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*University of Naples Federico II
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*European Society of Veterinary Pathology
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*European Society of Veterinary Clinical Pathology (ESVCP)
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Thanks also to the Organizing Committee of the 22nd Meeting of the European Society of Veterinary Pathology for their support and the useful information provided to us.

On behalf of the Organizing and Scientific Committees, and of the Department of Veterinary Pathology and Animal Health and as Dean of the Faculty of Veterinary Medicine of Naples University Federico II, I am very pleased to welcome you to Naples for the 23rd Annual Meeting of European Society of Veterinary Pathology.

This is the first ESVP congress in the South of Italy, therefore it has a special significance and represents an additional reason to point out the central role of ESVP in the spreading the value for the scientific community of veterinary pathology in general, and of comparative pathology in particular. As matter of fact, an important goal of the Society is creation a strong link among as many as possible societies involved in veterinary pathology education thus promoting and strengthening veterinary cooperation in Europe.

The annual meeting of the ESVP has always represented one of the most important scientific events for the veterinary pathologists to discuss the results of their daily professional work. We expected that the selected topics represent and reflect the main research areas and the related papers will be interesting and modern contributions to improve our knowledge in many emerging fields of veterinary pathology. In this context, we hope that the Naples meeting will be able to answer the increasing questions about some perspectives mostly in comparative pathology.

Furthermore, in consideration of the extraordinary cultural and artistic wealth of this part of Campania, we are trying to organize the September event keeping in mind the union between Science and Art. The Organizing Committee is making any efforts that the mixture of Science and Art will be a high light in next meeting.

The meeting will also include a special session organized by the European Society of Veterinary Clinical Pathology and the European College of Veterinary Clinical Pathology jointly with Organizing and Scientific Committee of 23rd ESVP meeting.

As the last years, the annual meeting of the European Division of the C L Davis Foundation for the Advancement of Veterinary Pathology will precede ESVP meeting. My young co-workers and colleagues of the Department of Veterinary Pathology and Animal Health have been offering a remarkable support in the preparation and realization of this congress, especially in the preparation of the proceedings for which we are providing an ISBN number registration to allow the widest spread to be made sure in the scientific world. I would like to thank all persons involved in the organisation of the meeting and all institutions, whose economic supports contributed to make it easier .

*Prof. Franco Roperto
Dean of the Faculty of Veterinary Medicine, University of Naples
President of the Organizing Committee*

Dear Colleagues, welcome to Naples.

The ESVP has had many annual fall meetings in Italy, but until now they have always been in northern Italy. Now we are coming together for the first time in southern Italy, in the famous city of Naples. The difference to northern Italy is obvious, our curiosity to get to know the city and its people is great. We will meet enormous hospitality and friendliness as well as a very interesting history and astonishing buildings.

Above all, however, our goal is to have a friendly, open minded and scientifically most rewarding meeting which will give us new knowledge, new contacts and an overview of what is going on in veterinary pathology in Europe.

The organizers have made every effort to make this meeting a success. Now it is our task to make this come true.

*Manfred Reinacher
President ESVP*

MOLECULAR PATHOLOGY OF BOVINE PAPILLOMAVIRUS: OLD STORIES AND NEW FACTS.

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Bovine papillomavirus (BPV) is perhaps the most extensively studied animal papillomavirus. Six BPV types (BPV-1-6) have been characterised associated with specific lesions with different histopathological characteristics. Recently, thirteen additional types have been found, more than trebling the known number of BPV types.

BPV induces exophytic papillomas of cutaneous or mucosal epithelia in cattle. The papillomas are benign tumours and generally regress without eliciting any serious clinical problems in the host, but occasionally persist and provide the focus for malignant transformation to squamous cell carcinoma, particularly in the presence of environmental cofactors. This has been experimentally demonstrated for cancer of the urinary bladder and cancer of the upper alimentary canal in cattle feeding on bracken fern.

BPV is the only papillomavirus that jumps species. Infection of horses and other equids leads to the development of equine sarcoids. Equine sarcoids are not productive for virus progeny and very rarely regress.

The viral protein E5 is the major transforming protein of the virus and mediates numerous aspects of the viral pathogenesis both in bovine and equine tumours. E5 disturbs the cell cytoskeleton and the Golgi apparatus, activates numerous protein kinases, including growth factor receptor kinases, and down-regulates MHC class I. E5 therefore is responsible for both cell transformation and evasion of the host immune response. These functions of E5 have been observed not only in cultured cells but also in naturally occurring tumours, highlighting the important role of the protein in neoplastic transformation.

PLENARY LECTURE

VIRAL AND BACTERIAL SUPERANTIGENS

GIANNI MARONE

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Conventional antigens usually stimulate <0.001% of the naïve lymphocyte pool, whereas superantigens activate >5% of the naïve lymphocytes. Superantigens have the unique ability to interact with most T lymphocytes expressing antigen receptors from a distinct variable region gene family. Several naturally occurring proteins are superantigens for B lymphocytes. These superantigens possess unusual immunoglobulin (Ig)-binding activities that parallel the activation of lymphocytes by T-cell superantigens. Protein Fv, which is produced in the human liver and released in biological fluids during viral hepatitis A, B, C and E, binds to the variable domain of the heavy chains of Ig, irrespective of Ig class, subclass, and light chain type. One protein Fv molecule can bind six F(ab')₂ fragments of human IgM, IgG and IgE. Protein Fv binds to the Ig VH3+ in a domain outside of the antigen-binding pocket and is a B-cell superantigen. Protein Fv is the most potent IgE-mediated stimulus of human basophil and lung mast cells by interacting with IgE VH3+. Protein Fv functions as an endogenous Ig superantigen interacting with IgE VH3+ bound to FcεRI+ cells. Protein Fv also induces IL-4 release from basophils. It is possible that the production of IL-4 induced by protein Fv in basophils plays a role in the mechanism of increased serum IgE levels in some patients affected by viral hepatitis. HIV-1 gp120 belongs to the Ig superantigen family and human Ig VH3+ are ligands for gp120. gp120 obtained from divergent HIV-1 isolates of diverse viral clades from different geographical regions induced IL-4 and IL-13 release parallel to the secretion of histamine from basophils by interacting with IgE VH3+. Most clinical isolates of *S. aureus* synthesize protein A, a cell wall protein endowed with unique Ig-binding properties. This protein has a classical site that binds IgG Fcγ; in addition, protein A possesses an alternative site that binds the Fab portion of 15% to 50% of human polyclonal IgG, IgM, IgA and IgE. *Staphylococcus aureus* Cowan 1, which synthesizes protein A, and soluble protein A induced histamine release from basophils and mast cells by interacting with IgE VH3+. Protein L, a cell wall protein produced by the bacterium *P. magnus*, binds to the variable domain of the human Ig κ light (L) chains, but not to Ig heavy (H) chains, λ chains, or the CL domains of κ L chains. Protein L binds Ig irrespective of the H chain class, is mitogenic for B cells, and is an Ig superantigen. We demonstrated that protein L induces the release of proinflammatory mediators and cytokines from basophils and lung mast cells by interacting with the κ light chains of IgE bound to FcεRI.

In conclusion, our data provide a novel mechanism whereby viral and bacterial superantigens could likely cause inflammation.

WHAT'S NEW IN CANINE INFLAMMATORY MYOPATHIES

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Inflammatory myopathies (IM) are a heterogeneous group of disorders characterized by nonsuppurative cellular infiltration of skeletal muscle. IMs are relatively common in dogs and can be focal, affecting specific muscle groups such as in masticatory muscle myositis (MMM) and extraocular myositis, or a generalized condition such as immune-mediated polymyositis (PM), associated with an infectious agent such as occurs with *Neospora caninum* infection, or as a pre-neoplastic condition (Evans et al 2004). Biochemical and immunohistochemical studies have shown distinct differences between MMM and PM. Masticatory muscles contain a unique muscle fiber type, type 2M, that differs both biochemically and histochemically from fiber types present in limb muscles. A recent study suggested that an immunologically distinct microenvironment might also be present in masticatory muscles compared with limb muscles (Pumarola et al, 2005). In MMM, CD4+ T lymphocytes were present in greater numbers than CD8+ cells, T cells utilized both $\alpha\beta$ and $\gamma\delta$ receptors, and multifocal clusters of B lymphocytes were present. Antibodies against masticatory muscle type 2M fibers have been consistently associated with MMM. In PM, CD8+ lymphocytes were present in greater numbers than CD4+ cells, T cells used predominantly the $\alpha\beta$ receptor, and no B cells were found. While autoantibodies may be localized to the sarcolemma in a subset of dogs with PM, antibodies against masticatory muscle type 2M fibers are not present. In both MMM and PM, robust muscle regeneration is found, even in the presence of connective tissue expansion (Salvadori et al, 2005). The extensive regeneration demonstrated that muscle may survive this adverse environment if inflammation and fibrosis can be stopped or reduced. These studies provide information that may be useful in the development of new treatment strategies for canine IMs.

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WHAT CAN PEMPHIGUS VULGARIS TEACH US ABOUT EPIDERMAL HOMEOSTASIS

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The skin and mucous membranes undergo constant self-renewal which is governed by a fine interplay between proliferation and differentiation. This process requires a strict balance between recruitment of epidermal stem cells into the proliferative compartment and their subsequent commitment to terminal differentiation. Our own studies and recent literature are more and more unraveling the pivotal role of intercellular adhesion in cell cycle control, onset of terminal differentiation and the development of the cornified layer which all are prerequisites for proper function of the multilayered, tightly sealing epithelium. The most significant indications for these interactions we learned from our investigations of the pathogenesis of certain epidermal diseases such as the autoimmune skin disease pemphigus.

Pemphigus vulgaris (PV) is triggered by autoantibody binding to desmoglein (Dsg) 3, an intercellular adhesion molecule of keratinocytes. We recently described that PV antibodies trigger degradation of Dsg 3 and the associated plaque protein plakoglobin (PG) at the plasma membrane. This resulted in lack of PG-dependent c-Myc suppression which is crucial for the transition between proliferation and terminal differentiation in keratinocytes. Here we show that high c-Myc triggers hyperproliferation in human and canine PV patients despite expression of terminal differentiation markers and continuing stratification. Inhibition of c-Myc by small molecule inhibitors was sufficient to abrogate development of PV-IgG induced lesions in neonatal mice demonstrating the pivotal role of c-Myc overexpression in PV pathogenesis. Keratin 6, 14 and 15 mis-expression reduced α 1 integrin levels further and are compatible with lack of desmosomal strengthening, impaired wound healing and stem cell depletion. In conclusion, PV antibodies impose a mitotic signal on keratinocytes which not only impacts on intercellular adhesion but which retains them in the proliferative compartment. This subsequently leads to impaired wound healing and disturbance of the homeostatic balance. These data provide a novel insight into the pathogenesis of PV and open the possibility for alternative therapeutic strategies using small molecule inhibitors of c-Myc.

ABSTRACTS

REACTIVE AND NEOPLASTIC HISTIOCYTOSIS OF BERNESE MOUNTAIN DOGS: TOWARDS THE IDENTIFICATION OF PREDISPOSING GENES

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Canine histiocytic proliferative disorders are heterogeneous diseases that include reactive disorders such as cutaneous and systemic histiocytosis, and neoplasia such as cutaneous histiocytoma and localized and disseminated histiocytic sarcoma (malignant histiocytosis, MH). Their etiology and pathogenesis are unknown. They share many clinical and pathological features with Langerhans cell histiocytosis in human beings, for which the underlying causes have still to be discovered.

Reactive histiocytosis and MH are highly breed related : their incidence in Bernese Mountain Dogs (BMD) is high, and MH represents nearly 25 % of the cause of death in the French BMD population.

Our aim is to better characterize the nosology and physiopathology of reactive and malignant histiocytoses in BMDs and to identify the genetic causes of these conditions. We undertook the collection of histiocytosis cases reported in France in the last ten years. All these cases were submitted to histological and immunohistochemical characterization and to clinicopathologic investigations. On a large "French BMD" pedigree as well as a set of American cases and controls, genetic analyses are underway to detect predisposing genes. Altogether, these approaches are expected to map and clone the gene for MH in the BMD, leading to a better knowledge of histiocytic-associated diseases in canine and human.

LYMPHOCYTIC INSULITIS IN A DOG WITH JUVENILE DIABETES MELLITUS

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Reports of canine juvenile diabetes mellitus are rare (< 1% of canine diabetes cases), and only very few cases have been investigated histologically. A six-month-old, male, Donge de Bordeaux was presented at the National Veterinary School of Nantes, for polyuria, polydipsia and persistent hyperglycemia. Due to poor long term prognosis and because of the extensive management required, the dog was euthanised and submitted to a complete post-mortem examination. At necropsy, only a diffuse, moderate hepatic glycogen and lipid storage was observed. Histologic analysis of the pancreas demonstrated a severe multifocal lymphocytic insulitis, associated with Langerhans islet atrophy by loss of endocrine cells. Immunohistological characterization of the inflammation revealed a high predominance of CD3+ T-lymphocytes. Insulin and glucagon immunolabelling showed a massive and specific β -cell loss.

In this case the presence of lymphocytic insulitis associated with juvenile onset supports the hypothesis of an autoimmune damage to β -cells, as in type I diabetes of human beings, for which an accurate spontaneous model is still lacking. Pedigree analysis and familial screening are in process in order to explore the possibility of obtaining related affected dogs for further study of the genetic and immunologic characteristics of this disease.

IMMUNOHISTOCHEMICAL DETECTION OF THE ONCOPROTEIN KIT AS A PROGNOSTIC MARKER IN CANINE AND FELINE CUTANEOUS MAST CELL TUMOR

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Canine and feline cutaneous mast cell tumors (MCTs) are challenging because of often unpredictable biologic behavior. Histologic evaluation and proliferative activity appear as the most consistently predictive parameters. However the recently discovered role of the proto-oncogene c-kit and its product the KIT protein in canine MCTs lead to wonder about the prognostic significance of the immunohistochemical evaluation of KIT expression.

KIT staining patterns identified in tumoral mast cells were either membrane-associated (i.e. similar to normal mast cells), or abnormal (i.e. intense paranuclear, to diffuse cytoplasmic, with or without loss of membrane-staining). These patterns allow to define two categories of canine and feline MCTs : tumors in which membrane-associated staining predominates and those displaying a majority of abnormal KIT staining.

In a retrospective survival analysis of 24 canine and 24 feline MCTs, abnormal KIT staining was significantly associated with a decreased survival rate at 2 years (20% versus 100% in dogs ; 18% versus 85% in cats). Mean overall survival time was decreased (9 ± 8 versus 33 ± 4 months in dogs ; 7 ± 6 versus 26 ± 14 months in cats). On the basis of these results, we propose to use KIT immunolabelling for the routine prognostic evaluation of MCTs.

STUDY OF RETINA AND VISUAL PATHWAYS IN NATURALLY AFFECTED SCRAPIE ANIMALS

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Scrapie is characterized by a spongiform degeneration and PrP^{sc} accumulation in central nervous system (CNS). Other, although not specific, histological lesions, are astrogliosis, apoptosis and neuronal loss.

The histopathological study of retina in TSEs has been mainly focused on experimental models, being only few the studies on natural scrapie. The aim of the present work was to study the distribution of PrP^{sc} in several retinal layers in relation with the evolution of the disease and the evaluation of the presence of associated retinopathies. Additionally, an histopathological examination of the main visual pathways areas was carried out.

Histopathological and immunohistochemical studies on CNS samples (retina and brain) from 20 Scrapie cases (in preclinical, clinical or terminal stage of the disease) and 6 control negative cases were performed.

The main affected layers were the inner plexiform layer and ganglion cells. A scarce involvement of other layers, such as the photoreceptor layer, mainly in the first stages of the disease, was also demonstrated in this work. Furthermore, a high correlation between PrP^{sc} accumulation and glial fibrillar acidic protein was observed, clearest in terminal stages. Finally, the main affected areas were located in the Diencephalum (Pretectal region). Concerning the visual pathways, a higher affectation chiefly in the reticular formation and geniculate nuclei and in the later stages of diseases, was detected.

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The assistance in processing the samples provided by the National Reference Centre for TSEs and the TSE Regional Laboratory in Aragón technical staff is also gratefully acknowledged.

CHRONIC NERVOUS SYNDROME IN A CAPTIVE CHEETAH *ACYNONIX JUBATUS*

*Acín Cristina*¹, *Fernández de Luco Daniel*², *Monleón Eva*¹, *Monzón Marta*¹, *Rodríguez José María*³, *Martin Mariangeles*³, *Hortells Paloma*¹, *Bolea Rosa*¹, *Vargas Antonia*² and *Badiola Juan José*^{1,2}

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An adult female captive cheetah *Acynonix jubatus* (11 years) showing neurological symptoms characterized by ataxia, incoordination bilateral blindness and weakness was studied. The animal was treated with B complex, nandrolone, auxine A+B and omeprazol during the first stages of the disease and with dexametasona, B complex and marbofloxacin in the last stage of the disease. Finally the animal was sacrificed.

Macroscopic lesions weren't seen at the necropsy. Liver, spleen, kidney, mesenteric ganglia, pancreas, lung, heart and central nervous system (cervical spinal cord, obex, pons, cerebellum, mesencephalon, diencephalon and frontal and parietal cortex) were sampled for histopathological study. The samples were stained with haematoxylin / eosin and immunostained for PrPsc detection, using a monoclonal antibody.

In the cervical spinal cord and medulla oblongata, sclerosis and mononuclear perivascular cuffing was observed. There were also gliosis foci surrounding the damaged zone, and generalized gliosis all around the central nervous system. PrPsc immunodetection was negative in the central nervous and linforreticular systems. The presence of viral antigens is been studied as the possible etiologic origin of the disease.

GROSS PATHOLOGY FINDINGS OF PROXIMAL DUODENITIS JEJUNITIS IN A FILLY.

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A two years old filly with history of diarrhea and dysentery for a couple of days without any response to antibiotic therapy was referred to the teaching hospital of Faculty of veterinary medicine of University of Tehran. The gross pathology findings were including, Hyperemia and congestion of mucosal surfaces specially conjunctiva and vagina, Dilation of stomach and small intestine with large volume of tinged blood fluids, The mucosal surface of duodenum was highly congested, Lungs were red dark in color and several petechial and echimotic hemorrhages were observed on the dorsal surfaces, On the heart sub-endocardial hemorrhages were evident. Samples from intestinal ingesta, a long bone, liver, kidney and spleen were collected for bacteriological tests. Clostridium perferingens was isolated from bone marrow. In this case, the tentative diagnosis of proximal duodenitis jejunitis (PDJ) was made. Although Salmonellosis may suspect alternatively, no Salmonella spp. was isolated from any organs. Histopathological findings will be discussed in details and according to large animal internal medicine PDJ hardly responses to current therapies.

RETROSPECTIVE STUDY OF CANINE EPITHELIAL, MELANOCYTIC AND MESENCHYMAL TUMORS OF SKIN

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Out of the total number of canine tumors examined based on the biopsy and section materials over the last five years at the Department of pathology, skin tumors and mammary gland tumors were proved to be the two largest groups diagnosed in 33.4% and 36% of cases, respectively. Cutaneous tumors were diagnosed in 211 cases, i.e., in 123 (58.3%) male dogs and 88 (41.7%) bitches, of different breeds, averagely aged approximately 7 years. Among the total number of 211 skin tumors, 32 types of cutaneous neoplasms were diagnosed, with epithelial and melanocytic tumors being the most predominant (58,6%), followed by mast cell tumors and histiocytic tumors (22,3%) and mesenchymal tumors of the skin and soft tissues (19.1%). Epithelial and melanocytic skin tumors were the most frequent (123 cases), with four subgroups within the group: tumors with adnexal differentiation - 66 cases (53.23% of all epithelial and melanocytic skin tumors), epidermal tumors - 25 cases (20.16% of all epithelial and melanocytic skin tumors), tumors without squamous or adnexal differentiation - 16 cases (12.90% of all epithelial and melanocytic skin tumors) and finally melanocytic tumors - 14 cases (11.29% of all epithelial i melanocytic skin tumors). The age of dogs with epithelial and melanocytic cutaneous tumors ranged between 5 months to 15 years. Mast cell tumors and histiocytic tumors comprising the total of 47 cases and mesenchymal tumors of the skin and soft tissues (41 cases) were subjected to histological analysis, as well as immunohistochemical analysis if necessary, and the tumors were classified based on the WHO classification of the cutaneous neoplasms. The fact that the number of skin tumors diagnosed in 1999 was doubled in 2002 is of the particular importance, which may be, among others, the result of increasingly high interest of clinicians for biopsy-based diagnosis.

