimentin.

Discussion: Luminal-like types A and B share the same phenotype but differ in ERB-B2 expression, considered an important index for prognosis and tumour progression in canine mammary carcinoma as well as in human breast cancer. The apparent lack of correlation between type A or B and invasion must be confirmed on a larger number of cases, but evidence is reported in literature on the absence of correlation between ERB-B2 expression and vascular invasion. The association of most luminal-like type B phenotype with PR loss is noteworthy. Surprisingly the myoepithelial proliferation was negative to the known markers of myoepithelial cells that characterize the s.c. basal-like phenotype. This may indicate either the lack of this specific phenotype in canine pathology or the need for investigating other markers of myoepithelial growth. The use of a panel of antibodies to assess the phenotype of a malignant mammary tumour seems to be of practical use in canine as well as human pathology. In spite of the presence of myoepithelial cells characterizing the complex or mixed type of canine carcinomas, the final pattern assessment is influenced by luminal $CK19^+$ epithelial cells where the loss of steroid receptors and the acquisition of ERB-B2 expression seem the most important events of tumour progression.

Complex and mixed patterns of canine mammary tumours are defective of specific myoepithelial markers.

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Introduction: The presence of complex and mixed growths in canine mammary tumours is very frequent but their origin is still debated. The most frequent indication concerns metaplasia of fusiform cells of probable myoepithelial origin, but conclusive evidence does not exist. Mammary epithelium consists of two strictly associated cell types: luminal and abluminal, the former (secretory) showing immunohistochemical positivity for cytokeratins (CK) 8, 9, 18, 19, whereas the latter (myoepithelial), expresses CK 5, 6, 14 and 17. Indication exists in veterinary medicine on the use of anti-vimentin, α-smooth muscle actin (SMA), and S-100 protein to individuate myoepithelial cells, but their usefulness in differential diagnosis from other mesenchymal (fibroblastic and or myofibroblastic) growths is questionable.

Material and methods: In a series of 77 canine mammary tumours (41 simple, 9 complex carcinomas, and 27 carcinomas in mixed tumours) in which immunohistochemistry to CK 14, 5/6, vimentin and SMA was already available from other studies, the phenotype of complex and mixed patterns was reviewed.

Results: In all cases the staining of basal abluminal (myoepithelial) cells was positive to CK 5/6 and 14, and was appreciable as continuous in simple non-infiltrating carcinomas, whereas discontinuous in infiltrating carcinomas. The elongated to stellate mesenchymal cells proliferating in the complex or the metaplastic cartilagineous/osseous pattern of mixed tumours were not stained by CK 5/6 or resulted from faint/inconstant to negative by CK14. As for SMA, the positivity was similar to CK 5/6, more intense than CK14 in complex tumours, whereas negative in the mixed pattern. Anti-vimentin antibodies produced a strong signal in the basally located myoepithelial cells of simple carcinomas. The same strong staining was evident also both in the complex and mixed patterns as well as in stromal and vascular cells.

Discussion: A myoepithelial single cell layer is shown in simple carcinomas of the canine mammary gland and its continuous or discontinuous organization allows the objective discrimination between non-infiltrating and locally infiltrating tumours as in human medicine. Among the markers used for the identification of the myoepithelium (CK5/6, 14, and SMA), a scant CK14 to moderate SMA expression was found in the whorls of cells typical of the complex type, whereas absolute absence of SMA expression in the metaplastic areas (cartilagineous/osseous) of mixed tumours. This result may be explained either assuming a modification (metaplasia) of basal abluminal (myoepithelial) cells in producing the complex or mixed pattern, or in their genesis from other mesenchymal cells (myofibroblasts or stromal fibroblasts). The strong positivity to vimentin in both complex and mixed patterns, as well as in basally located (myoepithelial) cells, stromal fibroblasts and vessels, indicates a scarce usefulness of this marker for the identification of complex and mixed patterns as arising from a single cell type, namely the myoepithelial cell.

Comparative histopathological study of adrenal gland lesions between cattle and buffaloes in Ahwaz, Iran.

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Introduction: Adrenal glands being a member of the endocrine system, are important in regulation of various functions of the body. Thus, diseases of the adrenal gland have vital consequences on various functions of the body. The adrenal glands are composed of steroid secreting cells of mesodermal origin and catecholamin secreting cells of neuronal crest origin that become associated anatomically to varying degrees in different animal species. A lot of work has been done in humans and dogs, but very little information is available on the disease of adrenal glands of large animals, especially cattle and buffaloes. Hence, an abattoir survey was conducted to find out which lesions affect the adrenal glands of cattle and buffaloes slaughtered in municipal slaughter-houses of Ahwaz, Iran.

Material and methods: An investigation was carried out to study the histopathological condition of adrenal glands of cows and buffaloes. A total of 230 pairs of adrenal glands of buffaloes and 250 pairs of adrenal glands of cattle, 100 pairs form each of two animals suspected to pathologic on gross observation were collected. After recording the measurement and gross lesions, appropriate tissue was fixed in 10% formaldehyde, tissue sections were examined microscopically and various pathological conditions were diagnosed.

Results: Comparison of the lesion in cattle and buffaloes: invagination 7% and 12%; accessory cortical nodules 4% and 10%; nodular hyperplasia of cortex 21% and 18%; degenerative and inflammatory alterations 18% and 21%; telangiectasia 1% and 4%; adenocarcinoma of cortex 1% and 0%; haemangioma 1% and 0%; fibrosis of capsule 10% and 13%.

Discussion: Accessory cortical nodules are common in the adrenal glands of adult to aged animals and are found in capsule, cortex, and medulla. Many accessory cortical nodules arise either as an evagination of the cortex into the capsule and surrounding periadernal fat, or as invagination of the cortex into the medulla. Nodular hyperplasia also is common in the adrenal as well-defined spherical nodules in the cortex, or attached to the capsule. Hyperplastic nodules are usually multiple, bilateral and yellow, and they may involve any of the three zones of the cortex. Histologically, the nodules near the capsule resemble the zona glomerulosa, sometimes with areas that resemble zona fasciculata. The cells either are about the same size as those of the normal adrenal cortex or are hypertrophied.
Infectious and parasitic agents are frequently localized in the adrenal gland and elicit varying degrees of inflammation and necrosis. Focal inflammation is usually suppurative, arising in the course of bacterial septicaemia. Adenomas of the adrenal cortex occur most frequently in older dogs and only sporadically in horses, cattle, and sheep. Adrenal cortical carcinomas occur less frequently than adenomas and have been reported most often in cattle and old dogs, but also occur infrequently in other species. Adenocarcinomas and cavernous haemangiomas are diagnosed only in cattle. They can develop in any location in the body, but haemangiomas in the adrenal glands are reported rarely. A comparative study, performed by other researchers, on the above mentioned lesions between cattle and buffaloes show that most of the results are similar to this study.

A contribution to the study of the Bovine nephroblastoma with pulmonary metastasis.

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Introduction: Nephroblastoma is an embryonal tumour which arises from neoplastic transformation of metanephric blastema during nephrogenesis or from nephrogenic rests which persist postnatally. These tumours are common in young animals, especially pigs and chickens, but they are rare in cattle.

Material and methods: A renal neoplasia was observed in a regularly slaughtered 33 month old neutered male Piemontese bovine. In the ante-mortem examination the bovine showed a good state of health and nutrition without clinical signs. After slaughtering, the left kidney showed an increase in volume and a deformation of surface. The cortex had a variegated colour and some lobules presented haemorrhages with whitish lardaceous nodules. The cutting surface was diffusely irregular. A restricted number of lobules were normal, but showed deformation and degeneration of the parenchyma. In the neoplastic tissue with lardaceous features, fibrotic tissue was evident with boundless interlacing, which separated the lesion in distinct lobules. These lobules were diffusely irregular. The contralateral kidney was larger in size but without alterations. The lungs showed numerous macroscopical subpleuric nodules. No gross lesions were observed in other organs. Samples of kidneys, lungs, renal and mediastinic lymph nodes were taken for histopathologic and ultrastructural examinations.

Results: Histopathological findings showed multiform and distinct features of renal tumour. It presented prevalence and deployment of epithelial and mesenchymal element and haemorrhagic, necrotic and cystic areas, even if morphostructural peculiarities were constantly related to typology of embryonic nephroblastoma. Anaplastic and embryonal aspects were represented by poorly differentiated or undifferentiated cells with atypical mitotic figures with a large hyperchromatic nuclei and evident nucleoli. Cells were organized in cords, nests or irregular clusters in the mesenchymal component constituted of myxo-fibromatosus tissue or thick collagen bundles. Rarely, neoplastic epithelial elements were organized in pseudo-tubular formations, moreover very irregular, or in rudiments that resembled primitive glomeruli, without vascularization, morphologically atypical. Metaplastic areas of cartilage were also present. Histological findings in the lungs confirm the diagnostic hypothesis of a metastatic tumour from the kidney. In pulmonary metastasis, mesenchymal stroma was scanty and it consisted of thin bundles which penetrated the neoplastic epithelial tissue. Lymphoid tissues were normal. Ultrastructural findings showed two different populations of epithelial cells: some smaller, elongated and organized in irregular nests or clusters with lengthened and irregular indented nuclei, multifocal chromatin and scanty cytoplasm. Other cells were organized in cords or nests, with big nuclei that presented clear nucleoli and cytoplasm with scanty organelles.

Discussion: The diagnosis of a bovine nephroblastoma with metastasis to the lung is a rare finding and we consider that further investigations are necessary to clarify the epidemiology and pathogenesis of this tumour.

Caspase-dependent and -independent apoptosis in TNF-transgenic mice infected with the neurotropic Borna disease virus.

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Introduction: TNF-transgenic (tg) and non-transgenic (ntg) mice were infected experimentally with the neurotropic Borna disease virus in order to analyze the role of neuronal overexpression of TNF on the development of cellular degenerations after Borna disease virus infection.

Material and methods: TNF-transgenic and non-transgenic C57/Bl6 x BDF mice were infected neonatally with a mouse-adapted strain of Borna disease virus (BDV). Mock-infected and non-infected transgenic and non-transgenic mice served as controls. Mice were killed between 7 and 49 dpi. Brains were formalin-fixed and paraffin-embedded. Sections were stained with H&E or used for immunohistology (ABC-method), applying anti-BDV-nucleoprotein-antibody, anti-GFAP-antibody and anti-Caspase 3-antibody. DNA-fragments were observed by terminale desoxytransferase-mediated dUTP nick-end-labeling (TUNEL-assay). Real-time-PCR was performed to analyze the amount of total TNF mRNA transcripts.

Results: Total TNF transcripts were approximately 100fold higher in transgenic compared to ntg animals. However, significant increase of TNF mRNA was detected only in ntg mice after BDV-infection. Transgenic mice inoculated with BDV developed a moderate to severe nonpurulent meningoencephalitis consisting of perivascular and parenchymal mononuclear lymphohistiocytic cell infiltrates. The inflammatory infiltration started at 21 dpi. and increased until 49 dpi.. Increased numbers of GFAP-positive astrocytes were noted in all BDV-infected animals. However, tg mice brains revealed astrocytic nuclei with a significant larger diameter, mainly in TNF over-expressing brain regions, compared to non-infected and ntg BDV-infected animals. Furthermore, few apoptotic cells with features of activated astrocytes adjacent to inflammatory infiltrates in BDV-infected tg mice were detected in H&E-stained slides. In transgenic BDV-infected mice, the amount of caspase 3-positive and TUNEL-positive cells was significantly up-regulated. These cells revealed different morphologies and were approximately 7 to 15 µm in diameter. Surprisingly, most of the GFAP-positive cells showing apoptosis-like chromatin condensation lacked caspase 3 immunoreactivity. Interestingly, some astrocytes and microglial cells showed TUNEL-positive cytoplasmic reactions which might indicate phagocytosis of DNA fragments. In all BDV-infected mice, viral antigen was present throughout the brain in the neuropil, in neurons, astrocytes, ependymal cells and oligodendrocytes.

Discussion: TNF-transgenic BDV-infected mice developed a nonpurulent meningoencephalitis with strong microglial activation, reactive astrogliosis and significantly more caspase 3-, as well as TUNEL-positive cells, compared to non-infected animals. Furthermore, apoptosis-like programmed cell death could be detected in single GFAP-positive caspase 3-negative cells, indicating a caspase-independent apoptosis. The unusual hypertrophy of astrocytes as seen by swollen nuclei might be related to high TNF-levels combined with BDV-infection. This might lead to a higher susceptibility to an unexpected caspase-negative apoptotic programmed cell death.

Cytokine profile of TNF-transgenic mice infected with the neurotropic Borna disease virus resembles incomplete Th1 response.

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Introduction: Homo- and heterozygous TNF-transgenic (tg) and non-transgenic (ntg) mice were infected with the neurotropic Borna disease virus (BDV) and the effect of the TNF-overexpression on the development of the inflammatory lesions and the cytokine profile in the brain was analyzed in detail. The neuronal TNF-overexpression was achieved by linking the TNF gene to the promoter of the particular NMDA-glutamate receptor ε2 resulting in a selective TNF over-expression in the cortex cerebri, hippocampus, thalamus and striatum.

Material and methods: Mice were infected neonatally with a mouse-adapted strain of BDV and killed at day 21 and 42 post infection. Histology was carried out on formalin-fixed and H&E stained brain sections. Antibodies against Mac-1α, CD3, CD4, CD8, CD25, and CD45R were applied, using frozen brain sections in order to characterize invading inflammatory cells. mRNA transcripts of pro-inflammatory (TNF, IL-1α, IL-2, IL-6, IL-12, IFNγ) and anti-inflammatory (IL-4, IL-5, IL-10, TGFβ1) cytokines were quantified on total brain sections by RT-PCR.

Results: After BDV-infection, notable inflammatory mononuclear infiltrates and microglial activation were detected only in tg mice, mainly in homozygous animals. The invading cells consisted predominantly of T-cells, macrophages and B-cells. T-cells were in decreasing quantities composed of CD4+, CD8+ and CD25+ T-cells regardless of the mice´s transgenic status. TNF levels were approximately 100fold higher in tg compared to ntg animals, however, a significant increase of TNF mRNA values could only be detected in ntg mice after BDV-infection. Elevated mRNA transcripts were measured in all BDV-infected mice for IL-1β, IL-6, IFNγ , and IL-10. However, total mRNA values were significantly higher in tg than in ntg mice. IL-2, IL-4, IL-5, IL-12, and TGFβ1 mRNA levels were not up-regulated after BDV-infection in any group.

Discussion: Though cytokine transcripts were more elevated in tg mice after BDV-infection compared to ntg BDV-infected mice, the cytokine expression profile in general remained unchanged. The lack of IL-2 and IL-12 mRNA up-regulation despite the presence of CD4+ and CD8+ T-cells indicates an incomplete Th1 response. This might be due to a virally induced alteration of the immune response or could possibly be linked to the immature status of the immune system at the time point of infection.

Distribution and prevalence of PKD (Proliferative Kidney Disease) in wild brown trout in Switzerland.

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Introduction: Wild brown trout (Salmo trutta fario) populations were declining over the last decades in Switzerland. The emerging disease Proliferative kidney disease (PKD) is discussed as one responsible factor. PKD is caused by an infection with the myxosporean parasite Tetracapsuloides bryosalmonae. It leads to a granulomatous and proliferative nephritis and high mortality rates during the summer. To investigate the role of PKD in the decline of our wild trout population we initiated a long term survey to monitor the distribution and prevalence of the disease in Swiss river systems.

Material and methods: From 2000 to 2006, 6722 wild brown trout were caught in different rivers all over Switzerland. The fish were necropsied and investigated for the presence of T. bryosalmonae. Associated lesions were evaluated by histopathology and immunohistochemistry. Selected cases were examined by in situ hybridisation.

Results: We demonstrated the presence of PKD in more than 50% of investigated rivers, with the highest prevalence in the midland areas north of the Alps. In several rivers we showed a high prevalence of PKD positive fish in the summer followed by a marked decline in fish numbers towards autumn. Whereas lesions of fish in the summer were typically marked granulomatous interstitial nephritis with presence of intralesional parasites, lesions observed in autumn were generally milder, accompanied by fibrosis and no or only scattered parasites. Only young trout showed marked lesions, fish older than 1 year generally showed no or only minimal histopathological changes.

Discussion: Our results indicate that elevated water temperature during spring and summer coincide with the development of severe granulomatous nephritis caused by T. bryosalmonae in wild brown trout. PKD leads to increased mortality rates amongst young brown trout during the summer months. Surviving animals show a regression of pathological lesions indicative for elimination of the parasites from the fish host. After elimination of the parasite these fish do not seem to be affected by PKD in the following years indicating the development of a protective immunity. Taken together our results show that PKD negatively affects Swiss brown trout populations by causing high mortality and thus contributes to the decline of wild fish.

Multinucleated giant cells in a canine cutaneous mast cell tumour.

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Introduction: A 9-year-old, neutered, male, mixed breed dog had a cutaneous mass of unknown duration at the left abdominal wall. After surgical excision, the mass was submitted for histopathologic examination and a moderately differentiated mast cell tumour (grade II) with formation of multinucleated giant cells was diagnosed.

Material and methods: The provided tissue sample, already fixed in formalin, consisted of a cutaneous nodule measuring 5x3x2 cm, slightly risen and covered by sparsely haired skin. The cut surface was firm, yellowish-brown and lobulated. Paraffin sections were stained with H&E, toluidine blue, and Giemsa.

Results: Histopathology revealed a poorly demarcated, unencapsulated mass in the deeper dermis, partially extending to the subcutis. Neoplastic mast cells were loosely arranged in cords and sheets, situated in a fine collagenous stroma, focally surrounding and seperating skeletal muscle fibers. The neoplastic cells, ranging from 20 to 60 µm in diameter, were round to polygonal and had abundant amounts of amphophilic cytoplasm predominantly interspersed with small basophilic granules. Numerous multinucleated neoplastic giant cells (measuring up to 80 µm) were scattered throughout the neoplasm containing up to 6 nuclei, showing the same cytoplasmic and nuclear features as uninucleate tumour cells. Neoplastic cells displayed marked anisocytosis and anisokaryosis. Mitotic figures were rare (<1/10 HPF). Erythrophagia by tumour cells was occasionally observed. Numerous eosinophils and fewer plasma cells, macrophages and lymphocytes were dispersed throughout the neoplasm, the latter focally forming perivascular aggregates. Additionally, there were small foci of mineralization in the center of the neoplasm. By the use of special stains (toluidine blue and Giemsa stain) the intracytoplasmic granules of the neoplastic cells appeared purple.

Discussion: According to the current grading system (established by Patnaik in 1974) canine cutaneous mast cell tumours are classified into three categories (grade I-III) based on a histological scale. Although being mentioned in the former WHO classification, the formation of multinucleated tumour giant cells, as seen in this case, is neither described in the recent classifications of the current textbooks nor in the recent WHO classification as a feature of canine mast cell tumours. However, in his original grading system Patnaik noted the infrequent occurrence of giant cells in grade II tumours and the common presence of binucleated cells, giant cells and scattered multinucleated cells in grade III mast cell tumours. While the current grading system does not provide any information concerning the prognostic relevance of multinucleated giant cell formation in canine mast cell tumours, in cats, giant cells with single multilobulated or multiple nuclei are reported to be a common feature of pleomorphic cutaneous mast cell tumours. Despite their cellular pleomorphism, these tumours are only considered as behaviorally aggressive if they additionally show high mitotic activity. Thus, at least in cats, cellular pleomorphism alone (including the formation of tumour giant cells), is not regarded as indicative of aggressive behavior of cutaneous mast cell tumours.

Cowpox virus infection in a cat with transmission to man. Histopathology, negative contrast electron microscopy, immunohistochemistry and molecular phylogeny of the virus.

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Introduction: Cowpox virus (CPXV) infections in cats are endemic in Europe and Western Asia. The clinical disease, characterized by typical poxviral skin lesions (papula-ulcerating dermatitis), occurs on sporadic basis. Severe illness or even lethal outcome due to CPXV-induced pneumonia is the exception in domestic cats, but is well known in non-domestic cats from zoological parks, especially cheetahs. There is good evidence that wildlife rodents are the natural reservoir of the virus. Human infections usually result from direct transmission of the virus through skin contact, causing single lesions at the inoculation site. However, generalized involvement of the skin and even death can occur in immunocompromized individuals or in association with systemic corticosteroid treatment for underlying allergic diseases.

Material and methods: Tissues from a 4-month-old cat which developed a generalized fatal CPXV infection were collected at necropsy, fixed by immersion in 10 % neutral buffered formaldehyde, embedded in paraffine-wax, cut at 4 µm thickness and stained with H&E. In addition, immunohistochemical, negative contrast electron microscopical, and molecular biological analyses were performed.

Results: Fresh skin lesions were sparse and histologically characterized by severe ballooning degeneration of akanthocytes containing single to multiple eosinophilic intracytoplasmic inclusion bodies. Typical square shaped Orthopoxvirus-particles were identified by negative contrast electron microscopy in tissue from such fresh lesions. Older skin lesions were characterized by full thickness necrosis of the epidermis variably extending into the superficial and deep dermis and involving the hair infundibula and adnexal glands. Formation of syncytial cells within the epidermis and infundibula was a constant feature but often obscured by necrosis. However, viral antigen was still present as demonstrated by immunohistochemistry. Full thickness necrosis of the nasal mucosa and necrotizing pneumonia completed the pathological picture. In both organs, viral antigen was detected by immunohistochemistry. Molecular and phylogenetic analysis identified the virus isolated from the presented case as a CPXV. The virus was transmitted to the 18-year-old son of the cat´s owners who was scratched and developed a self-limitating, nodular-ulcerative dermatitis at the inner site of the left forearm with signs of generalized infection (fever, nausea, anorexia, painful regional lymph nodes).

Discussion: This case represents a rather rare example of a CPXV infection in a domestic shorthair cat because of the simultaneous occurrence of severe dermatitis, necrotizing rhinitis and pneumonia. The virus was clearly identified by molecular methods, as it formed a group with other recent CPXV isolates and reference strains. CPXV forms a potential risk to human health especially after eradication of the smallpox virus in 1979 and ceasing the vaccination programs afterwards.

Canine and feline ocular pathology: a review of 67 cases.

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Introduction: The aim of the study was to obtain an overview of the most common lesions within enucleated globes submitted to the diagnostic laboratory at the Royal Veterinary College, London and to correlate macroscopic with microscopic findings.

Material and methods: 55 canine globes and 12 feline globes were submitted between September 2005 and April 2007. Following fixation with either 10% formaldehyde or Davidson's solution, the globes were longitudinally sectioned with a microtome blade paramedian to the optic nerve and perpendicular to the long posterior ciliary artery.

Results: The most common reasons for enucleation included glaucoma, ocular inflammation which was unresponsive to treatment and irreversible loss of structure or function. In dogs, neoplasia was diagnosed in 10/55 cases (18%). Intraocular neoplasia was always primary and included uveal melanomas (four anterior and one choroidal), iridociliary adenomas and an iridociliary adenocarcinoma. The most frequently diagnosed non-neoplastic lesions encompassed primary glaucoma due to goniodysgenesis (24%) and secondary glaucoma due to lens-induced uveitis and/or trauma (25%). In cats, neoplasia was diagnosed in 7/12 cases (58%). The majority were primary uveal melanomas (five diffuse iridal melanomas and one atypical melanoma) and one was a metastatic adenocarcinoma. Macroscopic examination enabled the diagnosis of glaucoma, traumatic injury, lens luxation, retinal detachment, intraocular neoplasia and the detection of inflammatory exudate. Microscopic examination confirmed the macroscopically observed findings and allowed more precise evaluation of the ocular lesions with regards to pathogenesis and associated lesions.

Discussion: The majority of ocular diseases seen in dogs were non-neoplastic, whereas in cats, although fewer globes were examined, an approximately equal number of neoplastic and non-neoplastic diseases were diagnosed. Macroscopic examination of globes can provide important information about the underlying disease and a correlation between macroscopic and microscopic findings helps to identify consistent macroscopic features of particular ocular diseases. Precise diagnosis facilitates ongoing patient care with respect to the contralateral eye and/or the treatment of systemic disease.

Histopathological study and PCR confirmatory test in aborted fetal brains of cattle, sheep and goats sent to Central Veterinary Lab. of Iran (CVL) in relation to toxoplasmosis and neosporosis.

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Introduction: The principal agents causing protozoal abortion in ruminants are Neospora caninum in cattle and Toxoplasma gondii in sheep and goats. Neospora caninum is a coccidian parasite of animals. It is a major pathogen for cattle and dogs and occasionally causes clinical infection in horse, goat and sheep.

Material and methods: In this survey, microscopic lesions indicative for cerebral protozoal infection were observed in 49 fetal brains of 141 collected brains of cattle, sheep and goats. Nested-PCR examination of formalin-fixed, paraffin-embedded tissues used as a confirmatory test to identify the protozoal agents in brain sections.

Results: Toxoplasma gondii was detected by PCR in the brains of 86.95% aborted fetuses of sheep and 5 bovine aborted fetuses. Neospora caninum was detected as aborting agent by nested-PCR in one fetal brain of a sheep which had microscopic lesions indicative for cerebral protozoal infection.

The main histopathological findings in relation to protozoal encephalitis (multifocal non suppurative encephalitis) include: focal gliosis in 34.04% of fetal brains (41.7% in 24 cattle, 30.3% in 109 sheep, and 62.5% in 8 goats) and multifocal necrosis in 21.3% of fetal brains (20.8% in 24 cattle, 21.1% in 109 sheep, and 25.0% in 8 goats).
Five protozoal tissue cysts were found in fetal brains including: three toxoplasma cysts in ovine fetal brains and two sarcocysts in bovine fetal brains.

Discussion: Histopathological lesions indicative for cerebral protozoal infections were observed mainly in the cortex. Histopathological features of neosporosis in sheep closely resemble to those of bovine neosporosis and ovine toxoplasmosis. Microscopical lesions of bovine toxoplasmosis were the same as ovine toxoplasmosis. The protozoal cysts were mainly observed in the brain stems. This is the first report of Neospora caninum infection in sheep diagnosed in Iran.

Diffuse seminoma with tumour emboli in a dog.

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Introduction: An 11-year-old cross breed dog was presented for anorexia and weight loss. On physical examination, the dog was emaciated and showed a large ulcerated lesion on the right lower lip in addition to an enlarged right testicle. Euthanasia was done because of poor body condition. Primary testicular neoplasms are common in dogs greater than 6 years old, with a mean age at diagnosis of 10 years. The most common primary testicular neoplasms - seminoma, sustentacular (Sertoli) cell tumour, and interstitial (Leydig) cell tumour - occur with approximately equal frequency. Metastasis are uncommon, particularly in Leydig cell tumours. Testicular neoplasms except Sertoli tumours are rarely hormonally productive. Testicular neoplasms may be unilateral or bilateral and are often of mixed-origin, especially in cryptorchid testicles. The seminoma of the testis develops from germ cells before somatic differentiation. It arise from cells of the spermatogenic series, presumably from basal spermatogonia. The aetiology is not well understood, but cryptorchidism and increased age are well-known risk factors. These tumours do not secrete hormones and metastasize rarely.