**ANATOMO-PATHOLOGICAL AND FORENSIC ENTOMOLOGICAL
EXAMS FOR DETERMINING THE BURIAL TIME
OF SWINE CARCASSES IN A CRIMINAL CASE**

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Post mortem evaluation of a number of swine carcasses, using anatomo-pathological exam, was compared to entomological analysis to determine the burial time. In the selected area of denounced land a series of random holes were dug. Carcasses were found in different stages of decomposition, and entomological material was also found with and around the rotten parts: that fauna was composed of Diptera and Coleoptera. Where there was only bone tissues remaining there was an absence of entomological reports. In one case by use of entomological samples it was possible to identify a vertical burial stratification. Anatomo-pathological analyses in veterinary medicine can make use of entomological evaluation as a useful tool to investigate the illegal disposal of carcasses.

ACUTE SMALL INTESTINAL OBSTRUCTION BY A PIECE OF SPONGE IN A GERMAN SHEPHERD DOG

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A 2-year-old male, drug detector German Shepherd dog carcass, with a history of inappetence, frequent vomiting, lethargy, dehydration and loss of body condition was referred to pathology division of Teaching and Research Hospital, Faculty of Veterinary Medicine, University of Tehran for necropsy. In gross examination the carcass was emaciated. The stomach and upper small intestine were congested and edematous with serosal extensive petechial and ecchymotic hemorrhages and accumulation of reddish watery putrefied odorous fluid in lumen and transudation from the peritoneal surface.

The middle portion of jejunum was completely obstructed by a piece of compacted sponge (absorbent mass), approximately 5 cm in diameter.

Pressure necrosis of the mucosa at the site of lodgment of the sponge had occurred and led to perforation and peritonitis.

Fibrinosanguinous fluid (100 ml) in the peritoneal cavity and adhesion of the small intestinal loops by fibrinous exudates were noticed. Distal to the point of obstruction, the bowel was collapsed and empty. Pure E.coli was isolated from visceral organs. There were numerous small foci of necrosis in the liver. It is likely that the sponge was poisoned with a hepatotoxin.

This is the first report of small intestinal obstruction by sponge in dog.

PRIMARY AND SECONDARY CARDIAC TUMOURS IN DOGS AND CATS

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Tumours of the hearts are mainly presented as case reports. This study summarizes primary and secondary cardiac tumours in dogs and cats routinely investigated in our institute during the last two years.

83 of 212 dogs and 29 of 127 cats were suffering from neoplastic diseases. Hearts were investigated macroscopically with special respect to tumours. Standardized specimens from ventricular walls, septum, aorta and tumours were examined histopathologically and immunohistochemically (CD31, CD3, CD79, CD45, FeLV-antigen, vimentin, desmin, neurofilament, cytokeratin, NSE).

11 dogs showed primary cardiac tumours (hemangiosarcoma n=7, chemodectoma n=2, rhabdomyosarcoma n=1, neurofibrosarcoma n=1).

In 26 of 66 dogs metastases of extracardiac neoplasia were found in the heart (16/36 carcinoma, 6/13 lymphosarcoma, 3/8 hemangiosarcoma, 1/8 other sarcoma).

In 15 cases metastases were easily seen by routine investigation. However, small metastases were identified grossly only after detailed laminating (n=5) or histopathologically (n=6). In four dogs metastases (carcinoma n=2, sarcoma n=2) occurred exclusively in the heart.

In cats only five cases of secondary cardiac tumours (3/13 lymphosarcoma, 2/14 carcinoma, 0/2 sarcoma) occurred.

This study shows an unexpected high number of cardiac metastases in dogs, probably relevant in diagnosis and therapy. Clinical data available from these dogs will be correlated to the pathological findings.

ULTRASTRUCTURAL PATTERN OF THE ORGANS IN TURKEYS FED A DIET CONTAINING OXIDIZED FAT AND INFECTED WITH THE O78K80H9 PATHOGENIC SEROTYPE OF ESCHERICHIA COLI

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The 40 BUT-9 turkeys, for the first 8 weeks of life, were fed a mixture supplemented with a fat of peroxide value (PV) up to 5 and 150 mEq O₂/kg. After that period 10 turkeys from each PV level were infected through the air sacs with 1 ml of O78K80H9 pathogenic serotype of Escherichia coli suspension representing the 8 X 10⁶ bacteria (McFarland's scale). After subsequent 4 days, all birds were sacrificed and analyzed - ultrastructural examination: medial gluteal muscle and liver).

Mentioned above feeding turkey hens for 8 weeks leads to ultrastructural lesions most often in mitochondria, rarely in SER and RER and additionally to foci of necrosis and myelin-like structures in liver. Such feeding also causes a tendency to steatosis. The birds fed diets supplemented with oxidized fat and then infected with E. coli exhibited more explicit tendency to ultrastructural lesions in the mentioned organelles in comparison with healthy birds.

PATHOLOGICAL AND MOLECULAR BIOLOGICAL STUDIES OF BOVINE PAPILLOMA IN KOREA

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Since clinical bovine papilloma-like infection has been reported frequently in Korea, it is unclear about its exact prevalence, pathology and molecular epidemiology. To investigate the prevalence, pathology and molecular epidemiology of bovine papilloma infection, teats of 880 cows (432 from Holstein and 448 from Korean native cows) were collected from local abattoirs of 9 different provinces in Korea during January 2001 to November 2002. On gross and histopathological examinations, bovine papilloma lesions were observed in 296/880 (33.6%) teats. The prevalence of papilloma was 8 times higher in Holsteins (263/432) than that in Korean-natives. Histopathologically, teat papilloma was characterized by various degree of hyperkeratosis, severe hyperplasia of granular and prickle cell layers, and large, irregular and keratohyaline granules in granular cells. Immunohistochemically, bovine papilloma virus (BPV) antigen was scattered in the nuclei of degenerated granular and cornified cells. Twenty-three percent of papilloma in Holsteins whereas 3.8% in Korean native cows were positive for BPV by immunohistochemistry (IHC). Electron microscopically, BPV particles were found in 39.2% out of papillomas in Holsteins and 0.0% in Korean native cows. To increase the sensitivity and specificity of detection rate, PCR assay was developed with one primer pair to detect any BPV type and each six primer pair specific to BPV types 1 to 6, respectively. BPV DNA was amplified in 71.4% out of Holstein teat papilloma by PCR with BPV universal primer pair, whereas 21.4% out of Korean-native teat papilloma was positive. Of ten samples positive by universal primer pair, only two samples were positive for BPV type 1 and type 6, respectively. The nucleotide sequences between each PCR amplified product of field teat papilloma were compared with the other known BPV types. All 21 teat papilloma showed 36.7.76.0% nucleotide sequence homology in comparison with that of BPV 1.6 reference strains. From these results, bovine papilloma infection was prevalently occurred in Korea. Moreover, it was speculated that the variants of BPV were circulated in Korea.

LISTERIA MONOCYTOGENES INFECTION IN KOREAN NATIVE GOAT : A CASE REPORT

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This paper describes naturally occurring case of meningoencephalitis associated with *Listeria monocytogenes* in a 4 month-old Korean native goat. The goat was raised in a herd consisting of seventy kids, and fed on straw, grain feed, and garbage that included onions, cauliflower, and bean sprouts. Clinical symptoms in the animal included circling and recumbency before death. No conspicuous gross lesions were found except consolidation of the lung. Microscopically, multifocal abscesses were observed in brain stem and mid brain. Mild nonsuppurative meningoencephalitis was seen in the brain. In addition, moderate bronchopneumonia was shown. *L. monocytogenes* was isolated from brain stem and PCR assay, using brain stem, amplified *L. monocytogenes* listiolysin O region. It was presumed that this case was caused by intake of contaminated garbage.

REAL-TIME RT-PCR EXPRESSION ANALYSIS OF ESTROGEN-RECEPTOR α , ESTROGEN-RECEPTOR β , PROGESTERONE RECEPTOR, AND ERB-B2/NEU IN FELINE MAMMARY CARCINOMAS AND MATCHED

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The majority of human breast carcinomas (HBCs) are positive for estrogen- and progesterone-receptors (ER, PR) expression. High levels of sex-steroid receptors represent a good prognostic factor, whereas ER-negative HBCs often show increased expression of the oncogene Erb-B2/Neu, which is associated with a poor prognosis. Feline mammary carcinomas (FMCs) appear to be frequently ER-negative and/or PR-negative. The aim of the present study was to evaluate, at the molecular level, the expression of ER- α , ER- β , PR, and Erb-B2/Neu in FMCs by comparison with normal controls. Adjacent samples of normal breast tissue and matched primary breast tumours were obtained from 26 female cats. After total RNA extraction and reverse transcription, relative expression of each target gene was measured by means of quantitative real-time PCR. Beta-glucuronidase was used as reference gene. Log-transformed values of expression for each sample pair (FMC and matched control) were analysed in a Wilcoxon matched-pairs signed-ranks test. Expression of ER- α and PR was significantly lower ($p < 0.0001$) in FMCs compared to matched controls. ER- β and Erb-B2/Neu showed no significant difference between the two groups. Molecular evidence confirms that most FMCs are ER- and PR-negative, whereas it raises a question on the recently proposed role for Erb-B2 in feline mammary tumours.

THE HISTOPATHOLOGICAL EFFECTS OF SUPEROVULATORY DRUGS GnRH α /HMG/HCG ON THE ENDOMETRIAL TISSUES OF RAT.

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Using of stimulatory drugs such as : GnRH α / HMG / HCG for induction of superovulation and harvesting more oocytes is very common during Assisted Reproductive Techniques (ART) protocols. Studies and experiences have recently shown that the rate of successful implantation in stimulatory cycles is less than natural cycles. On the other hand, it is also shown that endometrium has a main role in implantation. At the present study we use an experience animal (Rat) as a model. We have investigated the possible effects of above mentioned drugs on ultrastructures of endometrial tissues (Luminal epithelium). For this purpose endometrial biopsies were obtained from female rats (N=15) which were undersuperovulation treatment and those rats which never given any drugs (N=15).

The specimens were processed for electronmicroscopic studies. Qualitative and quantitative (morphologic and morphometric) studies were carried out on electronmicrographs. The data have been compared using statistical methods.

Morphological findings evaluated based on three structures namely: nuclear Channel system (NCS), giant mitochondria (GM) and glycogen vacuoles. Additionally pinopod system and nuclear euchromatin. Morphometric findings has also shown that in case group, volume fraction of nucleus, RER, mitochondrium, glycogen to cell and euchromatin to nucleus had statistically difference significance. These results (based on present study findings) suggest that ovulation drugs have a negative effects on endometrial tissues at the time of implantation which may lead to low implantation rate.

Key words: *Luteal phase of endometrium, morphological changes, superovulatory drugs, IVF.*

FIRST REPORT OF PORCINE DERMATITIS AND NEPHROPATHY SYNDROME IN SWEDEN

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Introduction: Porcine dermatitis and nephropathy syndrome (PDNS) is an immune-mediated vascular disease affecting the skin and kidneys in nursery and grow-finish pigs. This sporadically occurring condition was originally described in the UK in the early 90ies and has subsequently been identified in many pig-producing countries. Herewith, the first PDNS case in Sweden is reported.

Material and Methods: A 12 weeks old pig was euthanized and necropsied, due to the development of round to irregular, red to purple macules and large patches on the skin, mainly located on the hind limbs, perineal area and ears.

Results: The post-mortem examinations revealed, besides the haemorrhagic cutaneous infarcts, a generalized lymphadenopathy and enlarged, pale kidneys with petechial cortical haemorrhages. Histopathologically a systemic leucocytoclastic necrotising vasculitis, mainly in medium sized arteries in the skin and a severe necrotising and fibrinous glomerulonephritis were observed. Additionally, a slight to mild interstitial pneumonia and mild to massive lymphocyte depletion in the lymph nodes and occasional giant-cells within follicular areas were seen.

Porcine circovirus type 2 (PCV2) antigen was demonstrated in the lymphoid tissues by immunohistochemistry (ABC-method).

Conclusions: The clinical signs, gross post-mortem and histopathological changes were similar to those previously described for PDNS. As in previous reports, the lesions were associated with PCV2 infection.

INFLUENCE OF THE YTYPE AND DURATION OF FIXATION ON THE AETIOLOGICAL DIAGNOSIS OF MAEDI-VISNA IN LUNG SAMPLES

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Histopathological diagnosis of Maedi-Visna virus (MVV) infection in the lung is based on the recognition of a typical interstitial pneumonia. In some cases showing mild lesions or complicated with exudative pneumonias, aetiological diagnosis is seen as necessary. Immunohistochemistry or MVV detection by PCR in paraffin embedded samples are commonly used methods. The influence of the different fixation techniques on the efficacy of these techniques is not known. In this work, lung samples from 5 sheep showing lesions consistent with the respiratory form of MV have been maintained in three fixatives (10% buffered formalin, Bouin solution, zinc-salts based fixative) for different periods of time, between 1 and 30 days. After paraffin embedding, samples were examined microscopically and PCR and immunohistochemistry were performed.

Lesions were easily detected by using the three fixatives. They were severe (n=2), moderate (n=2) and mild (n=1). MVV was detected in formalin and zinc-salt treated samples until the end of the experiment. However, in Bouin fixed samples the reaction could only be examined at 24 h of fixation due to the appearance of non-specific reactions in relation with the duration of treatment. By PCR, samples fixed in formalin later than 14 days were always negative. No amplification was obtained in Bouin fixed samples and results were variable in zinc-salts treated samples. The lung showing mild lesions was only detected by immunohistochemistry.

This work has been possible thanks to Project LE52/04, Junta de Castilla y León.

KINETICS OF IgM+ CELLS IN LINFOHEMATOPOIETIC ORGANS OF TURBOT (*Scophthalmus maximus L.*) EXPERIMENTALLY INFECTED BY *Enteromyxum scophthalmi* (MYXOZOA).

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While immune responses of fishes to numerous bacterial and viral pathogens are well documented, our knowledge concerning the immune response to parasites is scarce. It is known that after certain antigen stimulation, antibody-producing cells appear in the kidney and spleen and their numbers increase exponentially. The aim of the present study was to evaluate the kinetics of these cells in the linfomatopoietic organs of turbot experimentally infected by *E. scophthalmi*, a myxosporean parasite that causes heavy losses in marine aquaculture. Twenty-five healthy turbot and twenty-five turbot infected by effluent transmission were employed. Fish of both groups were sampled at days 20, 40, 55, 77 and 106 post-exposure (PE). Bouin-fixed, paraffin-embedded samples were used and immunoperoxidase staining was performed with a rabbit polyclonal antiserum against turbot IgM. Mann-Whitney test was applied in order to assess the difference in the number of IgM+ cells between the two groups of fish. The higher number of IgM+ cells was found in the group of infected turbot at the beginning of the experiment. Afterward, the percentage of IgM+ cells in the infected group decreased until the end of the experiment. These results suggest that infection by *E. scophthalmi* induces a local (in the digestive tract) and a systemic response to the parasite.

NASAL CONIDIOBOLOMICOSIS IN TEN HORSES: EPIDEMIOLOGICAL, CLINICAL AND PATHOLOGICAL STUDY

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Aim of the study: to report the clinical, epidemiological, and pathological findings of nine costarricenses horses with nasal conidiobolomycosis.

Material and methods: nine nasal biopsies were taken and processed for histopathological examination. They were stained with H&E, PAS and Gomori's silver.

Results: all cases came from the pacific littoral (dry forest), in seven the complain started during dried season. The age ranged from 2 and 20 years old. However, 5 were between 3 and 5. There were 5 females and 5 males.

The main clinical sign was a chronic nasal discharge blood tinged, and mucopurulent, in eight it was unilateral. Grossly the lesions were mainly described as a mucosal irregular surface with erosions. Histopathologically: In general, there was an inflammatory reaction predominantly eosinophilic. Besides, a multiple hypereosinophilic, 50-400 μm foci, with a longitudinal or transversal hyphae (9.0 to 13 μm) with the splendore-hoeppli phenomenon were found. The hyphae were also demonstrated by Grocott and PAS stain.

Conclusion: despite been rarely reported. The Conidiobolomycosis is a very important nasal disease in costarricenses horses, in fact, in study done during in almost a same period of time only 3 nasal squamous cell carcinomas, one rhinosporidiosis and one inflammatory polyp were found.

INTESTINAL PROSTHENORCHOSIS SP INFECTION IN SEVEN MONKEYS FROM COSTA RICA

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Aim of study: to described the clinical and pathological findings in two captive and five wild *Saimiri oerstedii citrinellis* monkeys associated with the nematode *Prosthenorchis* sp.

Results: the carcasses of 3 *S. o. citrinellis* and formalin fixed tissues from 4 other monkeys, 5 males and 2 females ranging from 6 months to 5 years of age, were examined. Clinical signs of the 2 captive monkeys included alopecia, anorexia and emaciation. The ileum, cecum and/or colon of all 7 monkeys contained several adult *Prosthenorchis* sp. Three of the monkeys had peritonitis due to cecal and colonic perforations. Microscopically, the probocids of *Prosthenorchis* sp were embedded in the intestinal mucosa and muscular layers, creating a fistula and a granulomatous reaction. A few fibrotic nodules were present in the serosa.

Conclusion: It is well known in the literature that the new world primates *Saimiri* monkeys showed a highest prevalence of infection with *Prosthenorchis* spp, which may be either refractory to infection or the intermediate hosts (cockroaches and beetles) which constitute part of their normal diet. In fact, all our cases were *Saimiri* monkeys regardless they were living in captivity or in the wild.

CUTANEOUS EQUIDAE NEOPLASMS IN COSTA RICA: A RETROSPECTIVE STUDY (1994-2004)

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Aim to study: was to describe the cutaneous equine neoplasms in Costa Rica.

Materials and methods: a clinical and pathological retrospective study from 1994 to 2004 was done.

Results: 250 equidae skin biopsies were processed, 88 cutaneous neoplasms were diagnosed 86 in horses and 2 in burros. The three most common observed were sarcoid, 54,5% 46 in horses and 2 in burros. The breed predilections were Criollos (60.8%). There were 26 males and 16 females. The average age was 4.4 years. In 21 horses the sarcoid was a single mass. The main anatomic locations were the limbs 15. Squamous cell carcinoma (17.0%), 10 Criollo breed. In 7 horses, the tumour growth was reported in white skin areas. The mean age was 8.84. There were 8 females and 7 males. Ten carcinomas (66.6%) were present in the head. Melanocytic tumours (13.6%). All in grey-white horses. The mean age was 11.6. There were 7 males and 5 females. In 8 cases (66.6%) the tumor was located at the anal region.

Conclusion: as it has been reported elsewhere we found the same three most common skin neoplasms however, our epidemiological data specially with sarcoids and squamous cells tumours have some differences.

MORPHOMETRIC ASSESSMENT OF CANINE LUNG CHANGES AS MONITORING TOOL FOR ATMOSPHERIC POLLUTION

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Epidemiological and experimental studies have demonstrated that urban pollutants can cause chronic inflammation and higher tumour incidence in the dog airways. To assess if objectively measured lung changes can be proposed as a model to monitor public health risks related to air pollution, a retrospective study has been undertaken on 200 formalin fixed paraffin embedded randomly selected samples of canine lungs archived from 1975 to 2005. Histological sections stained with HE, Perls for iron and Masson trichrome for collagen, have been observed in transmitted and polarised light to assess the deposit of particulate matter and the degree of interstitial fibrosis. All parameters have been graded subjectively and also measured objectively by image analysis, and related to the dog age, sex, breed, lung changes and the epoch of sampling. Pneumoconiosis ranged from 0,02 to 1,52% of lung tissue, and was represented by black dust or grey crystalline refractile particles within macrophages in the peribronchiolar and perivascular interstitium. There was significant correlation between pneumoconiosis degree as subjectively estimated or measured by image analysis, but there was not strict association with other considered parameters. Regardless of concurrent diseases, interstitial fibrosis ranged from 0,55 to 34,95% of lung tissue, and was associated with higher deposits of crystalline dusts.