Material and methods: Formalin-fixed, paraffin-embedded sections from selected blocks were cut in 5 μm sections. After drying and incubation in a 60°C convection for 30 to 60 min, slides were deparaffinized in xylene, hydrated through a series of graded alcohol to distilled water and stained with H&E.

Results: Macroscopically this tumour was diffuse and flaccid to semi-firm with a homogenous glistening gray/white appearance on the cut surface. Similar to Sertoli cell tumours, it was lobulated and compresses the surrounding testicular tissue. The texture of seminomas helps differentiate them from Sertoli cell tumours, which are usually more firm. This tumour had infiltrative pattern and no proven correlation existed between subtype and metastatic behavior. Histopathological report was consistent with diffuse metastatic seminoma. Tumour cells were highly characteristic and appeared as very large, polyhedral cells with sharp borders, vesicular nuclei, prominent nucleoli and scant basophilic to amphophilic cytoplasm. Multinucleate cells were common and mitotic figures were typically numerous and bizarre. Tumour was subdivided by loose stroma infiltrated by focal aggregates of lymphocytes. Individual cell necrosis with scattered histiocytes produced a classic "starry-sky" appearance, which is characteristic for, but not diagnostic of, diffuse testicular seminomas. Also there were numerous microembolism in the vasculature of testicles.

Discussion: Most case reports on tumour microembolism show a vascular tissue reaction characterized by intimal proliferation and fibrosis; bland tumour emboli without or with only scarce vascular reaction are rarely reported. A reactive tumour microembolism, as observed in the present case, might arise as a sequel of massive embolization.Thrombotic tumour-induced arteriopathy, by contrast, can be explained much easier and less speculatively by recurrent tumour microembolism and consecutive endothelial and fibrous proliferation within the vascular lumen. In the present case, microscopic tumour fragments were not found in any other tissues.

Leiomyoma of the vagina in a bitch (case report).

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Introduction: Leiomyoma is a mesenchymal and common tumour of the vagina of the bitch and less commonly, other species and resembles the same tumour in the uterus. These tumours are hormon dependent, as they often regress after ovariectomy and do not occur in neutered bitches. Leiomyomas of the vagina occur most often in middle aged bitches, where there may be single or multiple. The tumour derives from smooth muscle cells of the vagina and, in some instances, has occurred more often in association with chronic oestrogen stimulation from either cystic ovarian follicles or oestrogen-secretion ovarian tumours. Neoplasia in the vagina in humans is extremely uncommon. These lesions usually occur in women older than 60 years.

Material and methods: A 7-year-old bitch was referred to the small animal hospital at the school of veterinary medicine (Tehran university) for vaginal discharge. A biopsy was taken from the multiple nodules observed in the vaginal wall. The biopsies were referred to the pathology laboratory at the department of pathology at Tehran School of veterinary medicine. After fixation, thin paraffin sections were stained with H&E.

Results: Grossly the tumour masses had well circumscribed, non-capsulated discrete nodules, about 2 cm in diameter that arose from the vaginal wall. The tumour masses were globoid and sessile in base and protrude into the lumen of the vagina. The dog had been ovariectomized before being refered to the hospital. Under microscopic examination curving bundles of densely packed spindle cells with distinguishable cytoplasmic borders with elongated, cigar shaped nuclei as nests of smooth muscle cells among the abundant collagen fibers, which is a characteristic feature of leiomyoma, are observed. Mitotic figures were exceptional. In addition excessive collagen production was prominent in this tumour.

Discussion: The tumour occurred in the bitch in spite of being ovariectomized. Fibroma of the vagina is less common than leiomyoma, with which it may be confused. Smooth muscle tumours can be usefully demonstrated with a connective tissue stain such as Masson´s Trichrome for identifying muscle cells.

Multiple myeloma in a dog from Southern Brazil.

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Introduction: Multiple myeloma (MM) is a rare malignant tumour of plasma cells described in humans and domestic animals that is associated with excessive secretion of immunoglobulin. This paper describes the findings of this tumour in a dog from Brazil.

Material and methods: A 12-year-old, female Boxer dog with a chronic history of ataxia and exhaustion was seen at the Veterinary Teaching Hospital, Universidade Estadual de Londrina, Southern Brazil. Clinical, laboratorial, radiological and serological examinations, and fine needle bone marrow aspiration were performed; appropriate therapy was applied for 47 days. However, the animal was euthanized on solicitation due to rapidly deteriorating conditions. Routine necropsy was performed; selected tissues were processed for histopathological evaluation.

Results: Clinically there was polyuria, polydipsia, and motor incoordination, with sensitivity to caudal abdominal palpation. Laboratory analysis demonstrated an initial hypercalcaemia, intermittent leukopenia (neutropenia and lymphopenia) along with thrombocytopenia and terminal microcytic normochromic and normocytic anaemia; creatinine and urea values were also elevated. Protein electrophoreses demonstrated hypergammaglobulinaemia with a monoclonal spike (7.21 g/dL; normal 0.06 - 0.14 g/dL). Fine needle bone aspirate showed a proliferation of plasma cells, hyperplasia of granulocytic series, hypoplasia of erythrocytic series, normoplasia of megakaryocytes and a myeloid : erythroid ratio of 4.15 : 1. Proteinuria, haematuria, bacteruria, and isosthenuria were observed by urine analysis. Radiography revealed multiple osteolytic lesions at the scapula and long bones, as well as the cervical, thoracic and lumbar vertebrates. Gross manifestations of disseminated disease were observed at the marrow of long bones, spleen, lymph nodes, and liver; a firm, white, solitary irregularly-shaped mass was observed at the base of the heart. Histology confirmed a plasma cell tumour of the bone marrow; the spleen, lymph nodes, and liver were also affected.

Discussion: The diagnosis of MM was based on a combination of exiting features: bone marrow plasmacytosis, elevated serum monoclonal gammopathy (M protein), radiological confirmation of osteolysis, histological observation of tumour in bone marrow and other tissues, associated with hyperglobulinaemia, hypercalcaemia, and cytopenia; these diagnostical findings are used to characterize this tumour. Although the diagnosis of MM can be also confirmed by light chain proteinuria, this analysis was not performed in this case since the results of the other diagnostic parameters utilized were consistent with this tumour. The anaemia observed in this case is a common manifestation of this disease in humans, but is not frequently seen in domestic animals. In dogs with MM, anaemia may occur due to blood loss by platelet destruction, presence of concomitant chronic disease, and accelerated destruction of erythrocytes.

Unusually high prevalence of equine lymphoma.

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Introduction: Lymphoma is rare in horses in spite that case reports are relatively common. The sporadic necropsies and abattoir surveys suggest that lymphomas represent less than 5% of all equine neoplasms. A higher prevalence rate is presented in the case of squamous cell carcinoma, skin fibrosarcoma, melanoma, ovarian granulosa cell tumour and pituitary adenomas.

Material and methods: 38 adult horses in the age of 1-27 years were presented for necropsy from January 2006 to June 2007. The necropsied horses were of different breeds and gender. The horses were dissected and sampled for histopathological and other laboratory examination as required. The routine formalin-fixed, paraffin-embedded tissue and H&E stain was used. In some lymphoma positive cases common immunohistochemistry methods for B and T cell differentiation were used additionally.

Results: Out of 38 necropsied horses 5 cases (13%) of equine lymphoma were found. No other neoplasms were observed. Four cases were of the multicentric form and one case was the alimentary form. Leukaemia was not present in any case.
All multicentric forms were characterized by irregular and various involvement of mesenterial and mediastinal lymph nodes with diffuse and/or nodular lymphoid infiltration of the liver, spleen and kidneys. In one case the conspicuous mediastinal tumorous mass and multiple nodular pericardial and myocardial involvement were observed. One case was associated with multiple serosal and mucosal haemorrhagies.

One case of alimentary form was characterized by irregular thickening of the intestinal wall and transmural lymphoid infiltration affecting the caudal part of duodenum, jejunum and the cranial ileum. The mucosa of the intestinal segment appeared eroded and ulcerated. The observed 13th thoracal vertebra fracture was associated with lymphoid obliteration of the osseous tissue.

Discussion: We are not able to clear up the high number of equine lymphoma cases observed during the short periode of 18 months. The lymphoma forms and the tumorous cell phenotypes of our cases correspond with literature data. Our results also showed that equine lymphomas can cause various unexpected pathological lesions with various clinical signs that could be the cause of a diagnostic confusions.

An uncommon case of Cryptococcosis in a cat.

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Introduction: The presented case describes an infection with an unusual species of Cryptococcus in a young-adult male cat.

Material and methods: Specimens of skin, skeletal muscle and lymph node were investigated by histopathological and mycological methods including Grocott stain, PAS reaction, mycological culture, biochemical differentiation, PCR and gene sequencing.

Results: The histopathological findings consist of a widespread granulomatous dermatitis, panniculitis and myositis of the left foreleg as well as inflammatory changes of the regional lymph node. Small roundish fungi within macrophages were detected using Grocott stain and PAS reaction and were identified as Cryptococcus magnus by using mycological methods of investigation. This result was confirmed by PCR and sequencing methods.

Discussion: Infections with obligatory pathogenous fungi such as Cryptococcus neoformans and Histoplasma capsulatum are differential diagnoses. However, the histomorphological differentiation of fungal elements could be problematic. Cryptococcus neoformans can easily distinguished by its mucin capsule, whereas the differentiation of Cryptococcus magnus and Histoplasma capsulatum proves to be more difficult due to their similar form and size. Cryptococcus magnus is generally considered nonpathogenic. It belongs to the yeast genus Cryptococcus and can be found on leaves or in arctic glaciers. Very few cases of Cryptococcus magnus infections in humans and only a single case in a cat have been published.

European veterinary pathology on the base of the congresses of the European Society of Veterinary Pathology in 1997 - 2006.

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Introduction: The main role of this society is to integrate the community of veterinary pathologists, as well as to promote and to provide education in the field of pathology. The organization of annual congresses is one of the tools used for achieving these aims. The purpose of the present study was to show the direction of activities of European veterinary pathologists and the pattern of knowledge presented by them, in the light of oral presentations (plenary lectures and short presentations) and posters contributed during annual ESVP meetings in the last decade.

Material and methods: The research materials consisted of scientific proceedings from annual meetings of the ESVP, which were held in the years 1997 - 2006.

Results: The analysis of scientific proceedings of ESVP meetings, covering the period 1997 - 2006, reveals that, relatively, the most frequent subject of interest for European veterinary pathologists was pathology of various organs (566 presentations). Among all issues that were raised, the most intense research activity was observed in the field of neuropathology. In this area of interest, the issue was the subject of 25.62% of all presentations. This number consisted of four plenary lectures, 81 oral presentations and 60 posters - making 145 presentations in total. Infections and parasitical diseases were only slightly less popular. These diseases were the subject of 548 presentations, which accounted for 30% of the total number of oral presentations and posters. About 1/6 of the presentations in this field were related to pathomorphological aspects of parasitical diseases. The field of oncology received a lot of attention among researchers and topics of this field were presented 404 times, which accounted for 21.37% of all presentations. Each meeting also included issues in toxicologic pathology. In this field, the form of posters slightly prevailed (74 - 6.90%) over oral presentations. Although there were 62 oral presentations, yet while expressed in percentage points, they accounted for 7.69% plenary lectures and for 7.58% short oral presentation. The presented research most frequently and, to a similar extent, concerned farm animals, accompanying and experimental animals, whereas was significantly less frequently focused on free-living and exotic ones that were subjects of 139 presentations, which accounted for 7.36% of all contributions. The number of posters in this field was twice that of oral presentations. The discussed topics included the role of the computer and the Internet in pathomorphological research and in student education. Attention was paid to telepathology as a tool with the potential to create international diagnostic centers.

Discussion: During the decade analyzed, there were 52 plenary lectures presented, 765 oral presentations and 1072 posters. Altogether, 1,889 presentations were made between 127 and 238 per year. Additionally, the subject matter discussed at the annual meetings was analyzed and the trends in the development of veterinary diagnostic pathology and broad pathology education were indicated. It was proved that veterinary pathology enhances knowledge in the field of veterinary medicine by fulfilling a cognitive and diagnostic role. Detailed presentation of this subject was accepted and will be published in Veterinary Medicine - Science and Practice.

The pathomorphological pattern of the liver in yellow-necked mice (Apodemus flavicollis, Melchior 1834) living near the pesticide tomb during its existence and two years after its liquidation.

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Introduction: Discovery of organochloric pesticides persistence in the environment and their dangerous toxicological influence on living organisms caused their withdrawal in most developed countries; in Poland, DDT pesticides have been banned since 1976. The remanding stocks were deposited, mostly in concrete tombs, but sometimes they were buried without any protection or markings. The aim of this investigation was to study how the morphological pattern of the livers of yellow-necked mice (Apodemus flavicollis, Melchior 1834) reflected environmental pollution by a pesticide tomb (PT).

Material and methods: In the autumn of 2004 and 2006 (2 year after PT eradication), 80 yellow-necked mice (10 animals per group) were caught in four zones of increasing distance from the PT. The area between the PT and the lake shore was divided into four consecutive zones: I - slope of the PT hill, south-east exposition, II - area between road and fish pond, being a flat ground among trees, III - continuation of zone II in the direction towards the lake, IV - control, a dam about 4 km off PT. Mice were examined macroscopically. While performing post-mortem examination, three sections for microscopic and one section for ultrastructural examination were taken from the liver. Material for histopathological evaluation was fixed in 10% neutralized formaldehyde. Paraffin slides were stained with H&E and Sudan III according to the method by Lillie Ashburn and according to PAS by McManus. The amount of microscopic lesions found in the liver were analyzed statistically (software Statistica PL, StatSoft) using Student's t-test for dependent samples, by comparison of the average values for the same groups of animals at different years and different groups in the same year. Ultrastructural examination of the liver was also conducted. The material was fixed in 2.5% glutaraldehyde in a 0.2 mol/L phosphate buffer of pH 7.4 and embedded in Epon 812. The ultrathin sections were contrasted with uranyl acetate and lead citrate. Ultrastructural analysis was conducted using an Opton 900 PC TEM (Germany).

Results: Most microscopic and submicroscopic lesions of the highest intensity were found in the livers of animals living in the vicinity of the tomb. The amount and intensity of the lesions diminished with increased distance to the tomb. The lesions showed destructive and adaptive character. The number of morphological lesions and the degree of their intensity were slightly higher in the liver of yellow-necked mice living in the PT area in 2006 in comparison with 2004. The distribution and character of the lesions showed the participation of environmental pollutants in their creation.

Discussion: Pathomorphological examination of the livers of yellow-necked mice revealed the influence of xenobiotics from the pesticide tomb on the surrounding environment and showed that changes in the livers of the animals can be used as biomarkers of environmental pollution. The study showed that two years after liquidation of the pesticide tomb, its influence on the surrounding environment is still significant.

Effect of immunosuppressive doses of dexamethasone on morphological pattern of the liver in the dog.

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Introduction: In some cases immunosuppressive-acting doses of Dexamethasone (DXT) are administered to dogs. The aim of this study was to determine morphological lesions in the liver of the dog after the administration of such doses of the above mentioned drug.

Material and methods: The experiment was conducted on 6 clinically healthy dogs (females, mixed breeds, aged 2 - 4 years). Liver samples from these dogs were taken by surgical biopsy for morphological examinations (group 1). After one month the dogs were administered 1 mg DXT/kg b.m. i.m. throughout 4 consecutive days (DXT 0,2 % pro injectione, Eurovet Animal Health), and subsequently after 4 days liver samples were taken using the same biopsy technique (group 2). Collected specimens were divided into 3 parts and fixed in a solution of 10% buffered formaldehyde, Carnoy and 2.5% glutaraldehyde. Paraffin sections were stained with H&E and by Masson's method (after routine fixation). Sections fixed in Carnoy were stained for glykogen using PAS-dimedon method. The liver for ultrastructural examination was embedded in Epon 812. The ultrathin sections were contrasted with uranyl acetate and lead citrate and analysed using an Opton 900 PC TEM (Germany).

Results: All dogs from group 1 exhibited signs of parenchymatous degeneration in the liver to a small degree. The livers of 3 dogs from this group showed the presence of fat vacuoles in individual hepatocytes. Apoptotic bodies were observed in one case, in two cases the proliferation of connective tissue was seen. The liver of all dogs from group 2 characterized hepatocytes in various sizes. In one case fat vacuoles were present in the cells. Another case showed hepatocytes with coagulation necrosis. All dogs showed proliferation of connective tissue around the central veins. All animals from this group also showed a steroid-induced hepatopathy, which expressed significant hepatocyte oedema and irregular glycogen accumulation in cells. In the liver samples of group 1 dogs, the hepatocytes ultrastructurally did not show any significant lesions. But in the liver of the animals exposed to DXT numerous, usually small lipid droplets, vacuoles and sometimes myelin-like structures were frequently found within cytoplasm of hepatocytes. In numerous mitochondria degradation of their cristae and rarefaction of their matrix, both of diverse intensity were observed and sometimes these organelles were proliferated or hypertrophied. Sporadically the extension of RER canals was noted. Some hepatocytes were partly or entirely necrotic. The increase in the contents of glycogen within the cytoplasm of hepatocytes was observed. Binuclear hepatocytes were quite frequently found.

Discussion: The research has shown that the immunosuppressive dose of DXT induces morphological lesions, in particular destructive ones, within the hepatocytes in the dogs. Those changes indicate the presence of steroid-induced hepatopathy in dogs, caused by immunosuppressive-acting therapeutic doses of DXT. Considering the frequency of this therapy used in veterinary practice, it would be wise to remember the potential dangers associated with its abuse.

Proliferation and apoptosis in ovine cells after experimental infection with bovine leukemia virus (BLV).

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Introduction: Bovine leukaemia virus (BLV) belongs to the family of oncogenic retroviruses which include human T-cell leukaemia virus (HTLV) I and II, and simian T-cell leukaemia virus I and II. These retroviruses share a common genomic organization and are associated with non-neoplastic lymphocyte disorders, lymphoid neoplasia and/or progressive myelopathies. To investigate the possible role of virus expression on lymphocyte proliferation, expression of proliferating cell nuclear antigen (PCNA) and anti-apoptotic protein bcl-2 in lymphocytes and tumour cells in experimentally with BLV infected sheep was determined with the use of flow cytometry method.

Material and methods: Ten lambs were inoculated i.m. with blood PBMC of BLV infected cow. Flow cytometry analysis using monoclonal antibodies for lymphocyte subsets, PCNA and bcl-2 labelled with FITC or PE conjugates was performed. Antibodies against BLV in the sera were determined in AGID and ELISA tests. The presence of BLV in the blood lymphocytes and cells in neoplastic changes was detected by flow cytometry, PCR, nested-PCR and in situ- PCR.

Results: We observed that in some animals B-cell lymphomas with tumours in inner organs and lymph nodes and a great percentage of cells with IgM^+CD19^+ phenotype were developed. Expression of PCNA and bcl-2 protein in blood lymphocytes and cells isolated from inner organs of infected animals was significantly stronger than in the control group. Great activity of PCNA was determined in tumours. Bcl-2 protein protected cells from apoptosis and those cells were viable for a long time. We observed that in sheep experimentally infected with BLV the proliferation of lymphocytes with $CD19^+$ markers was induced.

Discussion: Retroviral infection caused spontaneous, uncontrolled lymphoproliferation of blood lymphocytes. In the spleen, lymph nodes and mesenteric tumours about 100% of cells were B-cell phenotype. Observations of cell PCNA activity showed that this cycline activity increased in the infected lymphocytes, as compared to the control one. Percentage of PCNA positive blood cells was significantly higher in the animals with persistent lymphocytosis and in lymphoma stage. Strong activity of PCNA, a major nuclear protein, has been shown to be associated with human leukaemia and malignancies. The lymphocytes with high expression of bcl-2 protein were spared from apoptosis. This suggest that the ability of BLV expression to prevent apoptosis may be connected to its ability to arrest or delay cells in G0/G1 of the cell cycle. A number of in vitro mechanisms used to arrest cells at the G1 checkpoint can prevent cell death. For example, G1 cell cycle arrest, induced by expression of the cycline-dependent kinase inhibitor 21 can prevent apoptosis following DNA damage. Cell cycle arrested by a retroviral protein has been demonstrated for the HIV Vpr protein. In HTLV-1, attention has been focused on the anti-apoptotic and cell cycle regulatory properties of the transactivating protein Tax. Tax expression has been associated with altered expression of some cyclins and cyclin-dependent kinase inhibitors.
Proliferating cell nuclear antigen may play a role in the process of lymphoid transformation as a result of bovine leukaemia infection in sheep. This nuclear antigen may have a practical prognostic value in human tumour therapy and in small animals malignancies.

Proliferation and apoptosis in foetal ovine brains following experimental transplacental infection with cytopathogenic and non-cytopathogenic biotypes of Bovine viral diarrhoea virus.

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Introduction: Bovine viral diarrhoea virus (BVDV) exists in two biotypes, namely the cytopathogenic (cp) and the non-cytopathogenic (ncp) biotype. In vitro the cp biotype kills cultured cells by apoptosis. Transplacental infection of pregnant sheep with BVDV can induce a variety of foetal abnormalities including severe brain alterations. So far, information about the possible role of apoptosis in the pathogenesis of pestivirus-induced brain lesions are not available. The aim of the present study was to investigate the proliferative activity, the occurrence of apoptosis and the distribution of viral antigen in brains of sheep foetuses after experimental transplacental infection with cp and ncp biotypes of BVDV.

Material and methods: Brains from ovine foetuses infected between day 63 and 68 of gestation with cp (42 foetuses, 1 lamb) and ncp BVDV (10 foetuses) fixed in formalin, Bouin's or Schmechel's fluid were examined. Brains from 15 non-infected foetuses and 1 lamb served as controls. Paraffin sections from the telencephalon and cerebellum were examined immunohistochemically by using the ABC method and antibodies to Ki67, activated caspase 3 and BVDV antigen. Proliferation of cells was evaluated semi-quantitatively by counting $Ki67^+$ cells. For quantification of apoptotic cells caspase 3^+ nuclei were counted using a semi-automatic system by determining the apoptotic index (AI) and the numbers of apoptotic nuclei per brain area (AN/A).

Results: Unexpectedly, in both groups of infected foetuses significantly higher numbers of AN/A were found in different layers of the telencephalon [cortical plate (CP) and ventricular- and subventricular zone (VZ+SVZ)] and in the cerebellum in comparison with controls. Additionally in foetuses infected with cp BVDV a significant increase of AI was present in the CP and intermediate zone of the telencephalon. In contrast to foetuses infected with ncp BVDV, significantly reduced numbers of $Ki67^+$ proliferating cells were seen in the SVZ+VZ of the telencephalon in foetuses infected with cp BVDV. In both groups of infected foetuses a positive topographical correlation between the presence of viral antigen and distribution of apoptotic cells was only found in less than 20% of cases.

Discussion: The results of this study demonstrate that transplacental infection of ovine foetuses with both cp and ncp biotypes of BVDV is associated with significantly increased numbers of apoptotic cells in the telencephalon and cerebellum. The reason for increased apoptosis in foetuses infected with ncp BVDV is unknown. It can not be excluded that indirect mechanisms such as release of cytokines (e.g. TNF-α, IFN-γ, IL-4) from activated brain macrophages may be involved in the induction of apoptosis. Reduced numbers of proliferating $Ki67^+$ brain cells found in foetuses infected with cp BVDV are possibly due to induction of apoptosis of progenitor cells during early post-infectious stages. A possible explanation for the finding that only in the minority of fetal brains a positive correlation between apoptosis and viral antigen was present could be that the viral antigen had already been eliminated during early post-infectious stages.

Proliferative and necrotizing pneumonia and severe vascular lesions in pigs naturally infected with porcine circovirus type 2.

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Introduction: Proliferative and necrotizing pneumonia (PNP) is a severe form of interstitial pneumonia of swine showing lymphohistiocytic interstitial inflammation with proliferation of type 2 pneumocytes and presence of many coagulates of necrotic cells in the alveoli. At the present time the main contributor to PNP in North America is PRRSV, whereas in Europe the same applies to PCV2. Severe vascular lesions (segmental to circumferential vasculitis, fibrinoid necrosis) were recently reported in connection with PCV2 infection. The aim of our study is to present the occurrence of PNP and severe vascular lesions in pigs naturally infected with PCV2 in Hungary.

Material and methods: Forty pigs (4%) had died in 90 days of age during the 1 month episode of respiratory symptoms in a large herd. The thoracal organs were examined with histological and bacteriological methods in 3 animals. Immunohistochemical (IHC) test was used for the detection of PCV2, PRRSV, and SIV in the lung tissue samples and mediastinal lymph nodes. Detection of cytokeratin was applied for the better visualisation of the type 2 pneumocytes.

Results: PNP was diagnosed in 2 cases and acute severe lymphohistiocytic interstitial pneumonia with the presence of large amounts of hyaline membrane, and acute haemorrhages were seen in one case. The bronchiolar epithelial cells were often degenerated and necrotic, and vascular oedema, fibrinoid necrosis and vasculitis with formation of vascular thrombi were evident in several blood vessels in all cases. In one case the mediastinal lymph node showed multifocal to coalescing necrotic areas. Fibrinoid vascular necrosis, vasculitis and severe thrombosis with direct connection to tissue necrosis were clearly observed in this animal. Large amounts of PCV2 antigen were found in the mediastinal lymph nodes and in the lungs in all cases. The virus was frequently observed in macrophages and affected blood vessels and even in coalescing necrotic areas of lymph node. PRRSV, SIV, bacteria or histological lesions suggestive for bacterial infections, were not detected in any of the cases.

Discussion: The results further suggest the causative role of PCV2 infection in PNP, and the importance of the vascular system in the pathogenesis of PCV2 associated diseases of swine.

Pathological and immunohistochemical studies of naturally occurring paratuberculosis in sheep.

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Introduction: Paratuberculosis is a chronic progressive bacterial disease of ruminants caused by Mycobacterium avium paratuberculosis (Map). The purpose of the present study was to perform a comparative evaluation of histopathological lesions and immunohistological changes in sheep, naturally infected with paratuberculosis.

Material and methods: Histopathological changes and degrees of positivity by Ziehl-Neelsen (ZN) and avidin-biotin-complex peroxidase (ABC) techniques were studied in 29 adult sheep, grossly or clinically suspected to suffer from paratuberculosis. Tissue samples including different parts of intestine, ileocaecal valve, and mesenteric lymph nodes, were fixed in 10% buffered formaldehyde and processed by standard paraffin wax technique. Three sections of 5 μm thickness were obtained from each tissue and mounted on glass slides. One section was stained with H&E, one with ZN stain, and the third section was subjected to ABC technique. The degree of positivity by the ZN and ABC methods was categorized as follows: (-) negative; 1-3 isolated points of positivity (acid-fast bacilli by ZN staining or brownish granules by the ABC method) observed in the section; (+) mildly positive; (++) moderately positive; (+++) highly positive.