KIDNEY PROTEOME ANALYSIS OF A NOVEL MOUSE MODEL FOR DIABETES MELLITUS

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Diabetes mellitus is the most common disease leading to end stage renal failure in industrialized countries and accounts for about 50% of dialysis patients. Transgenic mice expressing a dominant negative glucose-dependent insulintropic polypeptide receptor (GIPRdn) represent a novel model of diabetes mellitus and have recently been shown to develop diabetes-associated kidney changes. The renal proteome of 218-d-old female GIPRdn transgenic mice (n=5), showing severe hyperglycemia and hypoinsulinemia, was compared with that of wild-type littermate controls (n=4). Proteins derived from whole-kidney lysates were separated by 2D-gel electrophoresis, stained with Sypro RubyTM and analyzed quantitatively using the ProteomWeaver Software. From approximately 1,000 visualized spots, 33 differed in their abundance by at least a factor of 1.5 in kidneys from GIPRdn transgenic mice. Corresponding protein spots were cut from preparative gels, stained with colloidal Coomassie and identified by MALDI-TOF mass spectrometry. A subset of these proteins is associated with the diabetic metabolism and reflects e.g. up-regulation of enzymes involved in gluconeogenesis. A further subset includes smooth muscle contractile elements and proteases, while the function of the remaining proteins is unknown and subject to further studies. These data demonstrate the great potential of proteome analysis to determine novel pathogenetic mechanisms of diabetes-associated kidney disease.

LARGE SCALE ISOLATION OF GLOMERULI FROM MURINE KIDNEYS: COMPARISON OF DIFFERENT METHODS AND SOLUTIONS FOR METHODOLOGICAL PROBLEMS

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Mice are widely used as animal models in nephrological research. Transcript profiling or proteomic analysis of kidney glomeruli from mice require suitable methods for the isolation of intact glomeruli in sufficient numbers and purity, within acceptable time and under defined temperature conditions. Different from other species, it is difficult to isolate pure glomeruli from murine kidneys, using simple sieving techniques. In this study, two advanced methods were compared and improved: Glomerulus isolation by combination of sieving and microdissection (s/md), and magnetic isolation of glomeruli from kidneys perfused with spherical superparamagnetic beads. To avoid critical points of the perfusion technique (e.g. precipitation of beads, air bubbles, perfusion pressure fluctuations), we constructed a device allowing uncomplicated perfusion of nearly all glomeruli with sufficient numbers of beads under adjustable pressure conditions. Using the magnetic isolation, an average of 10,000 glomeruli per kidney with 95% purity was harvested within one hour. Performing the s/md method without collagenase pre-treatment, isolation of 300 glomeruli of nearly 100% purity was achieved within 30 minutes at 4°C. Both methods provide glomerulus isolates of high purity, but differ with respect to efficiency. If larger quantities of glomeruli are required, e.g. for proteomic analysis, magnetic isolation of glomeruli is recommended.

EVALUATION OF APOPTOSIS AND CORRELATION WITH HEAT SHOCK PROTEIN (HSP) 27, 72 AND 73 EXPRESSION IN CANINE INTRACUTANEOUS CORNFIFYING EPITHELIOMAS AND SQUAMOUS CELL CARCINOMAS.

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The events of cell stress and cell death are linked and some HSPs appear to play an important role in neoplastic process also inhibiting apoptotic pathways. This study was carried out on 9 Intracutaneous Cornifying Epitheliomas (ICE) and 13 Squamous Cell Carcinomas (SCC). Immunohistochemical expression of Hsp27, 72 and 73 was determined using commercially antibodies, while apoptosis was evaluated through TUNEL method and immunohistochemical expression of total- and active-caspase-3. We found numerous TUNEL-positive tumour cells, prevalently in SCC, but only few active-caspase3-positive cells, although there was a diffuse total-caspase-3 immunolabelling both in normal and neoplastic tissues. Hsp27 labelling was more intense in normal epidermis and in tumour areas showing squamous differentiation. Hsp72 and Hsp73 immunoreactivity were respectively more and less intense in ICE and SCC than in normal skin. We found a lack of correlation between TUNEL method and active-caspase-3 expression. Considering the limits of TUNEL assay in detecting only apoptotic cells, particularly in tumour tissues, we considered that detection of a scant number of active-caspase3-positive cells and Hsp72 overexpression might be suggestive of an inhibition of apoptosis in these skin neoplasms, that might be important for oncogenesis and tumour growth.

EVALUATION OF HEAT SHOCK PROTEIN (HSP) 27, 72 AND 73 EXPRESSION AND APOPTOSIS IN NORMAL AND DYSCHONDROPLASTIC PIG ARTICULAR CARTILAGE.

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This study was carried out to investigate the role of Heat Shock Proteins (HSPs) and apoptosis in normal articular cartilage and in the pathogenesis of dyschondroplasia, a disease characterized by a failure of chondrocyte differentiation. Four normal and eight dyschondroplastic medial humeral condyles of abattoir pigs, were processed for histopathological and histochemical (Alcian Blue, Masson's trichrome) investigations. Immunohistochemical expression of HSP27, 72 and 73 was determined using a streptavidin-biotin-peroxidase technique. Apoptosis was evaluated performing TUNEL method. A strong HSP27 immunoreactivity was detected within the hypertrophied chondrocytes, in normal and dyschondroplastic articular cartilage, in clusters of proliferating chondrocytes adjacent to areas of chondrolysis, as well as islands of persisting denatured cartilage. None or weak immunoreactivity was detected against HSP72 and HSP73. Few TUNEL-positive chondrocytes were present in the hypertrophied layer of control samples; these positive cells seemed to increase in dyschondroplastic samples, while there were no positive cells in islands of persisting cartilage. Although preliminary, our results seem to remark the important role of HSP27 and apoptosis in swine articular cartilage metabolism and in the pathogenesis of swine dyschondroplasia: HSP27 functions in chondrocyte proliferation, differentiation, mechanical stress response and it could have a cytoprotective role in chondrocytes, protecting them against apoptosis.

SIALIC ACID AND GM3 GANGLIOSIDE EXPRESSION IN SOME PAPILLOMAVIRUS-ASSOCIATED URINARY BLADDER TUMOURS OF CATTLE

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Cows grazing on bracken fern-infested lands frequently develop bladder cancer (Pamukcu et al., 1976) thus suffering from chronic enzootic haematuria (CEH) BPV-2 DNA is present in the majority of naturally occurring urinary bladder tumours of cattle and that its major oncoprotein E5 is expressed in the cancer cells (Borzacchiello et al., 2003). The ganglioside content and pattern in thirty papillomavirus-associated urinary bladder tumours were investigated, for the first time, in cattle suffering from BPV-2 associated urinary bladder tumours..

The ganglioside content in normal urinary bladders was 28.6 ± 3.35 g lipid bound sialic acid per gram of fresh tissue. This value was higher in tumor bladder samples but very variable, 93.6 ± 45.27 g lipid bound sialic per gram of fresh tissue. In all normal and pathological samples the main ganglioside was GM3. It was about 75% of total ganglioside mixture in normal tissues and between 50 to 80% in tumor samples.

Both low and high grade urothelial carcinomas of the urinary bladder of cattle appear to metastasize less frequently than low and high grade urothelial carcinomas of man and dog. Factors involved in some invasive and metastatic properties of bovine urothelial cancers are not well understood.

The Authors emphasize the role of GM3 ganglioside in downregulating the metastatic potential of bovine urothelial cancers.

PRIMARY PULMONARY CARCINOSARCOMA IN A CAT WITH CARCINOMATOUS METASTASIS TO THE DIGITS

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Primary tumours of the lung are uncommon in cats and to date only one carcinosarcoma has been reported. The most common pattern is adenocarcinoma which has often been reported to metastasize to peripheral organs, including the digits. We describe here a case of a primary pulmonary carcinosarcoma with digital carcinomatous metastasis without the mesenchymal component. To our knowledge this has not been previously reported in cats. A 12-year-old male castrated European shorthair cat was presented for investigation with a history of lameness and swelling of multiple digits. At necropsy a lung mass was noted in association with metastases in the mesentery, spleen, lymph node, kidney, heart and digits. On histopathological examination of the lung the primary lesion was interpreted to consist of both neoplastic epithelial and mesenchymal cells rather than an epithelial malignancy with a desmoplastic stroma. The biphasic nature of the neoplastic tissue was confirmed by the immunohistochemical examination using a panel of antibodies for epithelial and mesenchymal cells. In the metastases only the epithelial component was observed.

AN OUTBREAK OF FATAL HEMOLYTIC ANEMIA, THROMBOCYTOPENIA, MICROHEPATICA AND IMMUNE-COMPLEX GLOMERULONEPHROPATHY IN A COLONY OF SPRAGUE-DAWLEY RATS

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We describe an outbreak of an unusual and invariably fatal condition in a group of outbred Sprague-Dawley rats housed in one facility. The disease was characterized by severe microangiopathic hemolytic anemia, thrombocytopenia, marked reduction in liver size, thrombosis involving multiple organs, and immune-complex glomerulonephropathy. Although each of these conditions, sometimes in combination has been previously described in the rat, we have been unable to find reference to a disease presenting with this constellation of abnormalities. The clinical, clinicopathologic, gross post mortem, histopathologic and ultrastructural findings are presented. The etiology remains unknown despite multiple investigations. Exposure to an unidentified toxin or infectious organism would seem the most likely consideration. Our current hypothesis is that the underlying abnormality is vascular, possibly involving an immune-mediated mechanism.

SHOOTING OF FOXES (*VULPES VULPES*) - A PATHOLOGICAL STUDY OF WELFARE ASPECTS

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In 2005 a ban was introduced, on welfare grounds, that means that is illegal to hunt foxes, or other animals, in the UK with more than two dogs. Other legal methods for the control of foxes include trapping, snaring, or shooting with a rifle or a shotgun. Of these, welfare aspects of shooting have been considered by Fox et al (2005). The British Association for Shooting and Conservation (BASC) wished to carried out a similar study to enable them to review their guidelines set out in their codes of practice, that were based on different conditions of distance and ammunition than that tested by Fox et al (2005).

The study was in two parts.

A Eight fox carcasses, killed as part of pest control, were shot with a shotgun at ranges of 20 or 30 yards (18.2 or 27.4 metres) using cartridges containing shot size No1 (3.6mm) or No3 (3.3mm).

The damaging effects of pellet strikes were assessed radiographically and at post mortem examination. There were no obvious differences in the distribution of pellets or damage caused by them dependent on either pellet size or range. Most pellets penetrated soft tissues but were retained beneath the skin on the far side of the carcass. Bony damage included fractures of the skull, limb bones and vertebrae.

Information obtained from shot fox carcasses was applied to analyse pellet strikes on paper targets shot under similar conditions.

B Ninety three paper targets indicating a printed outline of a fox and major bones and viscera were shot at ranges of 20, 30 or 40 yards (18.2, 27.4 or 36.6 metres) using No1 or No3 shot. The likely damaging effects of pellet strikes were assessed on the distribution of impacts on critical tissues, following specific criteria (based on a proposal by Fox et al, 2005):

- (I) Near Instant Kills - damage to brain, heart or aorta or cervical vertebrae.
- (II) Severely wounded [incapacitated sufficiently to allow subsequent dispatch] - fracture of two or more major limb bones or damage to thoracic vertebrae.
- (III) Wounded [not incapacitated enough to be sure of subsequent dispatch] - not falling into the above categories; including any number of abdominal hits.
- (IV) Missed - no pellets on target or passing outside outline or only hitting pinna, tail etc

About 80% of foxes would have died nearly instantly.

Seven foxes (about 8%) would have been injured/incapacitated so that it is almost certain they could have been subsequently dispatched in an acceptably humane manner.

Ten foxes (about 12%) would have been injured but not recoverable without the use of a dog to track the fox and dispatch it where needed.

Conclusions: In light of these results the BASC has reaffirmed that its advice on the shooting of foxes with a shotgun leads to a humane dispatch of the animal. Further studies along similar lines are planned to investigate lethality of these shot sizes at increased range and smaller pellet sizes, this may or may not lead to changes in the advice they give to their members.

Reference: Fox NC, Blay N, Greenwood AG, Wise D and Potapov (2005) Wounding Rates in Shooting Foxes (*Vulpes vulpes*)
Animal Welfare. In press

IMMUNOHISTOCHEMICAL INVESTIGATION ON CANINE MENINGIOMAS

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Immunohistochemical staining patterns were studied in brain and spinal cord meningiomas of the dog. Nineteen archived cases were selected and classified according WHO classification (Koestner et al. 1999). They included five transitional, five meningothelial, two fibroblastic, two angiomatous and five anaplastic meningiomas. To differentiate meningioma from other central nervous system tumors a panel of immunohistochemical markers (vimentin, S100, Glial Fibrillary Acidic Protein (GFAP), Pancytokeratin, AE1/AE3 cytokeratin) were checked. Moreover, E-cadherin and beta-catenin expressions were investigated in order to identify the role they eventually play in meningioma's morphogenesis. In all cases, vimentin labelling was strong and was detected in 100% of neoplastic cells. S100 expression was detected in 2 out of 19 cases (meningothelial and anaplastic type), showing to be mild and focal. GFAP, Pancytokeratin and AE1/AE3 cytokeratin were negative in all cases under study. E-cadherin was expressed in 3 out of 19 cases as focal, granular, cytoplasmic/membranous positivity. Whorl figures were E-cadherin positive in one transitional meningioma, one meningothelial and one anaplastic hystotypes, while neoplastic cells were negative in both the fibroblastic and angiomatous morphotype. Beta-catenin was not expressed. E-cadherin expression reflected the presence of homotypic interactions of neoplastic cells in the meningothelial as well as transitional hystotypes.

STUDIES ON THE ETIOLOGY OF NON-SUPPURATIVE ENCEPHALITIS IN PIGS

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A retrospective study was performed on 37 natural cases of etiologically unclear non-suppurative encephalitis in pigs. Brain samples were examined for presence of porcine circovirus-2 (PCV-2), porcine respiratory and reproductive virus (PRRSV), porcine enteroviruses (PEV), ovine herpesvirus-2 (OHV-2) and Borna disease virus (BDV) by different molecular biological and immunohistochemical methods. Histological examination of the brains revealed variable degrees of lymphohistiocytic encephalitis or meningoencephalitis predominantly characterized by perivascular mononuclear infiltrates.

One case could be attributed to PCV-2 infection. PCV-2 nucleic acid was present in the mesencephalon, the cerebellum and the medulla oblongata by in situ hybridization (ISH) using a PCV-2-specific digoxigenin-labelled oligonucleotide probe. Viral nucleic acid was mainly located in the cytoplasm of macrophages which were predominantly found in the meninges and around blood vessels. Real time PCR, however, detected PCV-2 DNA in additional 7 brain-samples. All these animals showed positive hybridization signals in the inguinal lymph node. 4 animals had PRRSV infections with virus demonstrated in lung samples. But neither by immunohistochemistry (IHC) and ISH nor by real time RT-PCR could PRRSV be detected in the brain samples. Furthermore, all brains investigated for BDV by IHC, and PEV and OHV-2 by PCR were negative.

PROMINENT LYMPHOCYTE TRAFFICKING THROUGH BOTH THE BLOOD-BRAIN AND BLOOD-CHOROID PLEXUS BARRIERS IN EARLY FIV INFECTION

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FIV infection in the cat is a model of HIV-1 infection in man. Studies in both infections have shown that virus enters the brain in the acute stages of disease yet little is known of the mechanisms of viral entry. The dissection of this stage is fundamental to the development of therapies that may prevent or modulate CNS infection.

The present study characterises the early sequential neuropathological changes following intraperitoneal or intravenous infection with FIVGL8, a strain known to enter the CNS in acute infection.

Following both routes of infection lymphocyte-rich perivascular infiltrates are noted within cerebral and cerebellar meninges, in the choroid plexus and spinal cord dura mater and within the epineurium of the sciatic nerve. In addition, following intravenous infection perivascular infiltrations are noted in parenchymal blood vessels primarily of cerebral white matter. Infiltrates are composed of CD79+ B cells and CD3+ T cells with the latter population containing a mixture of CD4 and CD8 + cells. The severity of lesions increases in intensity in the 8-16 week period following infection and then began to wane.

The pathology observed is comparable with that of early stage HIV-1 associated encephalitis and highlights that, as in HIV-1 infection, blood-brain barrier and choroid plexus brain barrier integrity are both compromised in early infection.

PLASMINOGEN ACTIVATOR SYSTEM EXPRESSION IN BOVINE MAMMARY GLAND: IMMUNOHISTOCHEMICAL AND BIOCHEMICAL EVALUATIONS

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Mammary gland proteolytic processes are mediated by plasminogen activator system (PAs). Aim of this work is to evaluate the expression of PAs in 50 mammary gland and 50 milk samples of Friesian health cows from a herd of Piemonte region. The samples were collected in different range of lactation (0-1.5; 1.3-3; 3-7; 7-10; more than 10). The milk fractions were analysed using a colorimetric assay for plasmin, plasminogen and plasminogen activator (PA). Formalin and glutaraldehyde fixed samples were investigated using a new polyclonal antibody raised against recombinant bovine urokinase-PA receptor by immunohistochemistry and immunogold techniques. All methods revealed increased levels of Pas with advancing lactation. These results represent the preliminary data. In order to better understand the effect of Pas, further investigations are in progress on mastitic tissues.

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SMALL RUMINANTS NEUROPATHOLOGY: A RETROSPECTIVE STUDY ON NEUROLOGICAL DISORDERS 1995-2004

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The increase in the number of scrapie affected animals in Italy since 1997 stimulated a retrospective investigation on neurological disorders of these species in the Department of Animal Pathology of Torino. A total of 200 cases were identified, mainly in adult animals. Histology, histochemistry and immunohistochemistry techniques permitted to classify the lesions. Diagnosis were divided in different groups: cerebellar abnormalities caused by Pestivirus (5%), non suppurative inflammatory lesions (19.5%), suppurative lesions (11.5%), mycotic encephalitis (1%), coenurosis (17.5%), degenerative lesions (12.5%), scrapie (10.5%), neoplastic lesions (0.5%) and not classified (5.5%). 19 % of animals didn't reveal any histological nervous lesions. The most important group is represented by inflammatory lesions, suppurative leptomeningoencephalitis, abscesses and Listeriosis. In the differential diagnosis of scrapie, Coenurosis represents the most frequent pathology causing chronic neurological disorders in small ruminants. This result suggests the importance of a constant surveillance especially on viral and parasitic infections of these species.

EXPRESSION ANALYSIS OF ANDROGEN-RESPONSIVE GENES IN THE PROSTATE OF VEAL CALVES TREATED WITH ANABOLIC HORMONES

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To identify indirect molecular biomarkers of anabolic treatments in veal calves, an animal experiment was performed using two combinations of growth promoters (consisting of boldenone undecylenate and estradiol benzoate, and of testosterone enantate and estradiol benzoate). We selected a set of 12 genes that are known to be androgen responsive in other mammalian species. The expression profile of this set of genes of prostate veal calves was analysed using a real-time RT-PCR approach. For each selected gene the corresponding bovine sequence was obtained and a gene specific real-time assay was optimised and validated. Messenger RNA levels were quantified in prostate samples and non-parametric analysis of variance showed significant up-regulation of three genes (MAF, ESR1 and AR) and significant down-regulation of four genes (HMGCS1, HPGD, DBI, and LIM) in treated samples when compared with untreated controls. To identify hormone-treated animals we performed a discriminant analysis that was effective in classifying treated and non treated samples with an accuracy of 93%. Our results indicate that identification of treatment with steroid hormones in veal calves by means of gene expression analysis is a feasible approach and could be improved increasing both the number of genes and the number of controls analysed.

MORPHOLOGICAL AND BIOCHEMICAL CHANGES INDUCED BY EXPERIMENTALLY ASBESTOSIS IN RATS

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Twenty Wistar rats as experimental group (E) and twenty as control group (C) were used. Group E was intraperitoneally injected four times at one month interval with a suspension of amphibole (antofilit and tremolit). One month after the last inoculation euthanasia was performed. Smears from peritoneal fluid and peritoneum (frozen slide technique) were stained by Wright and Crocker's methods for AgNORs. Histological examination from abdominal tissues and determination of lipid peroxides and proteins oxidation of blood, liver and epiploon were made. The peritoneal fluid showed increased number of cell/ μ l in E (E=1364+95; C=380+14) by increasing number of mesothelial cells and macrophages. In some areas of peritoneum, the mesothelial cells from E showed apoptosis, necrosis, dysplasia, increased mitotic index (E=3; C=0,4) and AgNORs number (E=6,9; C=2,5). In group E, not in C, the lesions were dominated by granulomatous and fibrous reaction in most abdominal tissues. The lipid peroxides have a significant increase in plasma (E=5,2; C=3,6 moles malonildialdehyde/ml), liver (E=4,31; C=2,71 moles MDA/mg protein) and epiploon (E=28,2; C=2,84 moles MDA/mg protein). Also, a significant increase of protein oxidation was noticed only in epiploon (E=14,99; C=4,69 moles dinitrophenylhidrazine/mg protein).

**PILOT STUDY ON PLASMA FILTRATION ADSORPTION DIALYSIS (PFAD)
IN A PORCINE MODEL OF ENDOTOXIC SHOCK:
ANATOMO-HISTOPATHOLOGICAL ANALYSIS OF ORGAN INJURY**

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The Plasma Adsorption Filtration Dialysis (PFAD) is a new technology in which the detoxification is improved by a complete blood dialysis, adopting a combination of convective and adsorption techniques. The therapeutic findings of PFAD were evaluated in comparison with Coupled Plasma Filtration Adsorption (CPFA), a dialytic machine that separates plasma from blood by means of a plasma filter. In order to prove the efficiency of these technologies, a severe and acute endotoxic shock was induced, by a bolus injection of LPS from *E. coli*, in ten pigs divided into three groups: group A receiving standard medical therapy [n=3, Survival Time (ST): 1hrs and 56min], group B receiving CPFA procedure (n=3, ST: 2hrs and 30min) and group C receiving PFAD procedure (n=4, 3 subjects survived beyond 36hrs). A complete post-mortem examination and histopathological analysis was performed. Groups A and B animals showed a diffuse hyperplastic, reactive lymphadenopathy, a massive neutrophilic margination in pulmonary alveolar capillaries and severe multiorgan congestion. Group C animals exhibited a mild reactive thoracic lymph node enlargement.

The results obtained highlight the protective role of PFAD in endotoxic shock and suggest a possible involvement in the treatment of different diseases, such as hepatorenal syndrome and acute liver failure.

OLLULANUS TRICUSPIS IN THE CAT STOMACH: HISTOLOGICAL EVIDENCE FOR ITS PRESENCE IN THE UK

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Ollulanus tricuspidis is a small nematode of the genus Ollulanidae, and has a worldwide distribution. It is found in the stomach of domestic cats and other felids, both captive and free-ranging. *O. tricuspidis* is difficult to see with the naked eye, and is associated with vomiting and weight loss. The parasite has a direct life cycle and is transmitted through the consumption of infected vomitus. Between 1980 and 2005, the parasite was found in the stomach of six cats, three at post mortem examination, and three on endoscopic gastric biopsy. Furthermore, 130 endoscopic biopsies submitted between 1996 and 2005, were examined retrospectively and one additional case was identified. Grossly, the mucosa of the stomachs examined post mortem were thickened, discoloured white/grey, and had a cobblestone appearance. The stomachs of the live cats were normal. Histologically, chronic gastritis was observed in three cases (one post mortem, two biopsies), while the remaining cases were microscopically normal. These are the first reports of Ollulanosis in British cats since 1927 and the first cases where the diagnosis was made by endoscopic biopsy of domestic cats.

DETECTION OF *CRYPTOSPORIDIUM* SP. IN DOMESTIC GEESE BY IN SITU HYBRIDIZATION

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An in situ hybridization (ISH) protocol was developed which allowed the specific detection of *Cryptosporidium* sp. in paraffin embedded tissues with a digoxigenin-labelled oligonucleotide probe targeting 18 S rRNA. This technique was used in organs from 20 juvenile domestic geese. The animals showed no clinical signs and only scanty histological lesions. Various developmental stages were easily discernible by distinct black signals at the epithelial cell borders in both conjunctiva (89%) and bursa of Fabricius (88%) of the geese. These findings add to the previously published knowledge that clinically inapparent cryptosporidiosis is common in domestic waterfowl. In contrast to literature data, our cases showed more frequent manifestation of the protozoa in the conjunctiva than in the bursa of Fabricius.

While traditional staining techniques, such as hematoxylin and eosin staining or periodic acid Schiff reaction, may produce unspectacular or inconclusive results, ISH proved an excellent tool to detect and identify even small amount of cryptosporidia in tissue sections.

Furthermore, the design of the probe allows detecting *Cryptosporidia* of any species and in any organ. Thus this technique could significantly contribute to an improvement of the diagnosis of cryptosporidial infections.

DETECTION OF PATHOLOGICAL PRION PROTEIN IN TONGUE OF SHEEP AFFECTED BY NATURAL SCRAPIE

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Recent studies on rodent models reported the detection of PrP^{sc} in tongue tissue after oral or intracranial experimental challenge with scrapie and transmissible mink encephalopathy (TME) sources. Although scrapie is not considered a risk to humans health, unlike bovine spongiform encephalopathy (BSE), it has been demonstrated that under experimental conditions sheep are easily infected by the BSE agent and that they carry abundant amounts of infectivity throughout most body tissues. A European Food Safety Authority Opinion recommends testing for PrP^{sc} presence and accumulation in ruminants tongue in order to facilitate risk quantification and assessment. In this study, we report on the detection of PrP^{sc} in the tongues of seven scrapie-infected sheep by immunohistochemistry and Western blot.

EVALUATION OF DIFFERENT PLASMID DNA DELIVERY SYSTEMS FOR ANTI-HER2/NEU IMMUNIZATION IN A TRANSGENIC MURINE MODEL OF MAMMARY CARCINOMA.

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Studies of anti-HER2/neu DNA vaccination showed the effectiveness of immunization protocols in models of transplantable or spontaneous tumours; scarce information, however, has been provided to identify the procedure of DNA administration more effective in activating immune system against spontaneously arising HER2/neu-positive tumours. We compared the effectiveness of different procedures of DNA vaccine delivery (intradermic injection, Gene Gun delivery and intramuscular injection alone or with electroporation) in a transgenic model of mammary carcinoma HER2/neu-overexpressing. We highlighted the role of DNA delivery system in the success of DNA vaccination showing that, among the analysed methods, intramuscular injection of the vaccine, particularly when associated to electroporation, elicits a better protection against HER2/neu spontaneous tumour and induces a Th1-like immunity.

CYTODIAGNOSTIC ACCURACY OF PLEURAL EFFUSION IN DOG AND CAT

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Cytology is very important clinical pathology ancillary procedures in diagnostic pleural effusions but often inconclusive diagnosis is related to the procedure of fluid collection. Aim of the present study is to assess the accuracy of cytodagnosis of pleural effusion in dog and cat under ultrasound guidance thoracentesis collection (USGT) or thoracentesis collection (T).

Pleural effusion of 20 dogs (11USGT and 9T) and 18 cats (8USGT and 10T) collected from clinical routine exams were compared for diagnostic accuracy. Fluid pathological samples were centrifuged (1500 RPM, 10 min.), pellets were smeared and MGG stained. In dog pleural effusion 3USGT and 3T inflammations, 6USGT and 3T neoplasia, 2USGT and 3T inconclusive were recorded. In cat pleural effusion: 5USGT and 4T inflammations, 2 USGT and 2T, 1T haemothorax, 1USGT and 3T inconclusive were observed.

Diagnostic accuracy in dog pleural effusion USGT was 9/11 (81,8%), T was 6/9 (66,6%) while in cat accuracy USGT was 7/8 (87,5%), T was 7/10 (70%).

Percentages were similar in two animal species compared with same method of fluid collection. The higher diagnostic accuracy (15%) of USGT vs. T was correlated to ultrasound guided collection that also decrease inconclusive casualness of sampling as could happen in saccate flogistic lesions.

CD44 POSITIVE CANINE GASTRIC CARCINOMA: VEGF-C EXPRESSION IN METASTATIC CELLS

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CD44v5 expression in canine gastric carcinoma is related to malignancy and metastasis but not in chronic gastric inflammation (Corradi A et al. 16th ESVP, 1998, Corradi A et al. 17th ESVP, 1999). CD44 is also related to LYVE-1, a lymphatic endothelial immunomarker. Recent studies has indicated lymphogenesis in solid tumor correlated with lymphatic metastasis and have identified VEGF-C as a growth factor that plays an important role in vascular permeability and lymphatic transendothelial neoplastic migration.

Aim of the study is to evaluate VEGF-C expression in CD44v5 immunopositive gastric carcinoma in comparison to normal gastric mucosa, chronic gastritis.

Immunocytochemical expression of VEGF-C was performed on 25 paraffin specimens of gastric mucosa of dog of different age, gender and breeds: 5 normal gastric mucosa, 14 chronic non-ulcerative gastritis (*Helicobacter* spp. positive case), 7 different gastric carcinoma (4 metastatic, 1 non metastatic, 1 gastric biopsy). The investigations were performed using antibodies vs. CD44v5, *Helicobacter* spp, and a complete immunomarkers panel specific for haematic and lymphatic endothelium.

All carcinomas were CD44v5 immunopositive and *Helicobacter* spp. immunonegative. Only epithelial neoplastic metastatic cells expressed VEGF-C. Gastric biopsy showed also VEGF-C immunopositive epithelial cells, suggestive of potential metastatic activity, but unluckily undetected for the small specimen dimension.

TWO CASES OF OESOPHAGOSTOMOSIS IN CHIMPANZEES: LESIONAL DESCRIPTION AND SANITARY IMPORTANCE

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Few informations are available about the clinical signs and lesions associated with Oesophagostomum infestation in wild chimpanzees (*Pan troglodytes*). We report two cases of oesophagostomosis in free-ranging chimpanzees and we describe lesions observed and sampled during necropsies.

After the accidental death of a first adult male chimpanzee from Kibale National Park (Uganda), the necropsic exploration the abdominal cavity revealed hundred of nodules from 10 to 50 mm each in the abdominal wall and on large intestine wall. Oesophagostomum worms were observed within the nodules. A 40 mm diameter abscess-like mass was also present on liver. Several nodules from the gut and abscess from the liver were dissected and thick pus-like fluid leaked out.

The second case, a twelve-year old chimpanzee from the HELP Congo project in Conkouati-Douli National Park (Republic of Congo), presented abdominal distension, acute pain, anorexia and weakness. Previous periods of lethargy, intestinal disorder and intense pain were reported within the six last months. Two days after the onset of acute symptoms, the animal died. At necropsy, two large masses were localised on mesentery and hundred nodular lesions (10-20 mm) were observed on intestinal wall (fig.1). Incision of some of the nodules revealed the presence of Oesophagostomum worms in a caseous, brown pus-like fluid (fig.2, 3).

Granulomatous and haemorrhagic lesions, caseation in some masses and presence of parasites were consistent with oesophagostomosis in both cases.

Microscopic lesions were typical parasitic granulomas, chronic, multifocal to confluent, located in abdominal and intestinal walls; granulomas were often centred on worms bathing in a necrotic substance (fig.4,5,6).

The inner part of the granulomatous lesion resulted of collection of many mononuclear inflammatory cells, mainly histiocytes, active macrophages including few small multinucleated giant cells, few lympho-plasmacytic cells and, surprisingly for these parasitic lesions, quite few eosinophils (fig.7, 8, 9, 10).

The outer part of the granulomas comprised a lympho-plasmacytic cellular infiltrate and a thick and dense fibrotic capsule (fig 4,8).

Inflammatory mononuclear cell infiltration was observed in the connective spaces in the vicinity of granulomas (fig.11, 12) and in the intestinal sub- mucosa (fig.13). Some associated vascular lesions were also observed, especially partially obstructive intimal fibrotic proliferations (fig.14).

The lesions observed in these cases are very similar to lesions reported in humans,

induced by several species of œsophagostomes including *Oesphagostomum stephanostomum*.

In reason of the clinical severity of human cases, the main underlying sanitary problem of this observation lays in the possibility of a human infestation from a propitious environment contaminated by apes parasitized by sexually matured œsophagostomes. Oesophagostomosis is a potential parasitic zoonose which each veterinary practitioner working with wild large monkeys should take care cautiously.

ENDOGENOUS LIPID PNEUMONIA IN A DOG

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Lipid pneumonia is a rare pulmonary disease, usually classified into two groups, depending on whether the oil/fat found in the respiratory tract is from an endogenous or exogenous source. Endogenous lipid pneumonia has been described in cats and other animals, but only one time in dogs. This study reports a case of a dog with a chronic respiratory disease, that had been previously treated against *Dirofilaria immitis*, with persistent cough and mucous expectorations. At necropsy, multiple white foci of solid appearance and irregular distribution were observed in both lungs, most of them in a subpleural location. Histopathologically, the case was diagnosed as an endogenous lipid pneumonia, on the basis of the presence of numerous foamy macrophages that filled the alveoli and contained small sudanophilic vacuoles. Endogenous lipid pneumonia has been related to a variety of causes, such as bronchial irritation or obstruction, or due to pulmonary parasitism. Injury to type II pneumocytes leads to overproduction of surfactant with a high cholesterol content, that enters the alveoli and is phagocytosed by macrophages, that acquire the foamy appearance.

GENE EXPRESSION INDUCED BY 17 β - ESTRADIOL IN BOVINE CULTURED STROMAL CELLS

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17 β -estradiol is one of the most effective sex steroids illegally used in animal production. It induces histologically hyperplasia and metaplasia of genital accessory glands epithelium. In male, estrogens act through the specific alpha cellular receptors located in the prostatic stromal cells which play an important role in the development and differentiation of the gland. The purpose of the present study is the identification of genes involved in bovine cultured stromal cells *in vitro* treated with 17 β -estradiol. Primary bovine prostatic stromal cells, grown in specific medium were treated with 17 β -estradiol at 100nM concentration. Total RNA was extracted from these cells and subjected to cDNA synthesis by reverse transcriptase. We evaluated by quantitative PCR method the expression of the genes encoding for bovine FGFR2, FGFR3, FGF-2, FGF-9 and FGF-7 that are regulated in the human prostate stromal cells by 17 β -estradiol. In this study we demonstrate that 17 β -estradiol regulates differentially the expression of estrogen receptor alpha, FGFR2 and FGF-2 . We also find out that these genes could be considered a molecular target to study the effect of 17 β -beta estradiol in experiment *in vivo*.

POST-MORTEM FINDINGS IN CETACEANS STRANDED ON THE ITALIAN COASTLINE

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Introduction: Stranded cetaceans offer a unique opportunity for collecting data on the health status of dolphin and whale populations living in the open sea. We report herein the post-mortem findings observed in 7 odontocetes found stranded between 2001 and 2004 on the Italian coastline.

Material and Method: Five bottlenose dolphins (*Tursiops truncatus*), one striped dolphin (*Stenella coeruleoalba*) and one unidentified delphinid were investigated. During post-mortem examination, their tissues were formalin-fixed and subsequently processed for histopathology. Suitable histochemical techniques were applied on some specimens, with selected tissue sections being also submitted to immunohistochemistry for Morbillivirus and Papillomavirus antigen detection.

Results: Pneumonia was the most commonly encountered lesion, especially *Halocercus delphini*-associated bronchopneumonia. Additional findings were nephritis, hepatitis and *Pholeter gastrophilus*-associated gastritis. Granulomatous meningoencephalitis by *Cladosporium* spp. was observed in a female bottlenose dolphin, while papilloma-like lesions were observed on the rostrum of another female bottlenose dolphin, with no Papillomavirus antigen being detected. Immunohistochemistry yielded no evidence of Morbillivirus infection in any of the investigated cetaceans.

Conclusion: In agreement with other authors, pneumonia was a very frequent disease condition also in the cetaceans included in this study, in which mycotic meningoencephalitis by *Cladosporium* spp. may be regarded as a remarkable pathological finding.

**IMMUNOSUPPRESSION DURING EARLY TIME POST-INFECTION
WITH FOOT-AND-MOUTH DISEASE VIRUS (FMDV)
IN SWINE DUE TO VIRAL INFECTION OF LYMPHOCYTES**

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Foot-and Mouth disease virus (FMDV) is a picornavirus that causes an acute vesicular disease of cloven-hoofed animals. In the acute phase of infection the animals suffer from skin vesicles, fever and loose of weight. In the present study we use the FMDV infection of swine to determine changes in the lymphocyte subsets occurred during the first 17 days of the infection. We report, for the first time, that FMDV can infect T and B cells at short period of time post-inoculation, corresponding with the peak of the viraemia. Lymph nodes showed severe depletion of T cells (CD3+) and B cells (CD45RA+). Using flow cytometry and immunohistochemical analysis, a significant lymphopenia that involves CD4-CD8+/-, CD4-CD8+ and CD4+CD8+ but not CD4+CD8- naïve Th lymphocytes were observed. In addition, a severe depletion of the vast majority of peripheral T cells in lymph nodes and spleen is observed. Depletion of SWC7+ cells was also observed in spleen and lymph nodes and a moderate number of inflammatory cells appeared in skin lesions. This selective depletion of T cells is not due mainly to apoptosis as visualized by TUNEL technique. However, eventually the immune system is able to eliminate the viral infection and reconstitute normal levels of T cell. These characteristics of acute phase infection likely play an important role in viral pathogenesis and may be important for improving vaccine formulations.

IMMUNOSUPPRESSION DURING EARLY TIME POST-INFECTION WITH FOOT-AND-MOUTH DISEASE VIRUS (FMDV) IN SWINE DUE TO VIRAL INFECTION OF LYMPHOCYTES

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KINETIC ANALYSES OF IMMUNE CELL POPULATIONS DURING PORCINE-ADAPTED BSE PRION INFECTION IN TRANSGENIC MICE EXPRESSING PORCINE PrP

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In transmissible spongiform encephalopathies (TSEs) the implication of the immune system in pathogenesis has been suggested. Also, a potential spread of BSE to other domestic animals such as pigs was pointed out. The bovine/porcine species barrier to BSE infection has been previously explored by generating a transgenic mouse model in which the porcine PrP protein was expressed (PoTg mouse). In order to evaluate the potential contribution of the immune system to disease development, we inoculated 1 mice by oral route with porcine-adapted BSE infective material. T and B lymphocytes and dendritic cell populations were analysed by flow cytometry and immunohistochemistry at different times post-inoculation (pi) in thymus, secondary lymphoid organs, blood and brain. We found that at very early times pi (15-25 dpi) all the analysed populations decreased significantly in Peyer's Patches. At early times pi (50-100 dpi) a similar reduction in the immune cell numbers was found in other lymphoid organs such as mesenteric lymph nodes. At later stages of the disease (250dpi), immune cells reached normal levels in lymphoid organs but not in spleen and thymus, where mainly the number of T cells was profoundly decreased. PrPres was detected in CNS at 400 dpi. The significance of these data for the role of the immune system in PrP diseases will be discussed.

EXPERIMENTAL STUDY OF APOPTOSIS INDUCED INFECTIOUS BURSAL DISEASE VIRUS ON SPLENIC TISSUE, USING TUNEL ASSAY.

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Infections bursal disease virus is an important immunosuppressive virus of chickens. The virus is ubiquitous and, under natural conditions, chickens acquire infection by the oral route. IgM cells serve as targets for the virus. The most extensive virus replication takes place in the bursa of Fabricius. The molecular structure of IBDV has been extensively studied; the genome consists of two segment (A and B) of double - stranded RNA molecules. The genome encodes five viral polypeptides, designated VP1 -5. The large segment A encodes VP2 -5 the smaller segment B encodes VP1 which has polymerase and capping enzyme activities. Infection results in lymphoid depletion and the final destruction of the BF(Bursa Fabricius) as the predominant feature of the pathogenesis of infections bursal disease. Besides necrosis, marked atrophy of infected BF without severe inflammatory response was also reported. This suggests the involvement of Apoptotic processes in the pathogenesis of the disease. The purpose of the present research is to evaluate in vivo rate of Apoptosis changes of the lymphocytes in splenic tissue. Therefore two group were chosen from among SPF chicken named TEST-IBDV and CONTROL. Each group consisted of ten male 21-day-old SPF chickens of leghorn breed, all of which received identical feeding and caring conditions. The TEST-IBDV group was infected with high-virulence infectious bursal disease Virus (VVIBDV) with IR-499 serotype by eye and nose drop method, and the CONTROL group was treated with salin normal eye and nose drop. Three days after the inoculation of the virus, clinical signs and over 80% mortality were observed among the TEST-IBDV group. During this time, sampling of splenic tissues was carried out within the two groups: The taken samples were sent to pathology lab to provide LM sections and routine staining H&E, TUNEL was applied. The results obtained from the microscopic studies in the H&E stained sections indicated the apoptosis changes in lymphocytes of splenic tissues. For the definite confirmation of these changes, TUNEL assay was applied, in which apoptotic cells present in the microscopic sections stained by TUNEL assay were counted in 5 fields with the magnification of 40, and the average number of the apoptotic cells were registered and compared with those of CONTROL groups. The number of the apoptotic cells in TEST-IBDV was found to be the larger than that in CONTROL groups. Based on the non-parametric tests Mann-Whitney Test significant results were obtained, whereby the mean difference of apoptotic cell number in the groups was $P < 0.005$. The present research in vivo showed that there is always a significant correlation between the virulence of the virus and the rate of apoptosis changes in the splenic tissue, and that apoptosis can be considered as a definite factor in the pathogenesis of infectious Bursal disease. The recent findings indicate that viral proteins VP2, VP5 and Cytokines like INF- and TNF- are among the major inductive factors in the development of the apoptosis in lymphocytes. In summary, IBDV infection in chicken caused increased apoptosis in the spleen, In the Bursa Fabricius, the apoptotic cells tended to concentrate in the medullary region, where as in the spleen they were randomly distributed.