Results: Of the sheep, 16 (55.1%) had microscopic lesions, associated with paratuberculosis, which were classified into four categories. Focal to multifocal lesions (6.9%) consisted of small granulomas in the lamina propria of ileum, especially in the ileocaecal Peyer's patches and also in the mesenteric lymph nodes. Changes in the ileum of one sheep were ranked as mildly positive by the ABC method only. In the lymph nodes results were negative with both the ZN and ABC method. Diffuse multibacillary lesions (31%) were characterized by a diffuse granulomatous enteritis with epithelioid macrophage infiltration, as main inflammatory cells, which were arranged in a mosaic-like appearance, and filled with numerous acid fast bacilli in ZN, were highly positive in ABC. There was no difference in the number of positive cases detected by the ZN and ABC method but the intensity of the positivity was much higher in the ABC technique. Diffuse paucibacillary (lymphocytic) lesions (6.9%) are of lymphocytes, as main inflammatory infiltrate, with some epithelioid macrophages or giant cells, which contain few if any mycobacteria. Scanty numbers of acid fast bacteria were seen only in the mucosa of ileum of one case by ZN staining whereas 2 cases were positive by ABC method. Diffuse intermediate (mixed) lesions (10.3%) were characterized by a diffuse granulomatous enteritis with infiltration of large numbers of lymphocytes and also epithelioid macrophages that showed varying degrees of positivity in ZN and ABC method. Mycobacteria were demonstrated by ZN and IHC methods in all these cases.

Discussion: Diffuse multibacillary lesions were the commonest type found in this study (31%), reported to be the classical lesion of paratuberculosis in sheep. The present study showed close correlation of the IHC method and the ZN stain, both methods detected almost all the animals with diffuse lesions; more cases with focal and paucibacillary forms were positive by IHC than by ZN. IHC is more sensitive than the ZN method; it detected a greater number of positive animals, by means of more visually striking reaction, which made the use of comparatively low magnifications possible. The importance of sampling the distal ileum and mesenteric lymph nodes to find microscopic lesions of paratuberculosis in sheep is emphasized.

Histopathological study of tibiotarsal rotation in ostrich.

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Introduction: This study describes the histopathological changes of tibiotarsal rotation (TTR) in ostrich. Cartilage abnormality may be present at the ends of all the long bones in the leg and has been reported in the humerus. The tibiotarsus is most frequently affected.

Material and methods: During 6 month, 10 birds (ostrich) from one ostrich farm around Tehran area, which were affected with TTR were necropsied. Tissue samples were chosen from leg bones. At least one tissue sample was removed from the proximal extremity of tibiotarsal bone of the affected leg. The age of these birds was between 3 to 10 weeks. Five sagital sections from each sample were prepared and stained with H&E for microscopic study. Suitable sections, which included the growth plate and adjacent area were prepared. Histological sections of affected legs were compared with healthy bird as a control.

Results: The histological study revealed that 3 cases were affected with dyschondroplasia. The most important lesions observed in this disease contain 1) presence of praehypertrophic chondrocytes which are unable to separate this zone from proliferative chondrocytes accurately; 2) defect in blood supply; 3) poor differentiation of praehypertrophic chondrocytes to hypertrophic chondrocytes; 4) praehypertrophic necrotic chondrocytes, shrunken cells with pyknotic nuclei and eosinophilic cytoplasm.

Discussion: Histological and macroscopic changes revealed that 2 cases were affected with rickets. Affected bones were characterized by severe weakness and poor calcification. The microscopic study revealed an increased length of cartilaginous columns with an irregular cartilaginous column formation and finally a defect in bone mineralization. In one case (10%) the two lesions (rickets and dyschondroplasia) occurred together. Three cases were diagnosed as suspected ones. This group of birds lacked enough lesion to diagnose the disease. Finally, in one case despite of the presence of clinical signs, no lesion were observed in the microscopic evaluation.

Classification and differentiation of epithelial and mesenchymal tumours of mammary gland in dogs with histopathology and special staining.

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Introduction: Mammary tumour is the most common malignant neoplasm in the bitch. Classification of human mammary gland tumour is a good model for the classification of the neoplasm in the dog. Three primary methods are used to classify the mammary tumours: 1) histogenetic, 2) histological descriptive, and 3) prognosis. The classification presented here is the same as recently prepared by WHO-AFIP.

Material and methods: During the one year survey beginning in October 2004 canine mammary tumours of 10 dogs were removed surgically and studied histopathologically. 5 μm sections of the tumour mass were stained with H&E and studied microscopically. Another section, prepared from the same tumour, was stained with special procedure adjusted for demonstration of connective tissue (Masson´s trichrom) and for differentiation of epithelial and mesenchymal mammary gland tumours.

Results: Ten different neoplasms of canine mammary were diagnosed and confirmed histopathogically including: 3 mixed mammary gland tumours, 3 invasive papillary carcinomas, 1 solid carcinoma, 1 fibroadenoma, 1 in situ papillary carcinoma, and 1 in situ micropapillary carcinoma. Special staining of Masson´s trichrom revealed 4 mesenchymal tumours, containing 3 mixed mammary gland tumours, 1 fibroadenoma, and 9 epithelial tumours, 3 of which were mixed mammary gland tumours. In this staining method, the collagen fibers stained blue and epithelium red.

Discussion: This staining is not able to separate the epithelial and mesenchymal tumours, but can also help to differentiate whether this tumour is invasive or not infiltrative. The red stained epithelial parts of the tumour, which were observed in the blue stroma of the tumour, will be a good marker for the presence of an invasive tumour.

Epidemiological data of lymphoma in domestic carnivores.

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Introduction: The incidence of lymphoid tumours is high in domestic carnivores, representing an important percentage of investigated cases. Medical literature specifies that the annual incidence is estimated to 13 - 24 cases / 100,000 dogs and 41.6 cases / 100,000 cats.

Material and methods: A retrospective study of all lesions in domestic carnivores was performed, including the period between 1998-2006, with 3,313 dogs and 603 cats. 106 cases were diagnosed with lymphoma, with 93 in dogs and 13 in cats. Considering these data, an epidemiological study was performed, studied criteria being the lesions of lymphoid tissue and the morphology of lymphomas. Cytology (May-Grünwald-Giemsa), histology (Masson trichromic) and immunohistochemistry (indirect method avidin-biotin-complex-peroxidase) were used as diagnostic methods.

Results: A constant increase of the incidence of lymphomas in dog occur all over the investigated period. The incidence of lymphomas in cat was inconstant, except in 2006 when an explosive increase was observed. More than half of all the lesions (58%) and 7% of all malignant tumours were lymphomas. Multicentric lymphoma in dogs and alimentary lymphoma in cats were the most frequently observed, with 58% and 40%, respectively. Centroblastic lymphomas occurred in 28.23% of the total investigated cases, followed by lymphoblastic lymphoma (15.21%) and immunoblastic lymphoma (9.41%). B-cell lymphoma occurred in 40% of investigated cases, followed by T-cell lymphoma (20%). Males seemed to be more affected, with 66% in dogs and 69% in cats. The incidence was higher in Rottweiler, Doberman and Boxer, European cats being exclusively diagnosed with lymphoma. Dogs and cats beeing around 4-and 12-years-old, respectively, exhibited the highest frequency of the disease. Survival time in treated dogs included a period between 4 and 12 months, untreated dogs surviving 1-4 months after diagnosis. Feline lymphoma had a faster evolution, survival time being uncorrelated with the treatment. 40% of cats survived 4 weeks after diagnosis and 60% 8 weeks after diagnosis. Sometimes death occurred rapidly in 1-2 days after the appearance of clinical signs.

Discussion: The annual increase of diagnosed lymphomas was due to progressive increase of number of investigated patients. On the other hand a bigger aggressiveness of aetiological agents might be possible. High frequency of lymphomas in males can be connected with anabolic hormones, which induce protein synthesis and therefore stimulate carcinogenesis indirectly. Viral aetiology might be associated with male dominant behaviour, males being suspected to come into contact with viral particles. The high number of lymphomas in European cats can be correlated with the high frequency of this breed in our country.

Efficacy tests of some treatment plans in the Walker 256 carcinosarcoma in Wistar rats.

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Introduction: Dacarbasine is one of the cytostatics used in diverse forms of cancer treatment (malignant melanoma, Hodgkin disease, soft tissue sarcomas, neuroblastoma). Yet quite extensive used, it manifests some toxic effects. To reduce these secondary noxious, we have done an experiment on animals with induced carcinosarcomas, combining dacarbasine with adjuvants (shark cartilage, zinc, arginine, phenylalanine, hystidine, vitamins C, B6, B2, methionine, malic acid, tryptophan, adenine, tyrosine, biotin), many of these being recognised as antioxidants.

Material and methods: The experiments were done on Wistar rats with Walker 256 carcinosarcoma. Dacarbasine was administrated alone or in association with the adjuvant tested products (at the same time as the tumour innoculation, or after its macroscopical appearance). Part of the animals was used to determin survival time and the others were sacrificed after 25 days from the first administration of the products. Some biological and molecular data were recorded: tumour evolution, cell cycle, detoxification system (cytochromes P-450, cytochromes b5, glutathione S-transferase, reduced glutathione) and oxidative stress (malondialdehyde, ceruloplasmine and thyols).

Results: The flow cytometry studies proved that the association between dacarbasine and the adjuvant products is efficient, mainly after the tumour`s appearance, with specific action on the S phase. We observed: a partial synchronisation of the malignant cells (75% of cases), a lower DNA synthesis and an accumulation of the cells in the G0/G1 interphase, with a corresponding change of tumour aggressivity. The tested products exhibited a moderate effect over the level of malondialdehyde and ceruloplasmin oxidative indexes, both reffering to the controls and dacarbasine alone; thyol albumines were not affected. Values of detoxification system were also affected, especially for cytochromes P-450. Tumour evolution (weight, volume) was quite similar both for the group treated with dacarbasine alone and those treated with dacarbasine and tested products.

Discussion: The results prove the presence of certain synergic effects between dacarbazine and the tested products, demonstrated by the changes of some investigated parameters, but also lead to the idea that the administration of adjuvant products, as the antioxidants, may be done in well justified cases, taking into account the individual particularities.

Pathological findings and immunohistochemical diagnosis of canine visceral leishmaniosis in naturally infected dogs.

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Introduction: Canine visceral leishmaniosis (CVL) is an endemic disease of the fox, other wild-canids, dogs, cats, horses, seals and human beings in Mediterranean countries, the Middle East and parts of Africa and South America.

Material and methods: A total of 25 dead dogs, displaying clinical signs consistent with canine visceral leishmaniosis were included in this study.

Results: At necropsy, the animals showed lymphadenopathy, hepatosplenomegalie, chronic nephritis and skin lesions consisting of dermatitis (exfoliative, ulcerative, nodular or pustular) and alopecia. Histopathologically, three different types of lesions were observed in the liver. In mild changes, individual or clustered macrophages within sinusoids of the liver were observed. In moderate changes, mild to moderate infiltration of the portal areas by mixed populations of macrophages (generally haemosiderin-laden), lymphocytes and plasma cells was observed. In addition, small granulomatous foci, consisting of macrophages loaded with amastigotes, lymphocyte and plasma cells, were scattered randomly within the hepatic parenchyma. In severe changes, the liver showed portal and periportal fibrosis with mononuclear cell infiltration as well as lymphoid granulomas in the hepatic parenchyma. In the kidneys, lesions consisted of a nonsuppurative interstitial nephritis and a chronic glomerulonephritis. In the spleen and lymph nodes, cortical and medullar sinusoids were enlarged by macrophages loaded with amastigotes and haemosiderin. The skin lesions were characterized by perifollicular dermatitis and /or perivascular to diffuse dermal infiltration as well as erosive-ulcerative changes. Immunohistochemically, using peroxidase and fluorescein labelling, amastigotes were observed within the cytoplasm of infiltrating macrophages, especially in the lymph nodes, spleen, liver, skin and kidney.
Moreover, a dual infection of CVL and hepatozoonosis was detected in three cases. Triplet infection of CVL, hepatozoonosis and toxoplasmosis was detected in one case. These co-infections were confirmed by histological and immunohistochemical findings.

Discussion: In this study, the typical morphological appearance of the amastigotes of Leishmania sp. was useful for the histological diagnosis of CVL. For definitive diagnosis of CVL, immunohistochemical methods were used successfully in tissue sections. However, in histological examination of many cases, haemosiderin impeded the appearance of amastigotes within macrophages, especially in lymph nodes, spleen and liver. Therefore, immunohistochemistry should be carried out in suspected cases of CVL after haemosiderin pigments are histochemically removed from tissue sections.

The immunosuppression caused by CVL can promote the occurrence of co-infections with other agents such as Ehrlichia, Babesia, Dirofilaria and Neospora in endemic regions. Similarly, simultaneous infections of hepatozoonosis and/or toxoplasmosis together with CVL were also described in this study.

Visceral leishmaniosis and parapoxvirus infection in a Mediterranean Monk Seal (Monachus monachus).

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Introduction: Visceral leishmaniosis is an endemic disease of the fox, other wild canids, dogs, cats, horses and human beings. The genus Parapoxvirus of the family Poxviridae consists of highly epitheliotrophic DNA viruses, causing cutaneous and systemic diseases in animals and man.

Material and methods: A female Mediterranean monk seal, aged about 20 years, found in January 2005 on the coast at Bodrum, Turkey, was taken to a rehabilitation centre. The animal, which showed weakness and respiratory symptoms, died despite treatment with antibiotics and vitamins. Necropsy was performed. For detection of parapoxvirus infection and leishmaniosis the avidin-biotin-complex-peroxidase and the indirect immunofluorescence method were used. In addition, PCR for parapoxvirus was performed on homogenized tissues of trachea and lung.