ANALYSIS OF VIRULENCE MECHANISM OF HELICOBACTER FELIS IN A GERBIL MODEL

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Introduction: The virulence mechanisms of *Helicobacter* (*H.*) *felis* are still largely unknown.

Objective: To follow up the sequence of events and to search for morphologic indicators of virulence mechanisms after experimental infection in gerbils.

Materials and methods: Gerbils were infected with *H. felis* ATCC49179 and euthanized after different time intervals. Samples from the stomach were taken for histology, PCR and transmission electron microscopy (TEM). Immunohistochemical stainings were done for hydrogen-potassium ATPase, *Helicobacter*, caspase-3, NF- κ B and Cox-2.

Results: Segmental loss of parietal cells extending from the limiting ridge started at 7 days post infection in some animals. It was present in all animals by 35 days. At the transition zone the bacteria were spatially associated with apoptotic cells and cells expressing Cox-2 and nuclear translocation of NF- κ B. TEM of this area revealed necrotic parietal cells and parietal cells with plasma membrane damage. Bacteria were adhering to parietal cells through close contact between microvilli of the host cell and periplasmic fibrils and flagellae of the bacteria.

Conclusion: This is the first time adhesion of *H. felis* to gastric parietal cells is reported. Membrane damage, necrosis and early inflammatory mediators appear to be directed linked to presence and adhesion of the bacteria.

MANIFESTATIONS OF PORCINE CIRCOVIRUS TYPE 2 INFECTIONS IN AUSTRIAN PIGS

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In the present study a panel of tissues from PCV-2 positive pigs showing different clinical forms of the infection were examined by in situ hybridization to evaluate the quantity and distribution of PCV-2 in correlation with histological lesions and clinical data. Pigs with PMWS showed comparably strong signals in inguinal lymph node, lung, intestine and spleen; in liver and kidney PCV-2 nucleic acid was also detected in all cases, albeit generally in lower quantity. A high percentage of pigs with PMWS had lymphoid depletion, interstitial nephritis, interstitial pneumonia and/or bronchopneumonia and enterocolitis. PCV-2 positive pigs, which were not diagnosed as PMWS, revealed up to moderate quantities of PCV-2 predominantly within the centers of lymphoid follicles of different tissues but not consistently in other tissues. Histological lesions were usually mild or absent. In general, PCV-2 showed a strong tropism to lymphoid organs, as well as to organs containing considerable amounts of lymphoid tissue, such as lung and intestine. Kidneys were strongly positive, when they had severe interstitial nephritis, i.e. infiltration with mononuclear cells. The virus-positive cells were most frequently representatives of the MPS, but in liver and kidney also epithelial cells were infected.

THE PERFECT ABORTION STORM: TOXOPLASMOSIS IN A SHEEP FLOCK

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A sheep flock were synchronized and bred to nine different rams. Thirteen ewes, separated and fed special diets, produced 13 healthy lambs, one stillborn lamb, one undersized lamb, and one lamb died from dystocia. Their mean gestation length was 145 days (breed normal 145 days). Of the remaining 28 ewes, 7 lost confirmed pregnancies, one died in dystocia, and 20 produced 35 mummified, stillborn, or weak lambs that died soon after birth and three live but stunted lambs. Their mean gestation length was 138 days (range 129-144). A pathologist examined five lambs, three stillborn and two mummified fetuses. Placentas had classic lesions of toxoplasmosis with necrosis and mineralization of cotyledons, and precolostral sera from heart blood or body fluids from three dead lambs were seropositive for toxoplasmosis. No possible contact with cats could be demonstrated, but the obvious segregation of the disease between the two groups of this flock suggested a point source of oocysts for the affected ewes. Corn bought from a feed cooperative and fed only to the affected portion of the flock beginning 55 to 88 days of gestation contained dark, admixd material that contained *Toxoplasma* oocysts. The feed cooperative paid reparations to the owner.

THE PATHOLOGY ASSOCIATED WITH ANGIOSTRONGYLUS VASORUM INFECTION IN THE RED PANDA (*AILURUS FULGENS FULGENS*)

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During a period of three years, four red pandas (nos. 1-4) died in Copenhagen Zoo. One animal (no. 1) was found dead without previous clinical signs, whereas the remaining three pandas died following treatment for angiostrongylosis with moxidectin combined with broad-spectrum antibiotics and high doses of steroids. A diagnosis of angiostrongylosis was obtained in pandas nos. 2-4 by the identification of *Angiostrongylus vasorum* larvae in faecal samples.

All four pandas were submitted for necropsy. Apart from histology, tissues were subjected to immunohistochemistry in order to identify specific fungal elements.

In three pandas (nos. 1-3), firm conglomerates of nodular masses were scattered throughout the periphery of both diaphragmatic lung lobes. The masses were heavily mineralized and the cut surface appeared greyish-white and fibrotic. In the fourth panda (no. 4), multifocal necrotizing gastritis, multifocal apostematous nephritis and pneumonia, and unifocal apostematous lymphadenitis (In. mesenteriales) and pancreatitis were found. All four animals had enlarged pulmonary lymph nodes. Histologically, several pulmonary arteries of pandas nos. 1-3 were thrombosed and contained adult worms, eggs, and larvae. Organized thrombi within arteries, mineralization, focal areas of osseous tissue, and fibrosis were found throughout the lung tissue together with conglomerates of granulomas and hyperplasia of type-II pneumocytes. Eggs and larvae were frequently contained centrally within the granulomas. Within the cortical and trabecular sinuses of pulmonary lymph nodes, granulomas with a content of larvae were frequently seen. In the lung tissue of panda no. 4, only a few larvae, granulomas, and focal mineralization were observed. However, in that animal acute necrotizing mycotic gastritis, pancreatitis, pneumonia, lymphadenitis, and pancreatitis were diagnosed histologically. Immunohistochemically, concomitant aspergillosis and candidosis were found to be the cause of the gastric ulcerations, whereas aspergillosis alone was the cause of the pulmonary abscesses. Candidosis was immunodiagnosed to be the aetiology of lesions in the mesenteric lymph node, kidneys, and pancreas.

There are several reports on parasitic infections in the lung and heart of the red panda (e.g. dirofilariosis, metastrongyloidosis, and strongyloidosis), however, neither cases nor the pathology of angiostrongylosis seem to have been reported previously. Apart from a heavy pulmonary mineralization, the lung lesions of angiostrongylosis in the red panda are comparable with lesions in foxes and dogs naturally and experimentally infected with

A. vasorum. In dogs, angiostrongylosis has been associated with hypercalcaemia. Therefore, it is tempting to speculate that mineralization of the lung tissue was secondarily caused by increased unregulated production of 1,25-dihydroxycholecalciferol by activated macrophages in the pulmonary granulomata. A similar pathogenesis of metastatic mineralization is known in humans and cows with granulomatous diseases. Although pandas apparently may die from the infection per se, also the treatment of infection is very problematic, which is the experience from treatment of dogs infected with *A. vasorum*, too. Sudden death of dogs following anti-parasitic treatment is thought to be related to a heavy release of worm antigen causing an anaphylactic reaction. Moreover, as seen in one of the present pandas (no. 4), subsequent systemic mycoses may also develop following treatment.

Summary: *The pathology associated with angiostrongylosis in four red pandas is described. Apart from lesions related to A. vasorum, one of the pandas also revealed acute lesions of systemic candidosis and aspergillosis.*

STUDY ON COPPER DEFICIENCY IN MARKAZI PROVINCE IN IRAN

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Aim of the study: To investigate the occurrence of copper deficiency in Markazi province (300 km . far from Tehran).

Materials & Methods : 129 blood samples of suspected (aborted or having lambs with inability to stand up) and normal sheep with different breeds were taken from the flock within the six cities of Markazi province . At the same time soil and forages were collected in situ . During the study 4 suspected dead lambs were observed and immediately transferred to the Institute . For evaluate the copper concentration using Atomic Absorption Spectrophotometry ,the blood samples , the liver tissue of suspected dead lambs ,soil and forages were transferred to the Research Center of Animal Science in Karadj . The spinal cord and brain were removed from the carcasses and fixed in to formalin saline in order to get the slides for histopathology .

Results : By using the statistical analyses applying 95% and 99% confidence interval there were no significance between suspected and normal serum . However there were differ significant in breeds and cities ($P < 0.01$) Also the copper concentration in soil, forages and liver tissue had not shown the deficiency comparing the standard normal data . Sections of spinal cord and brain of 3 carcasses were checked under microscope , Except one case which had malacia and prevascular cuffing and infiltration of mononuclear and plasma cells in meninges of the mid brain. There were minor demyelination and axonal degeneration were observed in two other cases .

Conclusion : We concluded the pasture areas and the flock of sheep in Markazi province do not have copper deficiency although four years ago had been reported by Veterinary Station Center . It is presumed that the other trace elements my be the problems of these areas.

POLYARTERITIS NODOSA IN SHEEP

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Polyarteritis nodosa (PAN) is a systemic vasculitis of unknown etiology affecting small- and medium-sized arteries of multiple organ systems without pulmonary arterial involvement. Cases of PAN have been reported in animals but in sheep, only one case has been described. We report three cases of PAN in sheep from different flocks showing locomotor clinical signs. At necropsy, the most significant findings were subcutaneous edema and vascular thromboses and aneurysms in subcutaneous tissue of hind-limbs, ovaries, uterus, adrenal glands and liver. Intrarenal branches of the renal arteries showed visible nodular thickening. These lesions were always bilateral. Histologically, small-to medium-sized muscular arteries of the kidneys, ovary, uterus and skin were consistently involved in the three sheep but vasculitis occurred in other organs (liver, gall-bladder, skeletal muscle, spinal cord, adrenal gland, mammary gland, spleen). Arteries within the lung were normal in all animals examined. Vascular lesions included focal fibrinoid necrosis, rupture of the internal elastic lamina and transmural infiltration of inflammatory cells, mainly lymphocytes and macrophages, extending into the perivascular tissue. In the kidney, many arteries showed narrowing or occlusion of the lumen and marked fibrosis. The distribution of arterial lesions was segmental with normal arterial profiles adjacent to affected areas. Immunohistochemical studies showed that the inflammatory infiltrates in PAN lesions in sheep were mainly composed of CD3 (T lymphocytes) and VPM32-positive cells (macrophages).

THE INTERACTION OF FELINE IMMUNODEFICIENCY VIRUS WITH A CELL CULTURE MODEL OF THE FELINE BLOOD-BRAIN BARRIER

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Feline Immunodeficiency Virus (FIV) infection of cats is an established model of HIV-1 infection in man. Like HIV, FIV is neurotropic, and central nervous system infection occurs early in infection. In association with early infection, there is prominent leukocyte trafficking across the blood-brain barrier. In order to study the interaction of FIV with the blood-brain barrier, we have developed an in vitro model of the feline BBB using primary cultures of feline brain capillary endothelial cells (FBEC) and feline astrocytes grown as a co-culture on opposite sides of Transwell filters. FBEC were characterised by uptake of DiI-Ac-LDL, and immunocytochemistry to identify von Willebrand Factor. Feline astrocytes were characterised by immunocytochemistry to identify GFAP. Functionality of the BBB model was assessed by measurement of tight junction formation between FBECs, using transmission electron microscopy (TEM), transendothelial electrical resistance measurements and paracellular permeability assays. The presence of tight junction proteins in the FBEC cultures was confirmed by Western blots. The ability of FIVGI-8 to infect cells of the feline BBB was assessed by immunocytochemistry, TEM and measurement of reverse transcriptase activity in BBB cells exposed to FIV. FBECs exposed to FIV developed cytoplasmic vacuoles, and virus particles were present in these vacuoles when viewed using TEM. Feline astrocytes were positive for FIV antigen by immunofluorescence. No productive infection was detected in either cell type by quantitative ELISA. The ability of FIV to disrupt the tight junctions between FBEC was measured by FD-4 permeability assays. Cell-free FIV did not affect tight junction integrity up to two days post infection. This BBB model system provides an ideal opportunity to characterise the interaction of FIV with the feline blood-brain barrier.

E-CADHERIN EXPRESSION IN CANINE MALIGNANT MAMMARY TUMOURS: RELATIONSHIP TO OTHER CLINICO-PATHOLOGICAL VARIABLES

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The relationship between E-cadherin epithelial expression, as detected by immunohistochemical methods, and other clinico-pathological characteristics of canine malignant mammary tumours was studied in 76 tumours surgically removed from 45 female dogs. The immunostaining was assessed on the basis of the estimated percentage of epithelial cells with membranous staining. Reduction of E-cadherin expression was found to be significantly related to size and ulceration but not to skin or underlying tissue fixation. A significant reduction of E-cadherin expression was found in solid tumours, tumours with lymph node metastasis, necrosis and infiltrative growth. Histological grade was marginally associated with E-cadherin expression ($P=0.069$). The significant relationship between E-cadherin expression and other known factors of poor prognosis suggests that the loss of E-cadherin expression may have prognostic value in canine malignant mammary tumours.

MAST CELL TUMOR

A CASE REPORT

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Mast cell tumour (MCT) is one of the most common skin tumours of the dog. It has been estimated that they represent 6% of all tumours and account for approximately 13% of all cutaneous tumours in the dog. Several breeds of dogs are predisposed to develop this kind of skin tumours, including Boxers, Boston terriers, bull terriers, weimaraners, and Labrador retrievers. Tumours are usually multiple and may involve any regions of the body skin. Most tumours occur in middle-aged dogs and there is no sex predilection. We report a case of multiple cutaneous MCT in a 9-years old Boxer male, during a 4-years period. The dog was previously submitted to surgery for excision of an MCT for three consecutive years, at the age of 6, 7 and 8 years old, respectively. At the age of 9 years the dog was presented with 17 cutaneous lesions, all diagnosed as MCT. The aim of our study is to describe the morphologic features of the MCTs and to evaluate their immunoreactivity profiles of a proliferation marker. Thin sections were obtained from formalin-fixed, paraffin-embedded tissues and routinely stained with haematoxylin and eosin. Tumours were graded according to Patnaik's histopathologic grades, and were also evaluated for the presence of necrosis. The cell proliferation index of these tumours was assessed with a standard avidin-biotin complex (ABC) immunohistochemistry protocol, using a monoclonal antibody directed against Ki-67 (MIB-1) as the primary antibody. The results have shown that all the grade I MCTs (10/10) had an average percentage of cells positive for Ki-67 below 7% and, with exception of one tumour, necrosis was absent. Grade II and III tumours had an average percentage of cells positive for Ki-67 higher than 26% and in 7 out of ten tumours, necrosis was present. The number and grade of these tumours seems to increase with age. The proliferation index of these tumours seems to be correlated with the tumour's grade, however, no significant correlation was determined between the presence of necrosis and tumour grade or the proliferation index.

PNL2, A NOVEL MONOCLONAL ANTIBODY FOR MELANOCYTIC TUMORS: IMMUNOHISTOCHEMICAL STUDY OF ITS REACTIVITY ON CANINE MELANOMAS AND COMPARISON WITH MART1.

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PNL2 is a recently generated monoclonal antibody that recognizes melanocytic cells and melanomas. Although the antigen recognized by PNL2 is not known yet, recent studies on human melanomas have confirmed its usefulness as a diagnostic marker.

In this study, we compared the immunoreactivity of PNL2 and MART1 (A103) on benign and malignant melanomas and on different non-melanocytic tumors.

Validation of PNL2 on canine melanomas was performed by Western blotting. PNL2 and MART1 immunoreactivity was tested on frozen samples of canine melanomas and on formalin fixed, paraffin embedded specimens of a wide panel of melanocytic and non-melanocytic tumors and normal canine tissues.

No staining was observed in cells other than melanocytes in normal tissues. All non-melanocytic tumors tested (carcinomas, sarcomas, fibrosarcomas, leiomyosarcoma and PNST) stained negative.

Melanocytic tumors stained positive with both PNL2 and MART1, regardless their benign vs malignant nature and their primitive vs metastatic origin. Staining was generally most intense and diffuse in epithelioid cell phenotype. In skin melanomas, PNL2 staining was more prominent in cells at the dermo-epidermal junction.

In contrast to human studies, no PNL2 immunoreactivity of granulocytes was observed. In conclusion, PNL2 is a valuable tool for diagnosis of formalin-fixed melanocyte tumors.

IMMUNOHISTOCHEMICAL OF CAPSIDIC PROTEIN p27 (CA-p27) IN TARGET TISSUES OF CAEV SERONEGATIVE GOATS.

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Caprine arthritis-encephalitis (CAE) is a persistent lentiviral disease. CAE eradication programs are mainly based on serological screening of goats. Aim of our study was to immunohistochemically investigate the presence of viral particles in tissues from seronegative goats. Samples of lung, udder and bone marrow were obtained from 29 regularly sloughed goats, formalin fixed and processed for histology. Microtomic sections were HE stained and immunohistochemically stained with mAb 1A7 anti-capsidic protein CA-p27. Histologically, interstitial pneumonia was present in 22/29 lungs and moderate BALM hyperplasia in 11/29. Interstitial, lymphoplasmacytic mastitis was detected in 21/29 cases, and moderate mammary fibrosis in 12/29. Bone marrow samples were composed of normal adipose and fibro-vascular tissues. Ca-p27 was immunohistochemically detected in 12/29 goats within bronchial (8/12) and mammary epithelium (7/12). More rarely, stromal fibrocytes, macrophages and endothelial cells in both organs stained positive. Bone marrow stromal cells were positive in 7/12 cases.

CA-p27 immunodetection in BMSCs, bronchial and mammary epithelium is consistent with previous results on seropositive goats. Our observations on seronegative animals suggest that CAE virus can subsist in reservoir cells of goats without detectable serum antibody. Further investigations (viral isolation) will elucidate if these results indicate the presence of CAE provirus or defective virus.

CANINE AURAL CHOLESTEATOMA: HISTOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF THREE CASES.

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Canine aural cholesteatoma (AC) is a rare, destructive lesion of the middle ear that parallels human acquired cholesteatoma. We report histological and immunohistochemical features of cholesteatomas in three dogs of different breeds. A diagnosis of AC was emitted on CT examination and Total-Ear-Canal-Ablation/Lateral-Bulla-Osteotomy was performed in all cases. Specimens from surgery were processed for histology. Micrometric sections were immunostained with monoclonal antibodies against cytokeratin (CK) 14, 16 and 19 and Ki67 (MIB1) and compared with samples of non-cholesteatomatous chronic otitis (NC-CO) and normal external auditory canal (EAC). Histologically, cholesteatomatous cysts were lined by 20/25 layer thick, keratinized squamous epithelium, resting on dense fibrovascular stroma, infiltrated by lymphocytes and plasmacells.

Immunohistochemically, normal EAC epithelium stained CK14 positive; CK16, CK19 and Ki67 negative. NC-CO epithelium stained CK14 and, focally, CK16 positive. Cholesteatoma epithelium was intensely CK14 and CK16 positive and CK19 negative. MIB1 immunolabelling was not significantly different in CO and AC.

According to previous observations on dogs and humans, cholesteatoma is a form of epidermal cyst. In our cases, cholesteatoma epithelium overexpressed CK16, consistently with the proliferative nature of the lesion. Low Ki67 expression suggested a low growth rate, consistently with the hypothesis that acquired cholesteatoma is a slowly progressive disease.

E. COLI SEPTICEMIA IN A FOAL

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Three-days-old, male foal was presented for the autopsy. The animal died with a signs of septic condition during the course of the antibiotic therapy. Autopsy revealed severe signs of septicemia with miliary necrotic nephritis, hemorrhagic focal colitis and serofibrinous peritonitis. Disseminated peritoneal, subepicardial and subendocardial hemorrhages were seen. Liver and spleen (reticular hyperplasia) were enlarged, and the lungs were congested.

Microscopically, severe bacterial, necrotic, multifocal lesions were seen in the kidneys, lungs and liver. Bacterial colonization was found also in the brain along with mononuclear perivascular encephalitis. Bacteriological examination revealed bacteria *E. coli*.

This case shows that some time may be difficulties in differentiation between the *Actinobacillus equi* septicemia and *E. coli* septicemia. Autopsy findings may be almost the same in both cases, but the histopathological examination of the kidneys showing extraglomerular bacterial colonization may be helpful in establishing correct diagnosis.



EXPRESSION OF ESTROGEN RECEPTOR BETA (ER β) IN CANINE SPONTANEOUS PROSTATIC CARCINOMA

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Epithelial cells in human prostate express type estrogen receptor (ER β). ER β demonstrates an anti-proliferative activity and its expression is reduced in human prostatic carcinomas according to the grade of malignancy and inversely proportional to the prognosis. Since canine prostatic carcinomas are highly malignant, we hypothesized a possible loss of ER β in these tumors. Moreover, the role of ER β in canine prostate has not yet been investigated.

In this study we evaluated the ER β expression in 10 neoplastic and 2 normal formalin fixed, paraffin embedded prostate from adult intact male dogs. Immunohistochemistry was performed employing a rabbit anti-human ER β polyclonal antibody. The percentage of ER β positive cells was quantitatively assessed considering at least 500 neoplastic cells in each section.

ER β expression was strictly nuclear and it was detectable in all epithelial cells of normal prostate. Conversely, a marked decrease of ER β was observed in all carcinomas. Noteworthy, the percentage of neoplastic cells lacking ER β expression in canine prostatic carcinomas was higher than that reported for the human counterpart. This finding demonstrates that prostatic neoplasms of the dogs, even rare, are more undifferentiated than the human ones.

SIGNIFICANCE OF THE INTRINSIC APOPTOTIC PATHWAY FOR CANCER

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Deregulated apoptosis is a condition considered to be relevant to onset and progression of cancer and to therapy failure. This is an overview on recent findings on the intrinsic apoptotic pathway and its significance for cancer. We will also shortly outline our current approach in view of these findings.

Apoptosis is mediated through the extrinsic and the intrinsic pathways, both relevant to cancer, which activate a cascade of latent cytoplasmic caspases causing cell death. The intrinsic or mitochondrial pathway is triggered by internal stimuli that can collectively be labeled as "cellular stress". DNA damage and activation of oncogenes induce apoptosis through this pathway. We can distinguish several levels of regulation of this pathway.

The p53 family plays a central role among the upstream regulators, in particular through p53 effects. The further members of the family, p63 and p73 are present in several isoforms with different functions in development and cell differentiation. New findings indicate that similar isoforms also exist for p53 and that there is cross-talk between family members. The prognostic value of p53 overexpression in tumors, as detected by immunohistochemistry, is well established. New findings indicate a prognostic value for p63 and p73 as well.

Mitochondrial membrane permeability increase causing efflux of factors such as cytochrome c from the intermembrane space into the cytoplasm is a crucial step in this pathway. Membrane permeability is controlled by the Bcl-2 family of molecules (addressed further below). Cytochrome c contributes to the assembly of the apoptosome, a molecule complex necessary for activation of caspase-9. Active caspase-9 in turn activates downstream caspases, which execute apoptosis by cleaving specific cellular substrates. The last downstream regulation mechanism intervenes at this point with the inhibitor of apoptosis proteins (IAPs: XIAP, cIAP1, cIAP2, ML-IAP, Survivin, and others), which can inhibit activated caspases. Smac/DIABLO and Omi/HtrA2, two further pro-apoptotic molecules released from the mitochondria, act as inhibitors of the IAPs. Prognostic value has been proposed for most of the molecules involved in these downstream events.

The Bcl-2 family is a large group of molecules sharing so-called Bcl-2 homology (BH) domains. It consists of three classes: the anti-apoptotic (Bcl-2, Bcl-XL, Bcl-w, Mcl-1, A1) and the complete pro-apoptotic (Bax, Bak) members are in a balance status and neutralize each other maintaining the outer mitochondrial membrane sealed. The complete pro-apoptotic members are necessary for signalling of apoptosis through the mitochondrial pathway, as shown by experiments with double knock-outs. The third

class, the BH3-only molecules (Bad, Bid, Puma, Noxa, Bmf, Bim, and others) act as sensors of diverse cellular stresses and their activation shifts the balance towards apoptosis mainly through sequestration of the anti-apoptotic members. The different BH3-only molecules are activated by several different mechanisms. Roles for BH3-only molecules have been established in lymphocyte maturation. New exciting findings indicate different affinities of these molecules for the anti-apoptotic molecules and explain the different apoptosis inducing potency of the individual molecules. Ample reference to the prognostic value of Bcl-2 molecules in cancer has been made in the literature.

Knowledge on the intrinsic apoptotic pathway in canines is very scant. The complexity and redundancy of this pathway calls for a global approach suited to assess its state in canine cancer. Specifically, we think that a immunohistochemical screen taking advantage of the tissue array technique and comprising as many molecules involved as possible should help define molecules important for tumorigenesis and progression to more malignant phenotypes.

LIVER AND SPLEEN RUPTURE IN HYPERIMMUNE SERUM PRODUCING HORSES

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Aim of study. The purpose of present study was to determine the rate and importance of liver and spleen rupture in hyper immune serum producing horses.

Materials and Methods. A retrospective study was performed on 50 necropsy reports, which was made during a period of 5 years, from 1998 until 2003 on dead horses in Razi institute. These horses were used for a long time in production of snake/ scorpion antivenoms.

Introduction. Rupture of the liver/spleen is an occasional accident in animals occurring usually as a result of trauma. Diffuse hepatic disease with enlargement, like amyloidosis, in which the substance is friable and the capsule taut provides a predisposition to rupture. In hyperimmune serum producing horses hepatic amyloidosis frequently develop, presumably as a reaction to repeated injection of foreign protein. In the spleen amyloidosis of an obvious degree is not common; when present it affects the white pulp and may spare the red pulp completely. Rupture of an enlarged liver/spleen frequently leads to fatal internal hemorrhage.

Results. Necropsy of horse carcasses revealed that; in four cases (8%) liver rupture and in one case (2%) spleen rupture caused severe internal hemorrhage leading to death. These organs were enlarged, friable and hemorrhagic. In histopathological examination by applying routine Hematoxylin & Eosin (H&E) dye, there were severe hemorrhage, necrosis and massive deposition of extra cellular eosinophilic hyaline materials, amyloid, in the livers (in the cases of liver rupture) and with lesser degree in the spleen (in the case of spleen rupture). But, in the kidneys the amount of amyloid deposition was much less than liver and spleen. By applying special dye, Congo red (CR), extracellular eosinophilic deposits that compressed surrounding tissues were constantly CR-positive.

Discussion. The results of this study revealed that; in process of snake/scorpion antivenom production deposition of amyloid materials in horses' tissue mostly occurs in liver, spleen and kidney, respectively. This can be led to fatal liver/spleen rupture.

HISTOPATHOLOGICAL FINDING IN CHICKEN INOCULATED INTRATRACHEALLY WITH A /CHICKEN /IRAN /259/1998(H9N2) INFLUENZA VIRUS

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Aim of study. The objective of this study was to determine the type, severity and frequency of gross and histopathologic changes and tissue tropism in chicken following intratracheal (IT) inoculation of Iranian avian influenza virus (AIV) isolate [A/Chicken/Iran/259/1998(H9N2)].

Materials and Methods. Twenty, 5-week-old chicken (hatched from SPF eggs) were inoculated IT with Iranian AIV isolate. Another twenty chicken were inoculated IT with sterile chorio allantoic fluid. Five chickens from each group were randomly sampled on days 1, 3, 6 and 10-post inoculation (PI). They were weighed, bled then humanly sacrificed (euthanitized). Subsequently, necropsy was performed and samples of different tissues were collected for virus isolation, histopathology and immunohistochemistry studies.

Results. Tracheitis, pneumonia and tubulointerstitial nephritis were the most frequent specific histopathologic changes. Influenza nucleoproteins were demonstrated in epithelium of trachea, secondary bronchi and cecal tonsile of a chicken. Common non-specific histopathologic changes were lymphoid and reticuloendothelial cell hyperplasia in spleen, cecal tonsile and leukocyte cell infiltration in myocardium.

Conclusion. These results indicated that Iranian AIV isolate [A/Chicken/ Iran/ 259/ 1998(H9N2)] was epithliotropic in chicken. In IT route of inoculation, it has tissue tropism and pathogenicity for trachea, lung (pneumotropic) and kidney (nephrotropic).

DETECTION OF RENAL COCCIDIOSIS IN WATERFOWL USING DIFFERENT HISTOCHEMICAL STAINING TECHNIQUES

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Abstract:In this study, two of eight waterfowls representing renal coccidiosis were evaluated histopathologically. In this evaluation, it was aimed to find out the best technique for detection of *Eimeria* spp. agents. Histochemically, Zielh-Neelsen (ZN) staining was the best staining in other techniques evaluated in this study. Furthermore, in this study, histochemical stainings were made for detecting role of the mast cells in renal coccidiosis and the results were evaluated by light microscope.

AVIAN TUBERCULOSIS IN A PIGEON (COLUMBUS LIVIA)

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Introduction: Tuberculosis of poultry, also termed avian mycobacteriosis or avian tuberculosis is a contagious disease caused by *Mycobacterium avium*. Avian tuberculosis is a chronic infection which persistence in a flock once established and induces unthriftiness, decreased egg production and finally causes death. Although tuberculosis in commercial poultry in the Iran is rarely diagnosed. Tuberculosis still occurs sporadically in backyard poultry and game birds, and it remains an important problem in captive exotic birds.

Material and Methods: In June 2004, a - year- old pigeon was referred to the Poultry section, Science and Research campus, Islamic Azad university of Tehran. The bird was fatigue and depressed. Because of poor of body condition euthanasia and necropsy was accomplished. The tissue samples include lungs and nodular mass of right wing were picked up. The tissues fixed in buffered neutral formalin immediately after take a contact smear from masses. The smears were stained by zeihl neelsen method.

Results: The gross lesions were characterized by milliary nodules in the thorax to irregular grayish white nodule 1 cm in diameter, on the right wing. On cross section, the nodule contained a single soft yellowish caseous center surrounded by a fibrous capsule. The fibrous capsule was of variable thickness and consistency. There was no or milliary nodular mass on the other organs. Histopathologically was diagnosed multiple granuloma with a center of caseous necrosis without mineralization.

Conclusion: In the zeihl neelsen staining there were a lot of acid fast bacilli in the macrophages .

Avian tuberculosis has been reported in wild birds and is reviewed elsewhere. It is more common among birds in many zoologic gardens than in domestic fowl. Infectious usually result from *M. avium* serovar 1 or serovar 2. Tuberculosis in pigeon is important because it may spread *M. avium* to poultry flocks. In Iran the report of *mycobacterium avium* is rare but it is very important for world's health.

ATHEROSCLEROSIS IN EPIDIDYMIS OF UREMIC DOG

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Introduction: Atheroma is a disease of old animals. It has little effect for many years but serious chronic disease is often the end result or at any time there may be a dramatic fatal or near fatal episode due to sudden complications such as thrombosis.

Material and Methods: In October 2004 a 12-year-old male German shepherd dog with pain at the urogenital system was dead from uremia.

The tissue fixed in bufferal neutral formalin immediately upon removal from the body.

Results: At necropsy the gross lesions include Large prostate and Large 5cm in diameter abscess at it's cranial side with necrotic and purulent exudate, without any fistula. The kidneys were to some extent shrunken and pale tan. The stomach was hemorrhagic, erosive and ulcerative. The lungs were hyperemic.

In the histopathologic feature there was chronic diffuse prostatitis with mononuclear cell infiltration. Purulent exudate and necrotic debris in the lumen of abscess. In the kidneys fibrosis, glomerulosclerosis and mineralization of the basement membrane of some tubules demonstrated chronic renal diseases. In the stomach there were erosion and ulcer with hemorrhage and mineralization of crypts.

In the epididymis the atherosclerosis in the tunica media of Artery include necrotic center with cell debris, cholesterol crystals, foam cells and calcium and fibrous cap with smooth muscle cells, macrophages, foam cells, lymphocytes, collagen, elastin, proteoglycans.

Conclusion: Atherosclerosis can be diagnosed almost in old dogs and Lead to some cardiovascular fatal Lesions. In this case there is an atherosclerotic plaque in the epididymis which is rare and blood tinged flowd contents.

RETROSPECTIVE ANALYSIS OF HISTOPATHOLOGICAL FINDINGS IN FULL THICKNESS BIOPSIES FROM THE STOMACH AND INTESTINES OF DOGS WITH CHRONIC GASTROINTESTINAL DISEASE SYMPTOMS

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Aim of the study: In dogs with chronic intestinal diseases, i.e. inflammatory bowel disease, food allergy, lymphangiectasia and neoplasms, histopathological evaluation of gastrointestinal biopsies is an important diagnostic tool. In contrast to endoscopic biopsies, in which only the mucosa of stomach, duodenum and colon can be sampled, full thickness (transmural) biopsies obtained by laparotomy contain all layers of the gastrointestinal walls and can also be taken from jejunum and ileum. The aim of this study was to analyse the spectrum of histopathological lesions in transmural biopsies from 76 dogs with gastrointestinal symptoms such as diarrhoea, vomiting and weight loss lasting for more than three weeks.

Materials and Methods: In all dogs, haematology, clinical chemistry, parasitology, radio- and ultrasonography were performed, and full thickness biopsy samples from stomach, duodenum, jejunum, ileum and colon were obtained during laparotomy. Samples from 15 dogs euthanased because of diseases unrelated to the gastrointestinal tract served as controls. Beside routine paraffin histology, immunophenotyping was performed in lymphoma cases by using antibodies to T and B lymphocytes.

Results: In 17 dogs, chronic inflammatory lesions, i.e. lymphocytic-plasmacytic enteritis (LPE, n=5), eosinophilic gastroenterocolitis (n=11) or granulomatous gastroenteritis (n=1) were diagnosed. Diffuse intestinal T cell lymphoma was present in 5 dogs. In 38 cases, lymphangiectasia of the small intestinal mucosa and submucosa, frequently accompanied by mucosal oedema, was found. In samples from 2 dogs no histopathological lesions were seen, and in 14 cases no diagnosis was made because of poor sample quality.

Conclusion: In the majority of cases (n=60), examination of full thickness biopsies allowed to make a definitive histopathological diagnosis. The availability of transmural biopsies from all segments of the intestine including the submucosal tissue layer proved to be essential for the diagnosis in cases of intestinal lymphoma, LPE and granulomatous gastroenteritis. Histological appearance of samples from cases with eosinophilic gastroenterocolitis did not allow to draw any conclusions concerning the possible aetiology. Some of the dogs with lymphangiectasia, may have primary or secondary protein-losing enteropathy because 12 of them had hypoproteinaemia

p21 KO MICE SHOWS MORE RESISTANCE TO GAMMA RADIATION-INDUCED TUMOURS DUE TO EPIGENETIC PROCESSES.

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p21^{Waf1/Cip1} is the main effector of the tumor suppressor gene p53, when DNA damage has been produced in cell. Gamma-irradiation causes a break in DNA strand, activating the action of p53 and, consequently, p21, displaying cell-cycle arrest or apoptosis depending of the accumulated damage. In the present study, we gamma-irradiated p21-null, p53 null and wild-type mice, following a protocol to induce thymic lymphoma development. We observed that irradiated p21-null mice showed a lower tumor incidence and that the survival was prolonged in comparison to both irradiated p53-null and wild-type mice. Moreover, tumor spectrum was smaller for irradiated-p21-null mice, in comparison to both irradiated p53-null and wild type mice. We characterized by immunohistochemistry, that thymic lymphomas were conformed by transformed T-cells. Then, we analyzed by immunostaining the levels of free PCNA, and DNMT1 demonstrating that in absence of p21 in a p21-null background, free PCNA shows increased levels in comparison to samples of p53-null and wild-type tissues. We studied apoptosis levels in our samples, using TUNEL. Our results showed that in p21-null mice, apoptosis had higher levels than in both p53-null and wild-type mice. Finally, we analyzed epigenetic events (promoter methylation and histone deacetylation) which could explain the p21 silencing. We conclude, that in irradiated p21-null mice, global methylation levels did not diminish as well as histone acetylation. Taken together, gene expression control exerted by epigenetic events is the most important tool for cell for becoming these p21-null mice resistant to tumour development.

IMMUNOHISTOLOGICAL DETECTION OF MHC II ANTIGENS IN CANINE DEMODICOSIS AND FUNGAL SKIN DISEASES

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Aim of this study was the detection of MHC II antigens on different cutaneous cells in canine demodicosis and fungal skin diseases. MHC II proteins present peptides derived from extracellular proteins to immune cells in order to induce an immune reaction.

This study was carried out on 23 skin biopsies from dogs with demodicosis or mycotic skin diseases. The control group consisted of skin biopsies of 7 necropsied dogs without obvious skin lesions. The detection of MHC II antigens was performed with immunohistological methods.

The immunohistological assessment of the MHC II expression revealed MHC II proteins on all cell types of infiltrating inflammatory cells. The plasma cells, however, only showed expression in 18 of 23 cases. Furthermore, in 14 biopsies from dogs with demodicosis and in 4 biopsies from dogs with fungal skin diseases the follicular keratinocytes expressed MHC II. The control group as well as samples from dogs with other inflammatory skin diseases did not show MHC II expression on epithelial cells. The endothelial cells exhibited MHC II in 18 cases.

This examination shows that MHC II expression in the skin is elevated in canine demodicosis and dogs with mycotic skin infections. Potentially, the expression of MHC II on follicular keratinocytes can be regarded as typical for these diseases.

FELINE SPONGIFORM ENCEPHALOPATHY: RESULTS OF ITALIAN SURVEILLANCE PROJECT

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Feline spongiform encephalopathy (FSE) is a fatal, degenerative neurological disease of domestic cats, associated with the accumulation of an abnormal isoform of the prion protein called PrP^{sc}. The disease was first identified in Britain in 1990 and experimental data have suggested an association with BSE. Affected cats with FSE show neurological signs including behavioural changes and movement disorder not easily distinguishable from other feline neurological conditions. Diagnosis can be achieved only by histopathological examination of brain tissue and identification of typical spongiform changes. A surveillance system of FSE is set up in Italy to find cases of spongiform encephalopathy in the feline population. In this project 61 cat brains with neurological signs have been examined and no evidence of FSE has been proved on the basis of histopathology and immunohistochemistry but this research had allowed to identify other neurological disease of cats.

A CASE REPORT OF SQUAMOUS CELL CARCINOMA(SCC) IN SHEEP FROM SHAHREKORD(IRAN)

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Introduction: SCC is a malignant tumor of squamous epithelial cells in which the cells show differentiation to keratinocytes. It is relatively common, locally invasive and occasionally metastatic neoplasm of domestic animals .The tumor is uncommon in sheep and the most sites reported are frontal and ear, however in any species this tumor may arise from any site.

Materials and Methods: In may-2004 a skin biopsy specimen from middle part of right side of thoracic area of a 4 - year- old native ewe was submitted to the Pathology Department of, Faculty of Veterinary Medicine, University of Tehran. On the basis of history, the lesion was seen from 1 month ago. The tumor was growing slowly, heamorrhagic and infected with bacteria which caused discharge of pus. The dimensions of mass was 12×12×5 cm and grossly appeared cauliflower like with dark gray color.The tissue sample was processed routinely and stained with H&E.

Results: Histopathologically neoplastic cells with pleomorphic and hyperchromatic nuclei and high mitotic figures were seen and a few horn pearls were present. There was also invasion of neoplastic cells to blood vessels and lymphatics and adjacent tissues.

Conclusion: there is reported at least 2 cases of SCC from frontal site in sheep from shahrekord which is very high altitude region in Iran. In this unusual case tumor was aroused from a part of skin which is densely covered with moderate pigmented and dense wool.

HISTOLOGICAL CHANGES OF BOVINE CORPUS LUTEUM AFTER ADMINISTRATION OF DEXAMETHASON

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Introduction: The most important subject of this study is to survey the Histopathologic changes of corpus luteum after administration of corticosteroid in animals, which have been treated with PGF2 α and cattle with normal estrous cycle.

Material and method: In this research estrous cycle of 14 heifers were synchronized by two intramuscularly injection of PGF2 α (25 mg PGF₂, upjohn, co) at 14 days interval. These animals were divided into 5 groups. (Control, A, B, C, D). No drug was administrated in 2 heifers in control group. PGF2 α was administrated in 4 heifers in-group A and 3 heifers in group D on 9th day of estrous cycle. Four heifers in-group B received 4 mg Dexamethason on 8th day and 25 mg PGF2 α on 9th day of estrous cycle. Dexamethason was injected to one heifer (group c). Corpus luteum removed via laparotomy on day 11 of cycle in all animals except group D (in 12) and then these tissues fixed in 10% buffered formalin solution. The samples were sectioned with 7micron thickness and stained with H & E.