Results: At necropsy, a deep ulcerative lesion was observed in the skin of the left dorsolateral side of the head. The animal also had erosive-ulcerative lesions on the gingiva and the inner aspect of the lower lip. Superficial and visceral lymph nodes and the tonsils were oedematous and enlarged, with a dark red cut surface. Histopathological examination revealed that the mucosal lesions of the lip, gingiva, pharynx and tonsils consisted of areas of hydropic degeneration of epithelial cells, and erosions and ulcerations of the epithelial layer, with infiltration of neutrophils, macrophages and lymphocytes. Immunohistochemical labelling of parapoxvirus was observed, especially in the epithelial and mononuclear cells of the oral mucosa and tonsils. The trachea contained exudate with desquamated epithelial cells, neutrophils and macrophages, and showed small numbers of eosinophilic intracytoplasmic inclusion bodies in the epithelial cells. Viral immuno-labelling occurred in both the epithelial and desquamated epithelial cells as well as in infiltrating macrophages. The molecular diagnosis of parapoxvirus showed an appropriately sized product (594 bp) amplified from the sample and specific signals were observed. The spleen, tonsils and lymph nodes showed marked depletion of lymphocytes of parafollicular areas, together with reticuloendothelial cell hyperplasia. The cortical sinusoids were dilated and filled with macrophages containing amastigotes and plasma cells. Fluorescein and peroxidase labelling of amastigotes was observed in the cytoplasm of macrophages and reticular cells in the lymph nodes, tonsils and spleen and in the sinusoidal endothelia Kupffer cells, hepatocytes and macrophages of the liver.

Discussion: Parapoxvirus infections have also been reported in young grey seals (Halichoerus grypus) in Canada, harbour seals (Phoca vitulina) in the German North Sea and Weddell seals (Leptonychotes weddellii) in Antarctica. It was reported that parapoxvirus did not spread from infected young seals to adults. The adult seal infection described in the present report was probably related to the immunosuppressive effect of the visceral leishmaniosis. This report represents the first description of both visceral leishmaniosis and parapoxvirus infection in a seal.

Sigma-2 receptor-expression in urothelial tumours of the urinary bladder in cattle associated with papillomavirus type-2 (BPV-2) infection.

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In cattle, tumours of the urinary bladder are generally associated with a syndrome known as chronic enzootic haematuria (CEH) due to prolonged ingestion of bracken fern and with a prevalence of 90% in adult animals. Similar tumours are infrequent when bracken fern is not eaten and showing a prevalence of 0.01-0.1% reported in cattle.

Bracken fern (genus Pteridium) is believed to be the only higher plant proven to cause cancer naturally in animals. The fern contains toxic principles and mutagenic, immunosuppressive, clastogenic and carcinogenic chemicals.

In addition, previous studies pointed out a strong relationship between bovine papillomavirus (BPV) and brackern fern. It is believed that bovine papillomavirus type 2 (BPV-2) plays an important role in the bladder carcinogenesis associated with this syndrome.

Recently, it has been demonstrated that a correlation between sigma-2 receptor protein expression and histopathologic grade in human bladder cancer exists. Sigma-2 receptors are overexpressed in several tissues and cell lines. Although the biomolecular mechanism linked to this overexpression has to be elucidated, sigma-2 receptor is considered a potential biomarker for monitoring solid tumour proliferation.

The aim of our research was to investigate sigma-2 receptor expression and study the relationship, if any, with tumour tissue stage and grade as demonstrated in human bladder. All bladder cancer were associated with papillomavirus type-2 infection.

In this study we used the International Histological Classification of bladder cancer as reported in the WHO Blue Book of Humane Medicine.

Comparative pathogenesis of experimental SARS coronavirus infection in cats and ferrets.

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Introduction: Severe acute respiratory syndrome (SARS) is an emerging infectious disease caused by a coronavirus: SARS coronavirus (SARS-CoV). In June 2003, the WHO reported more than 8000 human patients with an associated mortality of 9.2%. Fatal human cases were presented with respiratory disease, primarily consisting of diffuse alveolar damage (DAD). The main histological features were hyaline membranes, fibrin, oedema and syncytial cells in the alveolar lumina, type II pneumocyte hyperplasia and mild interstitial inflammation. Subsequently, angiotensin-converting enzyme 2 (ACE2) was identified as an important receptor for the spike protein of SARS-CoV. The pathogenesis of SARS in humans is still poorly understood, which makes it difficult to develop therapeutic strategies or vaccines.

Material and methods: We experimentally infected cats and ferrets with SARS-CoV in order to study SARS pathogenesis, in particular the relationship between pathology, cell types expressing viral replication, and ACE2 distribution. Four cats and 4 ferrets were inoculated intratracheally with $1x10^6$ median tissue culture infectious dose of SARS-CoV isolate HKU-39849. At day 4 after inoculation the animals were euthanized and necropsied according to a standard protocol. Tissue sections of various organs were evaluated for lesions and viral antigen expression by histopathology and immunohistochemistry. Respiratory tract tissues of 3 non-infected cats and 3 non-infected ferrets were evaluated for ACE2 expression by immunohistochemistry.

Results: We found that both cats and ferrets developed DAD that was more severe in ferrets. No hyaline membranes or syncytial cells were seen. A novel SARS-CoV-associated lesion observed in cats but not in ferrets was tracheo-bronchoadenitis. SARS-CoV antigen expression was limited to the respiratory tract in both species. In cats, SARS-CoV antigen expression occurred predominantly in both type I and II pneumocytes and serous cells of the tracheo-bronchial submucosal glands. In ferrets, it occurred predominantly in type II pneumocytes. All cell types that expressed viral antigen also expressed ACE2, although not all ACE2 expressing cells were positive for viral antigen.

Discussion: In conclusion, SARS-CoV in cats and ferrets mainly targets the alveoli, resulting in DAD similar in character to that in humans. ACE2 expression is necessary but not sufficient for infection. Importantly, comparison between alveoli of cats and ferrets shows that differences in ACE2 expression are correlated with differences in pattern of infection. Finally, the occurrence of SARS-CoV-associated tracheo-bronchoadenitis has potential implications for the viral excretion and pathogenesis of SARS.

Asymmetrical localized muscular pseudohypertrophy in a Holstein-Friesian heifer.

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Introduction: Double muscling is a well-known phenomenon in cattle. Although occurring in many breeds the Belgian Blue is notorious for its double muscled conformation.
The genetic basis for the muscular hyperplasia can be found in an 11-bp deletion mutation in the coding region of the myostatin gene.

Material and methods: Recently the Faculty of Veterinary Medicine of Ghent received a pure bred newborn Holstein-Friesian heifer calf that grossly showed signs of double muscling. In contrast to the generalized muscular hypertrophy in the Belgian Blue, this heifer showed macroscopically an asymmetrical enlargement of several muscles which resulted in a dystocia foetalis during birth. The muscles belonging to the upper arm of the left forelimb, the right thigh and the muscles covering the sternal bone were twice the size in comparison with the other normal muscles.

Results: Microscopically the muscular enlargement was due to an increase in proteoglycan rich stroma, an increase in small intramuscular blood vessels and accumulation of adipose tissue between the fibrils and mild hypertrophy of the fibrils.

Discussion: Whether or not the hypertrophy is due to the same deletion mutation as in the Belgian Blue is currently under investigation.

Extragonadal teratoma on the skull of a kitten.

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Introduction: Teratomas originate from pluripotent germ cells that undergo somatic differentiation into two and usually three embryonic germ cell layers and thus are composed of multiple tissues foreign to the body in which they arise. Teratomas originate most often from the ovary or testicle because of their germ cell origin. Extragonadal teratomas have been described in several species, however only few are reported in cats. The present case describes an extragonadal teratoma located on the skull of a kitten.

Material and methods: A subcutaneous non-ulcerating mass of approximately 2 cm in diameter was found on the left temporal area near the base of the ear in a 2,5 months-old kitten. At the age of 4 months, the mass was severely enlarged (measuring 8x10x15 cm) and removed surgically. It was fixed in buffered formaldehyde (10%), processed for paraffin sectioning and stained with H&E.

Results: Macroscopically, the mass was moderately hard, well demarcated and covered by a thick fibrous capsule. On cut surface, the tumour was composed of solid areas intermingled with several cyst-like structures filled with greasy gelatinous material. Histological examination revealed the presence of ectodermal components such as numerous hair follicles, hair bulbs, cystic cavities which were lined by stratified epithelium containing keratin squames and hairshafts; endodermal structures such as cysts lined by pseudostratified ciliated epithelium, as well as mesodermal components consisting of multiple islands of hyaline cartilage, adipose tissue and spindle cell proliferation.

Discussion: Teratomas can be classified into either immature or mature types, based on the degree of maturity. As the described neoplasm contained no immature components and consisted of a lot of cystic tissue, the present case was diagnosed as mature cystic teratoma. In cats, five gonadal and two extragonadal teratomas have been described. The latter were localized intracranial and intra-abdominal. To our knowledge this is the first report of an extragonadal teratoma on the skull of a kitten.

Polyarteritis nodosa in a young short hair cat.

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Introduction: Polyarteritis nodosa (PAN) is a systemic necrotizing vasculitis of humans affecting small- to medium-sized arteries of multiple organs. Areas of arterial branching are predilection sites, and the inflammation results in grossly visible nodular thickening of affected arteries. Similar syndromes were described in other species as pig, dog and cat. PAN is most common in adults, and the pathogenesis is uncertain. Here we present the first case of PAN in a young short hair cat in Italy.

Material and methods: A 6-month-old male short-hair cat was presented with fever, anorexia, dehydration and neurological signs. The complete physical examination was unremarkable; laboratory tests were all normal except for a mild leucocytosis. The animal did not respond to symptomatic treatment and was euthanatized. At necroscopy megaoesophagus, small white nodules on heart and kidney and splenomegaly were identified. Nevertheless, all organs were sampled. Representative tissue sections were fixed in formalin, processed, sectioned, and stained with H&E. For immunohistochemistry antibodies against the following antigens were used: vWF, CD3, CD79α, and MAC387.

Results: Histologically, small- to medium-sized arteries within the heart, oesophagus, liver, kidney and testis exhibited inflammatory changes of varying severity and stage of development. Inflammatory cell infiltrates were associated with intimal proliferation characterized by increased number of spindle cells. In severe lesions fibrinoid necrosis of the tunica media was present. The distribution of arterial lesions appeared random and segmental with normal arterial profiles sometimes present adjacent to severely affected ones. Veins and capillaries were not affected. Arteries within the brain, lung, lymph nodes, spleen, stomach, pancreas, and intestine were normal. The pattern and character of the lesions in this short hair cat were very similar to those described in human cases of PAN.

Discussion: We present here a case of multisystemic PAN of a young cat in which the diagnosis was made after the death. In human beings the same lesions are frequently described in isolated organs where the diagnosis is made after the excision of the diseased organ following another diagnosis. The aetiopathogenesis of PAN is not yet established, but in human the multisystemic syndromes occur in patients with hepatitis B antigen in the serum, while in pigs streptococcal infection is one of the aetiological factors, associated with hypersensitivity vasculitis. In this case the occurrence of the most common feline infectious diseases was not identified, while a cell-mediated syndrome cannot be excluded as the causative agent of PAN.

Nephrotropism and nephropathogenicity in chickens experimentlally infected with avian influenza viruses of the H10N7 (A/turkey/England/385/79) and H10N4 (A/duck/Romania/4385/06).

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Introduction: In vivo testing procedures have already demonstrated the presence of avian influenza (AI) virus isolates being consistently highly pathogenic, although the subtype H10 does not usually associate with high pathogenicity. The pathogenicity of the avian influenza viruses of the subtype H10 is associated with the replication in the kidney. The purpose of the present study was to investigate the potential of viral isolates of the H10 subtype to infect chickens by intravenous route and to detect possible sites of virus replication.

Material and methods: Two groups of 10 six-week-old chickens maintained under BSL3 were infected intravenously with an AI virus of the subtype H10N4 (A/turkey/England/385/79) and of the subtype H10N7 (A/duck/Romania/4385/06), respectively, according to the protocol for the intravenous pathogenicity index (IVPI) test as established by Council Directive 92/40/EEC. Chickens were observed twice a day for clinical symptoms until death. Samples of kidney, pancreas, intestine, lung, trachea, liver, heart, spleen, bursa of Fabricius and brain were collected, formalin fixed and routinely processed for histopathology and IHC. Virus isolation and RT-PCR were also carried out.

Results: Depression, ruffled feathers, reluctance to move and whitish diarrhoea were clinically observed 48-72 h post-inoculation (pi). Death occurred 72-96 h pi. On postmortem, congestion of the visceral organs, mild serous peritonitis, and swollen and discoloured kidneys were observed. 8/10 birds infected with the A/turkey/England/385/79 (H10N4) and 4/10 with the A/duck/Romania/4385/06 (H10N7) avian influenza viruses died. Histologically, severe necrotic lesions of the kidney were observed in all dead chickens. Pancreas acinar cells necrosis was detected in only two birds infected with viruses of the subtype H10N4 and H10N7, respectively. Both necrotic pancreatic acinar cells and renal tubule epithelium were positive to viral nucleoprotein antigen by IHC.

Discussion: The results reported herein confirm that both the investigated H10 subtype AI viruses are nephrotropic and nephropathogenic for chickens following intravenous inoculation. They cause severe, diffusely distributed necrosis and nephritis as well as immunohistochemical positivity to viral nucleoprotein.

Concurrent atypical myopathy and equine grass sickness in two horses.

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Introduction: Atypical myopathy (AM) is a disease of unknown aetiology in grazing horses characterised by sudden weakness and recumbency caused by acute muscular degeneration. Access to pasture, pasture quality, meteorological conditions and young age are regarded as risk factors. Equine grass sickness (EGS) is characterised by dysfunction of the autonomic nervous system resulting in decreased gastrointestinal motility. Although involvement of Clostridium spp. and ingestion of a toxic compound have been suggested, the aetiology remains unknown. Here we describe 2 cases in which clinical signs and histological lesions of both AM and EGS were encountered in the same animals.

Material and methods: In November 2006, a pony mare (2.5 years old) and warmblood filly (18 month old) were found recumbent on distant pastures. The pony had a short period of dysphagia. In the filly, a distended paralytic bladder was observed. Both animals showed a striking myoglobinuria and were euthanized because of aggravating signs.

Results: At necropsy, several skeletal muscles of both animals showed acute degeneration. A moderate impaction of colon content was found in the pony. At histology, muscles in both cases showed severe acute monophasic myodegeneration. Oil red O staining of degenerated muscle fibres revealed a severe accumulation of intracellular neutral lipid droplets. Based on these findings a diagnosis of AM was made. After histological examination of trigeminal ganglia, chromatolytic neurons and neuronophagia were observed. Immunolabelling showed strong intracytoplasmic accumulation of synaptophysin in chromatolytic neurons. Chromatolytic neurons were also observed in the brainstem of both horses and colonic submucosal nerve plexus of the pony. Neuronal degeneration of cranial nerves is considered a characteristic for EGS.

Discussion: This is, to our knowledge, the first report of AM and EGS occurring in the same horses. In the population of horses in the region of Flanders, both AM and EGS are rare conditions. Therefore, it is most unlikely that concurrency of both disease entities is a coincidental finding. Comparing the risk factors of AM and EGS, some striking similarities indeed are seen, such as grazing, young animals and cool weather with ground frosts. Moreover, both diseases have been encountered in the same geographical areas.

In conclusion, AM and EGS share common epidemiological risk factors, which may account for the concurrency of both diseases in these 2 animals.

Epidemiology and histopathologic findings of skin tumours in pet rabbits in the USA and Germany.

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Introduction: Rabbits have increased in popularity as companion animals; however, the majority of publications on neoplasia in this species has been derived from animals kept under laboratory conditions. The goal of this study was to record and compare the epidemiology of skin tumours in pet rabbits on two different continents.

Material and methods: Specimens of pet rabbits from the US submitted to the surgical biopsy service of the University of Pennsylvania and of pet rabbits from Germany submitted to a private diagnostic pathology service in Munich, Germany were reviewed. Specimens were routinely processed for histopathological evaluation and stained with H&E. In cases in which a definite histological diagnosis could not be determined based on the H&E slide, immunohistochemistry was performed.

Results: In the US over a 16 year period 190 masses from 179 pet rabbits were submitted for histopathological examination. In Germany over a 3 year period, 391 tumours and tumour-like lesions from 280 pet rabbits were submitted. A total of 28 different lesions were diagnosed. In both countries trichoblastoma was the tumour most frequently diagnosed. Other frequent tumours and tumour-like lesions included collagenous hamartoma, lipoma, malignant melanoma, fibrosarcoma and malignant peripheral nerve sheath tumour. Except for Shope fibroma and lymphoma, the incidence of most tumours and tumour-like lesions did not differ between both countries. While in the USA Shope fibroma was diagnosed in 19 rabbits, no cases of Shope fibroma were diagnosed in Germany. In contrast, only one case of lymphoma was diagnosed in the US, while lymphoma was diagnosed in 45 rabbits from Germany. In both countries, fibrosarcomas, malignant peripheral nerve sheath tumours, myxosarcomas and lipomas predominately occurred in male rabbits. Collagenous hamartomas were exclusively seen in male rabbits in both countries.

Discussion: The difference in the incidence of Shope fibroma mirrors the distribution of Shope fibroma virus, the causative agent of this tumour. The authors speculate that a viral aetiology could also account for the marked epidemiologic difference of lymphomas between the US and Germany. Different viral agents such as human retrovirus 5 and Epstein-Barr-virus related herpesvirus can induce lymphoma in lagomorphs under laboratory conditions. Future work will assess, by use of molecular methods, the role of viral agents in the causation of lymphoma in pet rabbits.

Characterization of V-, J- and C-region-genes of the feline T-cell receptor gamma.

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Introduction: Lymphomas and leukaemias are important tumours of domestic cats. Sometimes it is difficult to differentiate these tumours from reactive lymphatic hyperplasia. To overcome this problem, molecular biological techniques may help. One of these techniques is the analysis of clonally recombined antigen receptor genes using PCR or southern blot. PCR has the advantage that it is fast, sensitive and inexpensive. With this technique the assembly of the genes for immunoglobulines and T cell receptors during the development of the lymphocytes can be demonstrated. This assembly is called the V(D)J-recombination. Monoclonality of the relevant immunoglobulin or T cell receptor is regarded as proof of neoplastic proliferation.

Material and methods: To be able to establish such a technique in the cat we had to sequence the genes of the antigen receptors. We chose the T cell receptor gamma chain as primary target. We used RNA extracts from spleen of different cats and a 5'-RACE as well as a 3'-RACE PCR protocol to clone and sequence the feline V-, J- and C-region genes.

Results: By using different RACE techniques we were able to clone and sequence four different V-region genes, wich can be clustered into two subgroubs. One of these genes seems to have at least 15 subtypes. Additionally, we found eight J-region genes wich can be clustered into three subgroups, and six variants of the C-region gene. Subgroups of J-region genes, all except one, had homologous genes in the dog. The V-region genes are less similar.

Discussion: These sequences show sufficiently homologous areas to be used for establishing consensus primers for molecular biological diagnosis of feline lymphomas and leukaemia.

Different genotypes of Porcine circovirus type 2 in immunohistologically positive Swiss pigs from 1986 to 2005.

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Introduction: Porcine circovirus type 2 (PCV2) is known to be the primary causative agent of post-weaning multisystemic wasting syndrome (PMWS). PMWS is the most important clinical manifestation of a range of porcine circovirus diseases (PCVDs). Since 2003, PMWS has been epizootic in Switzerland and has caused problems for numerous farms. Prior to this date, only few problems at the farm level have been reported, despite the fact that PCV2 was present in Swiss pigs since at least 1986, as identified through retrospective immunohistochemical staining of lymphoid tissue.
The three main questions in this study are 1) whether different strains of PCV2 with varying pathogenicity exist in the Swiss pig population, or 2) do we have a new strain chronologically associated to the epizootic breakout, or 3) a general shift in strain prevalence over time.

Material and methods: 165 immunohistochemically positive animals (PDNS cases not included) from 83 different farms collected in the Veterinary Pathology archive from 1986 to 2005 have been reviewed. Each animal was re-evaluated by using all available information, at both the individual and farm level. Lymphoid tissue was investigated by H&E staining and immunohistochemistry (IHC) with the monoclonal antibody F217 specific for PCV2.
A conventional PCR was designed to target conserved sequences flanking the variable region 218 - 355 base pair (bp) of ORF2, resulting in amplification of a fragment with a length of 137 bp (excluding primers). PCR products were confirmed by sequencing and comparison to existing PCV2 genomes from the NCBI GenBank using the BlastN© program.

Results: Of the 165 cases, 13 different strains were identified, of which two resulted in identical residues at the amino acid level. Closer examination of PCR products revealed three major amino acid differences, which assisted in differentiating two distinct genotypes (genotype 1 and 2). Analysis of samples collected prior to 2003 revealed 18 diseased animals (4 farm problems) positive for genotype 1. No genoype 1 strains have been subsequently involved in disease. Genotype 2 strains, conversely, were rarely involved in diseases before 2003, but, between 2003 and 2005, have been identified in 65 single cases (26 farm problems).

Discussion: The epizootic of PMWS in Switzerland, which commenced at the end of 2003, seems to be associated exclusively with genotype 2 of PCV2. Prior to this, genotype 1 appeared to be the dominant PCV2 genotype, causing mostly sporadic cases. Nevertheless genotype 2 isolates have been present in Swiss pigs since at least 1989. Whether this dramatic shift to genotype 2 in the epizootic period is due to changes in pathogenicity of the agent, to environmental factors or to selection of these strains by host properties, remains to be elucidated and is the aim of further research. This data also suggests that the pathogenicity of PCV2 varies between different strains.

Primary pineal tumour in a dog.

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Introduction: Primary pineal tumours are very rare in humans. In animals, they are mainly seen in rats, but there have been occasional reports in other species, such as the horse, cow, goat, chicken and cockatiel. Here we report on a first case in a dog.

Material and methods: An entire brain from a dog with a suspected tumour in the region of the pineal gland based on MRI results was submitted formalin-fixed for histological examination. The clinical finding was confirmed by gross examination and the neoplasm was examined by light microscopy on sections stained with H&E and various special stains. Immunohistology was performed to identify epithelial, mesenchymal, neuronal, neuroendocrine, glial and melanocytic cells and transmission electron microscopy was used to assess the ultrastructural features.

Results: Gross examination revealed a black-beige, marbled, soft neoplastic mass (cross section: 1.5 x 2.5 cm), extending over the entire length of the mesencephalon (rostral to caudal colliculi) and obscuring the pineal gland, the aquaduct and the commissure of the rostral colliculum. Histologically, the mass was composed of closely packed, relatively monomorphic cells, which were occasionally arranged in pallisading and rosette like patterns, within a scant pale eosinophilic stroma. Individual cells were approximately 20 μm in diameter and had an angular shape. Several melanin containing cells were observed disseminated throughout the mass. The most relevant immunohistological findings were the expression of cytokeratin by individual and clusters of neoplastic cells, in conjunction with a lack of synaptophysin expression. Ultrastructurally, the neoplastic cells showed occasional gap junctions and intracytoplasmic electron-dense cores. There were scattered cells exhibiting melanin granules.

Discussion: The WHO (1993) provides a classification of parenchymal pineal gland tumours in humans as pineocytoma, pineoblastoma and a mixed pineocytoma/pineoblastoma. In animals, the WHO classification (1999) mentions pineocytoma and pineoblastoma. Recently, papillary tumours of the pineal region have been described as a new entity in humans, most likely originating from specialised ependymal cells of the subcommissural organ.
In the canine case presented here, the morphological features, together with the immunoreactivity profile, suggest that we are not dealing with a pineocytoma or pineoblastoma, since these tumours are known to express at least synaptophysin in all species where they have been described. Instead, neoplastic cells exhibited cytokeratin expression and some ultrastructural features of ependymal cells. As a consequence, we consider that the present case represents the first papillary tumour of the pineal region to be reported in an animal.

Effect of oxytetracycline and lysozyme dimer on ultrastructural pattern of the hepatocytes in Siberian sturgeon.

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Introduction: The Acipenseridae are characterized by high resistance to unfavorable environmental conditions, mechanical injuries and diseases. However, during the intensive breeding they become susceptible to infectious diseases. Results of studies on the influence of oxytetracycline (OTC - antibiotic registered for ichtiotherapy in many EU countries) on the hepatocytes of fish species, have not yet been provided. Simultaneous antibiotic therapy and immunomodulation have not yet been described. In regard of the above facts, research on the influence of OTC and lysozyme dimer on the morphology and ultrastructure of the hepatocytes in Siberian sturgeon were carried out.

Material and methods: The experiment was carried out on 150 siberian sturgeons of 2,400 g (± 100 g) body mass (b.m.). The fish were divided into 5 groups (1, 2, K1, K2, K3: n = 30). Fish from the groups 1 and 2 were injected i.p. with 50 and 100 mg/kg b.m. of oxytetracycline (OTC) (Tridox L.A., Eurovet Animal Heatlh BV, The Netherlands). 24 hours after the injection, fish were bathed in water with addition of lysozyme dimer (KLP-602) (100 μg/l) for 30 min. The fish from the group K2 were given only antibiotic (100 mg/kg b.m.) and the fish from the group K3 were only bathed in KLP-602. The fish from the group K1 served as control. Six fish from each group were slaughtered at the same time (directly after the bath in lysozyme dimer and then after 3, 7, 14, and 21 days) and were examined macroscopically. Each time samples of the liver for ultrastructural examination were taken from 2 sturgeons chosen at random. The material was fixed in 2.5% glutaraldehyde in 0.2 mol/l phosphate buffer of pH 7.4 and embedded in Epon 812. Semithin sections were stained according to the Levis and Knight method and assessed microscopically to select the area for subcellular analysis. The ultrathin sections were contrasted with uranyl acetate and lead citrate. Ultrastructural analysis was conducted using an Opton 900 PC TEM (Germany).

Results: OTC at a dose of 50 mg/kg b.m. is the reason of the proliferation of mitochondria, sometimes their destruction and small extravasations. However, OTC at a dose of 100 mg/kg b.m. leads to the intensification of these lesions, desorganisation and sporadic necrosis of cytoplasm and oedema of endothelium in the blood vessels. These lesions were noted in several fish (mainly from the groups K2 and 2) even 21 days after OTC application. They were less intensive and more retrogressive in the fish bathed in lysozyme dimer.

Discussion: Toxic action of OTC most frequently refers to the tissues where the drug is found in the highest concentration, e.g. the liver. Immunomodulation can be expressed as immunosupression or immunostimulation. The latter phenomenon is used in farming of fish with a positive result. The use of sturgeon in the experiment allowed the estimation of the influence of OTC on the substructure of hepatocytes at the recommended (50 mg/kg b.m.) and an increased dose (100 mg/kg b.m.). The results also showed the protective role of KLP-602 on the hepatocytes in sturgeons, which became suppressive doses of OTC.

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Prognostic significance of microvasculature density in canine cutaneous melanoma.

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Introduction: Melanomas, which represent 10% of canine cutaneous tumours, are characterised by a frequently unpredictable clinical behaviour. To improve prognostical levels, some techniques have been developed, in addition to classical histopathology, for instance the immunohistochemical detection of proliferation markers (e.g. the Ki67 antigen) and, in human melanomas, the evaluation of tumoral angiogenesis. The purpose of this study was to determine whether angiogenesis, assessed by intratumoural microvascular density, is linked to the post-surgical prognosis and to other prognostical factors (histopathology and Ki67 index).

Material and methods: Retrospective survival study on 30 dogs with cutaneous melanoma. Each case was submitted to histopathological evaluation, determination of the Ki67 index and morphometry of the tumour microvasculature on CD31 (endothelial marker) immunostained sections.