Results: Few lymphocytes were observed in corpus luteum of animal's in-group C and control group (average 2.75 lymphocyte in each field of microscope in x400 magnification). Leukocyte aggregated foci were not seen in these groups but a lot of similar foci were diagnosed in-group A and D which were treated with PGF2 α (average 10.57 in each cm² of tissue section). Numerous leukocytes contain lymphocyte; macrophages and plasma cells in each of foci were seen (13.55, 6.5, 3.5) respectively. Leukocyte aggregated foci were not seen at group B which received dexamethason and PGF2 α . But few lymphocyte (average 4.75 lymphocyte in each field of microscope in X400 magnification) were observed. Many luteal cells had characteristics of necrotic cells. The corpus luteum of cows at group D was similar to group A, but the fibroblasts moderately were increased.

Conclusion: the research revealed that administration of dexamethason decreased the number of leukocytes in corpus luteum and also inhibit to produce the leukocyte aggregated foci which forms normally after administration of PGF2 α . For this reason dexamethason inhibit or decrease the luteolytic affection of PGF2 α in corpus luteum of ovary.

**POINT MUTATION IN THE PKD1 GENE:
MAIN CAUSE OF POLYCYSTIC KIDNEY DISEASE
OF PERSIAN CATS IN GERMANY**

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Previous studies have identified a C→A-transversion in exon 29 of the feline PKD1 gene resulting in a premature stop codon. In this survey a of sample persian cats in Germany was scanned for the prevalence of this mutation.

137 persian cats including four families were examined for polycystic kidney disease using ultrasonography or pathological examination. The described mutation causes a new restriction site. Therefore, PCR products of mutated and normal alleles can be differentiated by RFLP analysis.

None of the 71 cats without renal cysts but 94 % of the 62 Persian cats with renal cysts displayed the mutation. In all positive cases the mutation was found in the heterozygous state. The mutation was absent in 6 % of the affected persian cats. The ultrasonographic examination and the genetic testing are concordant in 97 %.

These data support the assumption that the stop mutation causes most cases of feline PKD. The distribution of the mutation within the families agrees with the autosomal dominant mode of inheritance. In homocygous cats the mutation seems to be a lethal factor. Not all cats with multiple renal cysts have the investigated mutation. In these cases the disease might be caused by another mutation or another pathogenesis.

MULTIPLE MYELOMA, PSAMMOMATOUS MENINGIOMA AND SENILE LIPOFUSCINOSIS IN A DOMESTIC CAT

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Introduction: A 15 year old, neutered domestic short hair male cat presented with severe hyperproteinemia and neurological symptoms presumably of cerebral origin. Post-mortem examination showed a multiple myeloma involving the bone marrow, liver and spleen, an intraventricular psammomatous meningioma and pronounced senile neuronal lipofuscinosis.

Results: The spleen and liver were slightly enlarged. Multiple scattered subcapsular hemorrhages, measuring 0.1-1.0 cm, were observed in both organs. The spleen had a lighter than normal, fleshy cut surface. There was a diffuse yellowish discolouration of the brain parenchyma and the nerve roots of the spinal cord. Situated in the plexus choroideus/tela choroidea, close to the hippocampus, a nodular, well-circumscribed mass measuring 0.8 cm in diameter was found, compressing the underlying brain parenchyma. The bone marrow was homogenously red and firmer than normal.

Microscopically, infiltration of neoplastic plasma cells was observed in the bone marrow, the splenic red pulp and the hepatic sinusoids. The neoplastic cells were well differentiated, only slightly pleomorphic, closely resembling normal plasma cells. Occasional binucleated cells were observed.

The intraventricular mass was a psammomatous meningioma made up of neuroid, spindle-shaped mesenchymal cells growing in streaks and concentrically arranged around calcium deposits.

Severe lipofuscin deposits were found in neurons of the cerebrum and spinal cord, in the Purkinje cells and in ganglion cells of the spinal nerve roots.

Discussion: Based on the severe hyperproteinemia and the pronounced infiltration of neoplastic plasma cells in the bone marrow, spleen and liver, a diagnosis of multiple myeloma was made. A hyperviscosity syndrome, caused by the high protein content of the plasma, manifested as dementia-like neurological signs, can occur in multiple myeloma and thus may contributed to the symptoms in the reported case. However, the meningioma probably had the greatest influence on the clinical picture as it was intraventricularly located and caused a clearly visible compression of the surrounding brain parenchyma. Senile lipofuscinosis is a common finding in aged animals, normally not considered clinically relevant, but as the lipofuscin deposits were unusually heavy in this case, they may not be a neglectable finding.

THYMUS EPITHELIAL CELLS APLASIA/HYPOPLASIA IN FOALS.

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Introduction: Diseases associated with immunological deficiency have twofold pathogenetic background. There are disorder caused by conditions of genetic development or defects aquired in utero. There is also the second type of immunodeficiency resulting from secondary aquired immunological disorders.

Material and methods: Material was taken from 11 foals, both sexes, different breeds. All foals died due to *Rhodococcus equi* infection. Thymus specimens were stained with HE, histochemical methods AB/PAS and for cytokeratine 3 microfilaments expression by using monoclonal antibody clone AEI/AE3 Dako Co.

Results: The thymus microscopic evaluation of 8 foals died due to generalized *Rhodococcus equi* infection revealed the changes in the development of medullary epithelial cells. In 4 foals there was complete absence of the medullary epithelial cells, proved by the lack of cytokeratine 3 expression, negative AB/PAS histochemical reaction and not found any Hassals' bodies. The abnormal thymus structure was associated with the epithelial cells aplasia. Thymocytes, which populated these thymuses were not finally differentiated.

In 4 foals, which also died due to *Rhodococcus* infection, the thymus epithelial cells were found and the organ structure were normal, with the division into the cortex and the medulla. The cytokeratine 3 expression revealed the rows of tiny, small epithelial cells, which were located in the thymus medulla. There were no Hassals' bodies formation, and faint staining with AB/PAS reaction were observed.

Conclusions: Results obtained in this research are indicating for the developmental failure in the thymus of foals died due to generalized *Rhodococcus* infection. The failure was manifested by the aplasia or hypoplasia of the thymus medullary epithelial cells. The further research is in progress.

THYMOMA - 2 CASES IN DOGS, 1 CASE IN THE CAT

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Introduction: Thymoma is the neoplastic tumor originated from thymus epithelial cells. The tumor is rarely diagnosed in living animals, because of its localization in the mediastinum which makes some difficulties in the clinical diagnosis.

Material and methods: Two presented cases of thymoma were obtained from post mortem examination, and one was found during mediastinal tumor surgery. Thymuses' specimens were stained HE, histochemically with AB/PAS method and for cytokeratine 3 expression by using monoclonal antibodies clone AE1/AE 3 Dako Co.

Results: The microscopic examination revealed two cases of the typical neoplastic proliferation of the thymus epithelial cells. In both animals /dog and cat/ with that type of thymoma the immune response disturbances were manifested by bacteriemia, which were also the primary cause of animals' death. In one case /dog/ the neoplastic proliferation of epithelial cells, as proved by the cytokeratine 3 microfilaments expression. The tumor cells were present in the vascular tissue with the numerous hemorrhages. The massive hemorrhage was the cause of the dogs' death, immediately after surgery.

Conclusion: the neoplastic proliferation of the thymus epithelial cells may be the primary cause of the animal death due to immune deficiency syndrome or other complications as the consequence of tumors localization

IMMUNOPHENOTYPING AND MARKERS OF PROLIFERATION AND APOPTOSIS IN TISSUE ARRAYS OF CANINE MALIGNANT LYMPHOMA

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Tissue arrays appear to offer considerable advantages for the screening of large numbers of tumor samples, especially relatively homogeneous tumor types. We investigated their use with canine lymphoma samples.

An array with two to four core biopsies from 20 cases of lymphoma was screened using antibodies specific for CD79a, CD3, Ki-67, active Caspase-3, and p53. Antibodies recognizing Caspase-3 and p53 were tested for specificity using canine recombinant proteins (immunoblots), formalin-fixed paraffin-embedded E. coli expressing the recombinant proteins, paraffin-embedded UV-irradiated cell cultures and human tumor tissues.

Immunotyping yielded 14 B-cell, 4 T-cell, 2 non-B, non-T-cell lymphomas. In cases with weak staining, results were confirmed by investigating the donor block. Labeling for Ki-67 resulted in a broad range of positive tumor cells (7-95%). The range of Caspase-3 positive tumor cells was much smaller, with values below 5% in most samples. Four tumors were positive for p53 immunostaining.

The small number of tumors investigated in this pilot study precludes an in depth analysis of the data. However, the findings indicate that the procedures employed are well suited for investigating lymphoma samples. Comparison of labeling results with the donor blocks and morphological classification of the lymphomas is ongoing.

EYELID SQUAMOUS CELL CARCINOMA IN CAT

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Squamous cell carcinoma (SCC) is a common tumor involving the skin and accounts for approximately 15% of cutaneous tumors in the cat and 5% of those in the dog. SCC usually afflicts older animals (mean age of 12 years in the cat, 8 years in the dog). Siamese cats are under-represented, as would be expected because of pigmented skin color. Generally, SCCs involving the facial skin of cats are locally invasive but late to metastasize. Surgery or Cryosurgery are most commonly used for treating these lesions. Clinical examination of one year American curl cat showed erosion in left upper eyelid and in the palpation the firm solitary mass in wall of eyelid was determined. The method for biopsy was punch biopsy. For infiltration anesthesia 15mg/kg(IM), Ketamine hydrochloride plus 0.1mg/kg(IM), Acepromazine and 0.25 mg/kg (SC), lidocaine(0/02 solution) was injected in left upper eyelid region. A piece of Sample of mass was referred to pathology laboratory and H&E staining was used. Histopathological findings in light microscopy showed hyperkeratosis in superficial zone of epidermis, but most important problem was destroyed of basal layer and invasion of stratum corneum(keratinocytes) or horn pearl into the dermis layer in many areas was rarely, cells had no mitotic figures, finally squamous cell carcinoma (SCC) with grade II, were confirmed.

THE MEASUREMENT OF T3 , T4 ,UPTAKE T3 AND TSH IN THOROUGHBRED HORSES IN TWO SEASONS(WINTER AND SPRING)

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Thyroid is one of the most important endocrine glands ,from which the vital, T3 and T4 hormones are secreted, physiological effects of these products in growth and development of bone , differentiation of nervous system and finally put in order the metabolic functions in the tissues.

In this survey, secretions of thyroid gland in 24 horses from thoroughbred races including T3 , T4,Uptake T3 and TSH have been studied in different ages and two seasons(winter and spring) by RIA . This study shows that, only amounts of T3 and T4 hormones are remarkably increased in winter rather than spring. Other factors influence the secretions of thyroid hormones are: altitude ,daylight and humidity in weather. TSH and Uptake T3 had no remarkable difference in two seasons which may be due to the blood free hormone.

MORPHOLOGIC CHANGES AND IMMUNOHISTOCHEMICAL DETECTION OF VIRAL ANTIAGENS IN THE ILEUM OF CALVES INFECTED WITH BOVINE VIRAL DIARRHEA VIRUS

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Bovine viral diarrhea virus (BVDV) is one of the most important viral pathogens of cattle in most cattle-producing countries. The morphologic changes, pattern of tissue distribution and cellular localization of viral antigens were studied in the ileum of 16 calves infected with bovine viral diarrhea virus. The gross and histopathologic lesions in the alimentary tract were similar to those described with mucosal disease and virulent BVDV type 2 infections. Microscopic lesions in the ileum included multifocal erosive and ulcerative ileitis, severe congestion and hemorrhage, lymphoid depletion of Peyer's patches, herniation of mucosal epithelium into Peyer's patches and crypt dilation filled with mucus, epithelial debris and leukocytes. Fibrinoid vasculitis was seen in ileum submucosa of two calves (12.5%). BVDV antigen was detected by immunohistochemistry (IHC) in macrophages, dendritic cells, smooth muscle cells, endothelial cells, as well as, epithelial cells of crypts and mucosal epithelium, other mononuclear cells including lymphocytes, plasma cells, fibroblasts and intramural ganglionic cells. No consistent correlation between the presence of BVDV antigen and the vascular lesions in the ileum was identified. The severity and distribution of the immunoperoxidase stain in the ileum was graded as highly positive (18.7%), moderately positive (56.3%) and mildly positive (25%). In conclusion, the results of this study show that IHC staining for BVDV in formalin-fixed, paraffin-embedded intestine is an effective method for the diagnosis of affected animals even in cases with advanced postmortem changes and in majority of affected calves, at least in the ileum, histological vascular lesions could not be used as diagnostic indicator for the presence of BVDV infection. The pattern and density of distribution and localization of BVDV antigen in the ileum was not consistent or correlated with the severity of characteristic microscopic lesions.

MHV-68 CHEMOKINE BINDING PROTEIN M3 MODULATES THE PULMONARY IMMUNE RESPONSE TO MHV-68 IN ITS NATURAL HOST

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Murid herpesvirus 4 strain 68 (MHV-68) infection of laboratory mice (*Mus* spp.) has been used as a model system to study gammaherpesvirus pathogenesis. However, the wood mouse (*Apodemus sylvaticus*) is the natural host of MHV-68 and we have recently performed comparative studies on the pathogenesis of MHV-68 pulmonary infection in laboratory bred wood mice and BALB/c mice. In wood mice, a brief phase of viral replication is followed by an intense, B cell-dominated immune response with intense viral latency. In BALB/c mice, however, MHV-68 seems to induce a prolonged macrophage and T cell-dominated response. In vitro studies have shown that the MHV-68 genome encodes for a broad-spectrum chemokine binding protein (ORF M3) which selectively binds numerous chemokines, but is functionally redundant in BALB/c mice infection. We therefore infected wood mice with a recombinant MHV-68 that lacked M3. This dramatically abrogated the B cell response, suggesting a role for M3 in B cell recruitment to the lung. Protein analysis using a chemokine antibody array system has shown that M3 selectively binds and inhibits chemokines involved in the recruitment of T cells, but does not bind B cell chemoattractant chemokines. Thereby, MHV-68 can recruit B cells in the primary site of viral replication and efficiently establish latency.

EQUINE IDIOPATHIC FOCAL EOSINOPHILIC ENTERITIS: CHARACTERISATION OF THE INFLAMMATORY REACTION

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Equine idiopathic focal eosinophilic enteritis (IFEE) is an inflammatory condition of the small intestine which has recently been described. So far, little is known about its aetiology and pathogenesis.

This study aimed to further characterise the inflammatory reaction. Twenty cases of IFEE and, for comparison, four cases of diffuse eosinophilic enteritis (DEE) were examined. Inflammatory cell populations were identified by light microscopy, including immunohistology for T and B cell markers, macrophages and activated endothelial cells. In IFEE, the submucosa exhibits a focal granulomatous infiltrate with a variable macrophage-eosinophil ratio and a lesser T cell-dominated lymphocyte component. The infiltrate extends into adjacent layers and often exhibits proliferating fibroblasts and new vessel formation. An acute component, represented by blood vessels with activated, MHC class II-positive endothelial cells, is generally seen as well. Additionally, a mucosal infiltrate of T (and B) lymphocytes, plasma cells, macrophages and eosinophils is present. Its composition and intensity, however, is similar to the mucosal infiltrate observed in DEE and in lesion-free small intestinal areas of IFEE cases.

Results indicate that IFEE represents a focally exacerbated inflammatory reaction in horses with DEE, but this has to be further clarified in future studies.

Scientific programme section appropriate for presentation:

Microscopic and submicroscopic pathology

POSTER PRESENTATION

NEUROVIRULENCE OF PSEUDORABIES VIRUS DEPENDS ON UL37-PROTEIN: HISTOPATHOLOGY AND TIME LAPSE RECORDING IN NEURONAL CELL CULTURE

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Pseudorabies Virus (PrV) is an alphaherpesvirus that causes Aujeszky's disease (AD). To study the role of viral proteins in neuroinvasion and intraneuronal transport, PrV mutants lacking structural and nonstructural proteins were instilled intranasally into mice. Mean survival times, kinetics of viral spread and host response were examined in the trigeminal circuit. All mutant viruses, except PrV- UL37 infected first and second order trigeminal neurons, as shown by immunohistochemistry. Deletion of the respective genes indirectly influenced the degree of nonsuppurative encephalitis and ganglioneuritis by longer survival times, whereas no neuronal apoptosis could be detected by immunohistochemistry against active caspase 3. By confocal time lapse recording intraneuronal, bidirectional transport of fluorescent capsids after infection of murine primary trigeminal cell culture was observed with wildtype PrV, whereas deletion of the UL37-protein inhibited intraaxonal capsid movement. Therefore, we conclude that the UL37-protein is essential for intraneuronal transport of PrV and might possess connective functions between viral capsids and microtubule-associated motor proteins like dynein and kinesin.

TRACHEAL ORGAN CULTURES ALLOW THE STUDY OF MORPHOLOGICAL CHANGES INDUCED BY FELINE HERPESVIRUS

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Feline herpesvirus-1 (FeHV) is a major cause of upper respiratory tract disease in cats. FeHV is primarily an epitheliotropic virus and induces extensive necrosis of the airway epithelium.

Tracheas were collected from cats euthanased for reasons other than respiratory disease and cultured for 24 hours prior to infection with a high dose of FeHV (strain B927). Cultures were maintained for variable lengths of time (24 to 120 hours). Viral load within the culture medium was measured by qPCR and virus reisolation. Morphological changes and expression of viral antigen were examined in tracheal sections by light microscopy including immunohistology as well as transmission electron microscopy. Tracheas developed typical focal epithelial changes; initially intranuclear inclusion bodies and cell rounding with loss of cell-to-basement membrane and cell-to-cell contact were seen. Cell death by apoptosis and/or necrosis followed. Finally, affected areas were completely devoid of epithelial cells. Maximal viral loads were measured at 72 hours post infection.

Results identify tracheal ring cultures as suitable models for in vitro studies of the effect of alphaherpesviruses on respiratory epithelial cells. There is evidence for cell-to-cell spread after initial infection of scattered cells, but further studies to evaluate functional changes and the modes of virus spread are needed.

PRIMARY INTRAOCULAR PRIMITIVE NEUROECTODERMAL TUMOUR (MEDULLOEPITHELIOMA) IN A HORSE.

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Primary intraocular tumours of neuroectodermal origin develop either from mature neuroepithelium, i.e adenomas, adenocarcinomas of the (unpigmented/pigmented) ciliary epithelium, or from primitive medullary epithelium (during differentiation of the optic vesicle or optic cup), i.e. medulloepitheliomas and retinoblastomas.

In animals, medulloepitheliomas are rare congenital tumours possessing multipotentiality and retained ability to differentiate into retinal, ciliary epithelial, vitreous or neuroglial cells. Medulloepitheliomas have been described in goldfish, cockatiels, llamas, feline, canine, and equine species. The majority of medulloepitheliomas is reported from young animals, classified as benign or malignant, non-teratoid or teratoid.

Here we describe a case of a primitive neuroectodermal tumour, i.e. a medulloepithelioma, in a 14-year-old mare. The anterior eye chamber and posterior uvea were filled with an endophytic, cup-shaped, opaque to pale whitish, soft tissue, composed of moderately vascularised, spongy, lamellar sheaths or irregular tube forming elements. Histologically, spindloid to spherical tumour cells, arranged in sheaths, tubules or nodules, formed Flexner-Wintersteiner, Homer-Wright and pseudo rosettes, often separated by large areas of necrosis, cellular debris and calcification. Immunohistochemistry for vimentin, GFAP, NSE, NF, cytokeratin, S100 protein, synaptophysin, retinal S protein and transmission electron microscopical studies principally supported the diagnosis of medulloepithelioma, but also showed several features allowing the diagnosis of retinoblastoma.

AN EXOTIC DISEASE IN EUROPE: EQUINE RHINOSPORIDIOSIS.

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Rhinospordium seeberi is the infectious agent that causes rhinosporidiosis, which commonly presents as polyps of the nasal mucosa or conjunctiva of humans and animals. *R. seeberi* is a water-borne organism and has recently been classified as a mesomycetozoa. Rhinosporidiosis is predominantly present in the tropics and especially southern India and Sri Lanka. Sporadic cases have been reported in Africa, the Americas and Europe.

A biopsy of a focally ulcerated polypoid lesion from the nasal mucosa of a polo pony imported into the UK from Argentina was submitted to the Department of Veterinary Pathology, University of Liverpool, for diagnostic histopathology. A chronic hyperplastic rhinitis with fungal-type organisms was diagnosed. Light and electron microscopy revealed that the organisms were consistent with *R. seeberi*. Using PCR and *R. seeberi* genome-specific primers, DNA was amplified and sequenced. The sequence matched published sequences for *R. seeberi*.