Results: There were 21 benign and 9 malignant melanomas. Our study confirms the prognostic value of both the histopathological analysis and the Ki67 index. A 5%-cut-off for the Ki67 index predicts death or survival at 24 months post-surgery. Microvascular density has a prognostical value to predict both the metastasis rate and the 2-years survival rate: 260 ± 29 vessels/mm^2 for dogs which have died from their tumour, versus 165 ± 27 vessels/mm^2 for surviving dogs; t-test, p=0.03. Two other microvascular parameters, which are correlated with microvascular density, differ between dead and surviving dogs: the inter-capillary distance (56 ± 3 micrometers, versus 79 ± 6; p=0.03) and the total vascular area (13.6 ± 4.5‰, versus 4.8 ± 0.8 ‰; p=0.03). These three vascular parameters are correlated neither with the histological malignancy nor with the Ki67 index.

Discussion: Microvascular density is an independent prognostical factor in cutaneous melanomas of dogs which predicts both the survival rate and the metastatic risk. In a therapeutical perspective, quantifying microvessel density may help identifying subgroups of malignant melanomas that are relevant to angiogenesis inhibition.

Aromatase expression in the cerebellum of dogs infected with canine distemper virus.

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Introduction: Aromatase, the enzyme that catalyzes the biosynthesis of oestrogens from precursor androgens, is suggestively involved in neuroprotection. Oestrogens are known to induce astrocytes to synthesize progesterone, which is a myelin protective neurosteroid. The present study investigated aromatase expression in the cerebellum of dogs infected with canine distemper virus (CDV), a disease characterized by demyelination in the white matter of the cerebellum.

Material and methods: Histopathological evaluation classified CDV infections as acute (n=6) and chronic (n=6). Immunohistochemical localization of CDV antigen in glial cells of the substantia alba confirmed the CDV infection. Acute and chronic cases were compared to control dogs (n=4) for the number of glial cells, number of aromatase immunoreactive glial cells, and the percentage of aromatase immunoreactive glial cells.

Results: The number of glial cells were significantly ($p < 0.05$) higher in chronic cases. Although the number of aromatase immunoreactive glial cells was significantly higher ($P < 0.05$) in chronic cases as a result of gliocytosis compared to controls, the percentage of aromatase immunoreactive glial cells was significant ($P < 0.05$) only in acute cases (36.52±7.14 %) compared to controls (12.38±1.22 %) and chronic cases (19.71±1.95 %).

Discussion: The results suggest that glial cells, suggestively astrocytes, response to invasion and persistence of CDV by increased oestrogen production to protect nervous tissues. Glial cell response by aromatase synthesis is more prominent in acute cases as the percent aromatase immunoreactive glial cells was higher in cerebella with acute CDV infection.

Equine testicular lesions related to invasion of nematode larvae.

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Introduction: Larvae of Strongyloidae nematodes have been reported to be able to erratically migrate to the vaginal cavity of the equine testis and to provoke characteristic inflammatory lesions.

Material and methods: In a 20-year experience of equine genital organs collected from slaughters or castrations, we found 12 cases of atypical inflammatory lesions in scrotal testes, which are herein described. In one case, these lesions were associated to the presence of three viable nematodes of the species Setaria equina in the vaginal cavity of the testis.

Results: In the vaginal cavity of the affected testes, hydrocele was constantly observed. The tunicae vaginales were generally thickened and numerous adhesions could be present. Furthermore, large elongated bloody to yellowish areas could be seen on the surface of testis and epididymis, especially at the cranial pole. The appendix testis was often included in these lesions or appeared enlarged, prominent and inflammated. Only in one case, at section, haemorrhagic tracts could be observed in the different areas of the testicular parenchyma. Histologically, they were considered haemorrhagic tracts in a developing granulation tissue. Frequent vasculitis and perivasculitis were observed along with infiltrates of lymphocytes, macrophages filled with haemosiderin, mast cells and eosinophils. Squamous metaplasia could be observed focally in the appendix epithelium and diffusely on the surface of the elderly granulomatous lesions. The testicular parenchyma could display mild to severe degeneration, peritubular oedema and focal interstitial lymphocytic infiltrates. Only in one case, at section, haemorrhagic tracts could be observed in the different areas of the testicular parenchyma. The epididymis showed enlargement of head or tail, oedema, interstitial inflammatory infiltrates, sperm granulomas and hyperplasia/squamous metaplasia of ductal epithelium.

Discussion: In agreement with literature, these lesions have to be considered as resulting from invasion of nematode larvae, but the finding of the parasites in scrotal equine testis is extremely rare. It has been hypothesized that worms could penetrate the appendix testis and reach the testicular parenchyma, but generally only their tracks could be seen, identified as elongated and atypical haemorrhagic or already organized lesions with frequent foci of squamous metaplasia. Different grades of orchitis and epididymitis may occur in relation to intensity and time of worm invasion.

Telomerase reverse transcriptase (TERT) expression in canine mammary tissues: A specific marker of malignancy?

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Introduction: Telomerase is a complex enzyme that elongates telomeric sequences at the ends of eukaryotic chromosomes, a process which is necessary for the maintenance of cell proliferation and survival. Telomerase reverse transcriptase (TERT), the catalytic domain of telomerase, is the rate-limiting factor for telomerase activity and is expressed in virtually all tumours, but not in most normal tissues. Therefore, TERT has been proposed as a diagnostic and prognostic marker for many types of cancer. In the present study we investigated the expression of TERT in normal and malignant canine mammary tissues.

Material and methods: A total of 104 mammary tissue samples were analysed: 50 tumours, 50 adjacent to malignant clinically normal tissues, and 4 normal mammary tissues from healthy dogs. H&E stained sections were used for diagnosis according to WHO classification. Canine TERT expression was investigated by both, IHC, using two different anti-human TERT antibodies, and RT-PCR.

Results: TERT was detected in 47/50 malignant tissues, 30/50 peritumoural tissues and in 1/4 normal healthy tissues by IHC. TERT expression showed three patterns of staining: nuclear, cytoplasmic and both, nuclear and cytoplasmic. In the malignant tissues TERT distribution was as follows: nuclear 22/47 (47%), cytoplasmic 10/47 (21%), nuclear and cytoplasmic 15/47 (32%). Using RT-PCR TERT was identified in 49/50 malignant tissues, 47/50 peritumoural tissues and in 2/4 normal healthy tissues. In two of the samples a TERT transcript of a different size was detected, which suggested the presence of an insertion.

Discussion: This is the first study that evaluates canine TERT expression in a large number of mammary tissue samples using both IHC and RT-PCR methods. The results obtained with one of the two antibodies used are being questioned, since a recent report demonstrated a strong reactivity of this antibody to nucleolin, a protein with an almost identical pattern of expression to TERT. Dog TERT was detected in the great majority of tissues, both malignant and normal. This finding challenges the conventional view that TERT expression is repressed in somatic cells and activated in neoplastic cells, but it is in absolute agreement with findings from human mammary tissues. Canine TERT detected in the peritumoural tissues may have been caused by the presence of occult microinvasion. Alternatively, TERT may indeed be expressed by normal mammary epithelium, which suggests a role of this protein in the homoeostasis of normal breast epithelial cells. However, it has to be determined whether such positivity also reflects increased telomerase activity. It is possible that TERT may be regulated in a post-transcriptional or even a post-translational level and give rise to a non-active telomerase. Indeed, in two samples we detected a TERT transcript of a different size. The nucleotide sequence inserted may potentially lead to a premature protein translation and to a non-active telomerase. Overall, our findings support the notion that TERT may not be a useful marker for canine mammary cancer.

Intestinal lesions in PMWS affected pigs in Brazil.

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Introduction: Postweaning multisystemic wasting syndrome (PMWS) is the most recognized syndrome associated with PCV2 infection and since the first description of the disease it has been responsible for a large amount of economic losses in pig production worldwide. Enteric pathogens causing diarrhea in growing pigs are an important problem in swine herds and PCV2 has also been associated with enteric diseases in pigs. The objective of this work is to describe the macroscopical and immunohistopathological findings in 79 PMWS affected pigs which showed clinical signs or macroscopical lesions suggestive of enteric disease.

Material and methods: This study included 79 pigs, confirmed (lesions and immunohistochemistry of PCV2) as PMWS cases, that were selected on the basis of clinical signs or gross findings suggestive of intestinal disease. Animals originating from 12 Brazilian farms were necropsied immediately after euthanasia. Fragments from mesenteric lymph nodes and intestines (small and large) were collected and processed by routine histological methods. Formalin-fixed, paraffin-embedded samples were examined by H&E and anti-PCV2 immunostaining (IS). Sections were processed for IS against Lawsonia intracellularis, Brachyspira spp., Salmonella spp., and porcine coronavirus. Double IS for simultaneous demonstration of PCV2 and cytokeratin was applied. Intestinal samples were submitted for bacteriological examinations for Salmonella spp. and Escherichia coli. Fragments from intestines were collected for scanning electron microscopy. Ziehl-Neelsen staining was performed on samples in which giant cells were seen.

Results: All the 79 pigs had microscopic changes and anti-PCV2 IS positive in samples of intestine and mesenteric lymph nodes. The main macroscopic findings were enlarged mesenteric lymph nodes, mesenteric oedema, necrotizing enteritis, lymphangiectasia and thickened ileal mucosa. Microscopically, there were variable amounts of lymphohistiocytic infiltrates in Peyer´s patches and mucosa, lymphoid hyperplasia, giant cells, intracytoplasmatic inclusion bodies, villous atrophy, lymphatic dilatation, oedema in mucosa and submucosa, dilatation and abscesses in crypts, and mucosal necrosis. Anti-PCV2 IS was seen in Peyer's patches, histiocytes within the mucosa, epithelial cells from crypts and endothelial-like cells from lymphatic vessels of the ileum and colon. Lawsonia intracellularis was observed by IS in 1 case and Brachyspira spp. in 2 samples. Escherichia coli was isolated in 5 samples. IS of Salmonella spp. was positive in 17 of 29 cases of necrotizing colitis. Salmonella positive samples were classified as Salmonella enterica serovar typhimurium. Double IS anti-PCV2 and cytokeratin confirmed the presence of PCV2 within epithelial cells in ileal and colonic samples. Scanning electron microscopy showed shallow crypts and atrophic and fused villi. Ziehl-Neelsen stained samples were negative.

Discussion: The main macroscopic, microscopic, and ultrastructural findings, as well as the PCV2 IS pattern suggested the participation of PCV2 in these lesions. In the presented study Salmonella spp. are the main pathogen found in growing pigs with naturally acquired PCV2 infection and necrotic enteritis. Since the role of Salmonella spp. in these cases of necrotic enteritis is still unclear, additional studies are required to clarify the interaction between PCV2 and Salmonella spp. in intestinal lesions of PMWS affected pigs. This work was supported by CAPES and CNPQ.

The loss of gamma 2 chain laminin-5 in canine mammary tumours as demonstrated by real time-PCR.

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Introduction: Laminin-5 (ln-5), a large heterotrimeric glycoprotein consisting of an alpha 3, beta 3, and gamma 2 chain, is a component of epithelial cell basement membranes which functions as a ligand of the alpha 3 beta 1 and alpha 6 beta 4 integrins to regulate cell adhesion, migration, and morphogenesis. While other carcinoma types exhibit an increased laminin-5 deposition, which has been suggested as an invasion promoting factor, the loss of laminin-5 in mammary cancer supports the view that mammary carcinomas do not utilize laminin-5 for invasion.

Material and methods: Based on an immunohistochemical study, the expression levels of laminin-5 in 73 female dogs with mammary neoplasia were investigated by Real-Time PCR and correlations with clinicopathologic variables were evaluated statistically. PCR amplifications were performed using a 7500 Fast Real-Time PCR System (Applied Biosystems). The gene expression stability over different samples was analyzed using the geNorm software.

Results: There was a loss of expression of gamma 2 chain laminin 5 in mammary tumours compared with normal mammary tissue in all groups. Multivariate analyses indicated that clinical stage, histologic grade, and outcome were independent prognostic factors ($P < 0.05$). The presence of clinical symptoms and the down-regulation of laminin 5 gamma 2 chain were identified as negative prognostic predictors in the univariate analysis ($P < 0.05$).

Discussion: Reduced expression of gamma 2 chain laminin 5 may indicate a poor prognosis and the current results suggest that mammary tumours do not utilize gamma 2 chain laminin 5 for invasion.

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The immunohistochemical study of maspin as a prognostic marker in canine mammary tumours.

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Introduction: Maspin is a serine protease inhibitor that inhibits tumour invasion and metastasis in human breast cancer and is consistently expressed by mammary myoepithelial cells. It is related to the serpin family with a tumour-suppressing function in breast cancer. It has been reported that maspin is expressed in normal mammary epithelial cells and is down-regulated during the progression of cancer. However, to date, there is very limited data on the clinical significance of maspin expression in canine mammary cancer.

Material and methods: The immunohistochemical expression of maspin was studied in formalin-fixed tissues from 54 benign and malignant tumours using a commercially available monoclonal antibody (anti-Maspin (Erviegas®) 1:50. The immunoreactivity was observed in the cytoplasm of these cells.

Results: There was a loss of maspin expression in mammary tumours compared with normal mammary tissue in all groups. Multivariate analyses indicated that clinical stage, histological grade, and outcome were independent prognostic factors. The presence of clinical symptoms and the down-regulation of maspin were identified as negative prognostic predictors in the univariate analysis ($P < 0.05$) when the results are compared with event-free survival by Analysis of Dependency method.

Discussion: Reduced expression of maspin may indicate a poor prognosis and the current results suggest that maspin may function as a tumour suppressor gene in canine mammary cancer.

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