R. seeberi is predominantly a human pathogen, with sporadic cases also reported in dogs, swans, cattle, cats and horses. The majority of cases seen outside of the tropics occur in hosts who have travelled from these regions.

THE FREQUENCY OF CANINE OSTEOSARCOMA IN BIOPTICAL INVESTIGATION FROM 01.01.1991 TO 31.12.2004.

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Osteosarcoma is the most frequent malignant bone tumor in dogs and it is reported to represent 80-85% of primitive bone tumors and 3-4% of all canine tumors. The vast majority of cases occur in old large breed dogs. Our purpose is to provide epidemiologic data on canine osteosarcoma based on the results of histopathologic examinations performed from Jan 1, 1991 to Dec 31, 2004 by the Department of Biopathologic sciences and Food and Animal Production Hygiene of the Veterinary School of the University of Perugia.

We examined 16550 canine tissues. A tumor was diagnosed in 8473 (51,20%) of these. The remaining 8077 (48,80%) had other types of lesions. There were 168 bone biopsies performed during this interval, 111 (66,07%) of which were tumors and 57 (33,93%) of which were inflammatory or degenerative disorders. Primitive bone tumors were divided into 91 malignant and 20 benign. The 82 cases of primitive osteosarcoma represent 73,87% of all primitive bone tumors examined.

HISTOLOGICAL, IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL FEATURES OF FELINE INTESTINAL MAST CELL TUMORS

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Feline Intestinal Mast Cell Tumors (IMCTs) differ for clinical, histological and prognostic features from the more common Cutaneous (CMCTs) and Splenic Mast Cell Tumors (SMCTs).

Some feline intestinal round cell tumors were analysed by histochemical (alcian blue, PAS, Giemsa, PTAH, Grimelius, CAE), immunohistochemical (tryptase, CD117, CD3 and CD79) and ultrastructural (TEM) techniques to identify sensitive diagnostic tools for IMCT and investigate its histogenesis.

IMCTs were characterized by variable histological patterns. Tumor cells often lack metachromasia, and rarely showed orthochromatic PAS positive cytoplasmic granules. Chymase activity (demonstrated by CAE reaction) wasn't detected in any tumor. Most IMCTs were variably immunoreactive for mast cell tryptase, while CD117 immunostaining was very faint or totally absent. Ultrastructurally most tumor cells had rare, heterogeneous, variably electron-dense granules associated with empty, often coalescing vacuoles, suggestive of undifferentiated or degranulated mast cells.

In this study none of the techniques used was satisfactory in every case. Tryptase was sensitive and specific, but it didn't stain every tumor; CD117 was not suitable as a marker for feline IMCTs; TEM allowed a definitive diagnosis, but rarely can be used in diagnostic routine. A combination of different techniques should be used in suspected cases to allow a definitive diagnosis of IMCT.

EFFECT OF THE HERBICIDE AZOPRIM 50 WP ON THE HEPATOPANCREAS PATHOMORPHOLOGY IN CLINICALLY HEALTHY AND WITH *ICHTHYOPHTHIRIUS MULTIFILIIS* INVASION CARP (*CYPRINUS CARPIO* L)

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The experiment was carried out during 96 h on carp (81 - 98 g), divided into 4 groups (n=10): 1 and 2 - control fish without exposure to the herbicide, 3 and 4 - the carp exposed to atrazine (Azoprim 50 WP, Organika-Azot S.A., Jaworzno, Poland) in 2 mg/l concentration. Fish from the groups 1 and 3 were clinically healthy and carp from the groups 2 and 4 became infected by *Ichthyophthirius multifiliis* (natural invasion). Macroscopically, retrogressive changes were the most frequently noted in hepatopancreas of fish from the groups 2 and 4. Circulatory disturbances were less frequent and progressive changes were sporadic. However, in the ultrastructural pattern of the liver lesions in mitochondria and RER were dominant. Morphological lesions were of 40 % more frequently observed and more intensive in carps with *Ichthyophthirius multifiliis* invasion and exposed to the herbicide than in clinically healthy fish.. Azoprim 50 WP in 2 mg of atrazine/l had pathogenic effects on carp. It led to morphological changes and generated intensification of these lesions in carp infected by *Ichthyophthirius multifiliis*.

PATHOLOGICAL AND VIROLOGICAL STUDIES ON SMALL RUMINANT LENTIVIRAL ARTRITIS IN SHEEP IN SPAIN

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A group of 12 sheep with articular processes in the carpal joint was studied. Arthritis was seen either unilaterally or bilaterally but normally it caused light clinical signs. Macroscopically, animals were mostly characterized by cool, swollen and non-painful carpal joints. All animals were seropositive to maedi-visna virus (MVV) as determined by recombinant ELISA. Sheep were killed and samples were taken for histopathology, microbiology and PCR and sequencing. Lesions consisted on proliferation of the synovial membrane, mononuclear inflammation of the synovial tissue, fibrosis and necrotic areas with calcification. Some lesions coexisted with MVV lesions in lungs and mammary gland. Positive cells to MV in cytological preparations were detected by immunohistochemistry (IHC) in 6 sheep. However, by using IHC and in situ hybridization (ISH) only three animals showed positive cells in carpal tissues. MVV was also detected by IHC and ISH in lung and mammary gland of the affected animals. Four sheep showed both presence of MVV and detection of *Mycoplasma agalactiae* antigens in cells from the affected joints. MVV was isolated in two animals and its sequence demonstrated to be much closer to the caprine arthritis encephalitis virus (CAEV) rather than to MVV. These findings will be discussed.

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DEVELOPMENT OF ANTIANGIOGENIC TUMOR THERAPY USING SEMLIKI FOREST VIRUS VECTORS

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Introduction: The angiogenesis dependency of tumour growth has led to increased interest in antiangiogenesis tumour therapies. With this in mind it was decided to test the efficacy of a recombinant Semliki Forest virus (SFV) suicide particle vector expressing Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2/Flk-1), an antigen upregulated on tumour endothelium, in the treatment of experimentally induced tumours in mice.

Materials and Methods: Using RNA isolated from mid-gestation mouse embryos, the gene for VEGFR-2 was isolated using RT-PCR and the extracellular and transmembrane domains of the receptor were cloned into the vector. BHK cells electroporated with SFV-Flk-1 RNA demonstrated strong positive immunofluorescence for Flk-1. CT26 colon carcinoma cells were cultured and tumours were induced in BALB/c mice following subcutaneous inoculation. Prior to the induction of tumours, mice were inoculated at weekly intervals up to five times with rSFV particles encoding VEGFR-2. Tumour growth in treated and control mice was measured and tumours were sampled for histological and immunohistochemical examination. Serum was collected from the mice before and after tumour induction.

Results: Tumour growth in mice treated with rSFV-VEGFR-2 was significantly reduced compared with controls. Decreased size of the tumours in vaccinated mice was correlated with reduced intra-tumoural microvascular density and circulating antibodies to Flk-1. The Flk-1 gene has been cloned into a SFV replicating vector and studies using a metastasing 4T1 mouse mammary adenocarcinoma model are in progress.

SOME HISTOPATHOLOGICAL ASPECTS OF CHLORINE TOXICITY IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Eight months old rainbow trout were found dead in a freshwater recirculation system. Ten days before the system was shut down during a blackout. The tanks had been filled with town water with fluctuating levels of chlorine. Even after sodium tiosulfate was added, chlorine levels were as high as 0.2 ppm. Fish had enlarged gall bladders and the carcasses appeared pale and anemic. Wet preparations of skin scrapes and gill biopsies were negative for parasites. In histopathological inspection the gills epithelium had foamy vacuolation and the chloride cells were swollen. Increase of golden pigments and azurophilic bodies within phagocytes and erythrophagia were prominent in the kidney and the spleen. Variable amounts of colorless cytoplasmic vacuolation and small hyaline droplets were seen in the hepatocytes and foci of phaged golden pigment were scattered in the liver. Elevated numbers of melanocytes in the skin and mild mural reactions in the myocardium were other histopathological findings.

The condition appeared to be primarily due to red blood cells destruction and is consistent with the marked anemia that was confirmed by clinical chemistry results. History and findings were consisted with met-haemoglobinaemia, which may be related to excess chlorine toxicity.

FIBROSARCOMA AND FIBROUS OSTEODYSTROPHY: COMPLICATION DUE TO ARTHRODESIS OF FETLOCK JOINT IN A HORSE

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A six-year-old Arabian stallion was presented with a disclosed right metacarpal bone from the fetlock joint due to an accident exhibiting a compound fracture. Following a complete clinical, paraclinical and radiological examination, it was decided to reduce the fracture and arthrodesis the fetlock joint.,

Under general anesthesia the cartilage of the distal metacarp and first phalange was curetted and the joint was fixed using a conventional plate. After two month, the wound broke down exposing the metal implants and animal did not support the leg any more. Euthanasia was induced and the animal was sent for autopsy evaluation.

A thick layer of compact, hard mass surrounded the implanted metal. Muscle atrophy and subcutaneous edema was prominent in the affected limb.

Histologically, there was two distinct portions. In one the normal constituents of the callus was not formed and the callous was composed of fibrous tissue and discrete islands of cartilage and woven bone. The central portion of the callus showed a cystic like degeneration. Most of the cortical bone was replaced by fibrous tissue. Discrete osteoid formation that were not mineralized was the other characteristics in this portion. The overall picture of this portion was suggestive of fibrous osteodystrophy of metacarpal bone.

The second portion was composed of fibroblasts arranged in a pinwheel pattern that had replaced cancellous and cortical bone. Some scattered giant cells were detected in this section. The neoplastic tissue had tended to replace the medullar cavity in one of the sections. There was no hemorrhage and osteoblasts were not detected in this portion. Therefore the microscopic findings were suggestive of fibrosarcoma in the metacarpal bone.

FIRST REPORT OF MEIBOMIAN GLAND ADENOMA FROM IRAN

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A three-years old male German shepherd dog was presented to veterinary teaching hospital with a papillary, protuberance and bulging outward from his eyelid margin. Clinical signs included conjunctivitis and keratitis due to contact of the mass with the cornea. Multiple biopsies were obtained from the mass and were sent for histopathological study.

Microscopically, multiple lobules of tumor cells separated by fibrovascular connective tissue and remnants of preexisting dermal connective tissue were observed. The tumor cells had pale eosinophilic cytoplasm and large hyperchromatic nuclei with different degrees of intracytoplasmic lipid vacuoles. The cells showed moderate pleomorphism and moderate numbers of mitosis.

The macrophages surrounding the gland contained intracytoplasmic refractile membranous bodies. Based on the gross morphology and histological features the tumor was diagnosed as Meibomian gland adenoma.

FIRST REPORT OF BLUE DOBERMAN SYNDROME FROM IRAN

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A one year old male Doberman was presented with marked alopecia which had become progressively more extensive over the last 4-6 months. The problem was first noticed at 2-3 weeks of age. Initially, there were patchy alopecia in the ear margins and around the eyes that had spread to involve the neck and proximal limb as far as the elbow joint. In affected areas, the hair loss was virtually complete apart from scattered residual tufts. Multiple punch biopsies of skin were submitted for histopathological examination. Punch biopsies were all similar in microscopic appearance. The hair follicles were mostly in telogen phase containing clumps of granular melanin. A scanty to moderate cellular infiltration was visible around some of hair bulbs, which melanin-containing macrophages were prominent. Based on clinical and histopathological examination the case was diagnosed as color mutant alopecia (Blue Doberman syndrome).

TELEPATHOLOGY IN VETERINARY DIAGNOSTIC HISTOPATHOLOGY

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Abstract - Telepathology (TP) is the process of diagnostic pathology at a distance through a suitable communication channel. At present, telepathology by electronic mail attachment is the most common communication medium, because it is relatively simple and incurs minimal cost. We assessed its validity and accuracy in routine veterinary diagnostic histopathology. Using 20 biopsy specimens, a total of 130 images were digitized by one histopathologist and sent as e-mail attachments from the Faculty of Veterinary Medicine of Naples to the Faculty of Veterinary Medicine of Messina (Italy) for interpretation. The median time taken to capture the images was 30 minutes for a case. The median time taken to make a TP diagnosis was few seconds for a case. The images were reviewed at the Unit of Messina and one histopathologist formulated his diagnoses.

He was able to give a definitive TP diagnosis in 18 of 20 cases. For the diagnoses of the remaining 2 cases, he required others images and finally the glass slides. The referring and consultant histopathologists reviewed all glass slides and achieved agreement in all cases using light microscopy. The telepathology through e-mail represents an easy, available and inexpensive tool for veterinary diagnostic histopathology.

PORCINE DERMATITIS NEPHROPATHY SYNDROME (PDNS): PCR-ANALYSIS OF PORCINE CIRCO-VIRUS 2 (PCV-2) EXPRESSION IN DIFFERENT ORGANS INCLUDING ISOLATED GLOMERULI OF 10 PIGS

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Data are presented from 10 pigs with pathomorphological lesions associated with Porcine Dermatitis Nephropathy Syndrome. Besides the systemic vasculitis leading to haemorrhages and necroses of the skin all pigs showed an acute necrotizing glomerulonephritis often accompanied by sclerosis of other glomeruli. By electron microscopy an accumulation of electron dens material could be seen in the glomerular mesangium of most animals. The aim of the study was to demonstrate PCV-2 in skin, lymph node, spleen, kidney as well as isolated glomeruli by polymerase chain reaction. The glomeruli were isolated from formaldehyde-fixed tissue of the renal cortex using a simple microdissection-technique. Samples containing about 100 isolated glomeruli in formaldehyde-solution per animal were used for analysis by TaqMan-based real-time PCR.

SURVEY ON PATHOLOGIC LESIONS OF LUNG IN SLAUGHTERED CAMELS (*CAMELLUS DROMEDARIUS*) IN IRAN

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Among domestic species, camel is more adaptable to tropical and sub-tropical climates. It can be fed with low quality roughage, so has a lesser food competition with other domestic ruminants. Camels are more resistant to most of bacterial, viral and parasitic diseases. There are a few investigations done on camel lung pathology in our country, so this study was conducted to find out macroscopic and microscopic pathological lesions in lungs of 100 camels slaughtered in Mashhad Industrial Meat Complex. Four hundred specimens were taken from lungs and referred to laboratory of histopathology. Tissue slides were prepared and stained with Heamatoxillin & Eosin to be evaluated pathologically. Results were as follow: emphysema (8%), atelectasis (11%), pleurisy (4%) anthracosis (56%), hemorrhage (11%), hyperemia (28%), parasitic lesions (17%), parasitic cysts (1%), bronchopneumonia (7%), fibrinous pneumonia (1%) and pulmonary edema (3%). Parasitic lesions due to *Diptalonus evansi* seemed to be more important. Colour and consistency changes in tissues bearing such lesions were observed in gross assessment. In two cases, dark-colored points which were diagnosed as vascular thrombi were seen. Cross-sections of parasites were observed among vascular thrombi in microscopic evaluation. In other cases, some lesions were seen inside the vessels which were due to presence of various parasitic agents. These lesions were as follow: chronic proliferative endoarthritis, prevascular cuffs in most of vessels, phlebothrombosis and interstitial pneumonia.

BOVINE CUTANEOUS MASTOCYTOMA IN THE EXTERNAL EAR

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Introduction: Neoplasms of the skin of the external ear are uncommon, but may originate from the pinna or from ceruminous glands. Any neoplasms of the skin can arise from auricular skin. Single or multiple nodular dermal proliferations of mast cells occur in all domestic species but are most common in dog and rare in cattle. Cattle of any age, including calves, may be affected, and no sex or breed predilection has been noted. This paper describes the macroscopic and histopathological features of a cutaneous mastocytoma found in the external ear of an adult cow.

Materials and Methods: In June 2002, an incisional biopsy specimen from a 6 years old Holstein cow was submitted to the department of pathology, faculty of Veterinary Medicine, University of Tehran. Tissue sample was processed routinely and stained with H&E, Toluidine blue and Giemsa.

Results: A firm nodular mass, approximately 3 cm in diameter was present at the base of the left external ear, which had protruded into the outer end of the cartilaginous part of the external auditory meatus.

Histopathological examination revealed neoplastic mass that lacked capsule and was well demarcated with dermal connective tissue. The tumor was composed of diffuse sheets or cords of round to somewhat polygonal cells with central round to oval nuclei, prominent, single nucleoli and a moderate to abundant amount of granular basophilic cytoplasm. The neoplastic mast cells were almost uniform in size and shape, with rare mitotic figures. These cells contained numerous metachromatic cytoplasmic granules that stained red and purple with Toluidine blue and Giemsa respectively.

Conclusions: The site, gross pathology, histopathological finding and the results of special stainings confirmed the diagnosis of a benign mast cell tumor of external ear as a rare report in the Cattle.

OUTBREAK OF ICHTHYOPHONOSIS IN FARMED SHARPSNOUT SEABREAM (*PUNTAZZO PUNTAZZO*)

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Ichthyophonosis is a systemic mycosis of fish caused by the fungus *Ichthyophonus hoferi* belonging to the class Ascomycetes. Such disease has been reported worldwide in different fish species and represents the most important fungal disorders in both feral and farmed teleosts. Severe losses (30%) were registered in sharpsnout seabreams (*Puntazzo Puntazzo*) farmed in sea cages in the South Tyrrhenian Sea. Specimens were analyzed for gross changes and, after necropsy, tissue samples were fixed in 10% formalin solution. At necropsy fish showed multiple differently sized, 1 to 5 mm, white spots or nodules involving all coelomic organs. Histological sections confirmed the granulomatous nature of the changes, showing the granuloma formation surrounding single fungal spores, 10-250 µm in diameter, or groups of spores. The macroscopical changes are not always detectable and cannot be used as a diagnostic feature, while the association between gross and microscopical pathological changes can provide a sure diagnosis. Moreover, to distinguish *Ichthyophonus* from other granuloma-inducing conditions, the characteristic germination of the fungus from the resting cells must be demonstrated in histological sections.

CANINE OVARIAN TERATOMA: CLINICOPATHOLOGICAL STUDY OF TWO CASES

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Ovarian teratoma is a germ cell neoplasm composed of different tissues derived from two or three germ layers. It has been reported in the bitch, but few clinicopathological studies were found. In a survey of over 600 canine ovaries, only two ovarian teratomas were discovered in two cyclic German Shepherd bitches. The first (I) was 5 years old and was admitted for vaginal bleeding 48 days after mating. The second patient (II) was 11 years old and nulliparous, and was admitted for a mammary nodule. During physical examination, in both cases, a left ovarian neoplasm was found by abdominal palpation and/or ultrasound. The bitches were ovariohysterectomized. Grossly, the teratomatous ovaries appeared irregularly ovoid (10x6 and 3x2,5 cm). The consistency was hard in some areas. On sections, cystic areas with hairs and sebaceous material were observed, together with solid areas. Histologically, different adult-type tissues were recognized as keratinized squamous epithelium, sebaceous and sweat glands, respiratory epithelium, nervous tissue, lymphoid tissue, fat, bone and cartilage. Functional ovarian remnants were also found close to the neoplastic area (II). The controlateral ovaries presented corpora lutea (I) and preovulatory follicles (II). The uterus was enlarged and contained reddish fluid, showing a normal involution of the placental sites, in case I, normal in II. Canine ovarian teratomas do not seem to interfere with ovarian function, but, if very large, physical complications could occur.

CHEMOTHERAPEUTICAL APPROACH IN A DOG AFFECTED BY TRANSMISSIBLE VENEREAL TUMOUR (TVT) AND LEISHMANIOSIS

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The association of canine TVT and leishmaniosis is not rare in Sicily, where both diseases are endemic. A 6 year old mixed breed dog was admitted for leishmaniosis (IFAT: 1600), with severe symptoms including anemia, thrombocytopenia, leukopenia, increased liver enzyme levels, epistaxis and melena, concomitant to genital, cutaneous and genigival proliferative TVTs. After hemotransfusion, chemotherapy with metronidazole (25mg/Kg daily per os), antihemorrhagics and hepatoprotectors, was administered for 15 days, with significant improvement of blood and liver parameters. A stronger antileishmanial treatment with N-methyl glucamine antimoniate (50mg/Kg daily im) was then given for 30 days. Leishmaniosis symptomatology ceased while TVT nodules showed no evidence of regression and appeared a little enlarged. Vincristine (0,025mg/Kg weekly iv) was then given for 4 weeks, until no more neoplastic cells could be found in cytological smears. Moreover leishmaniosis symptoms re-emerged, maybe due to the antiproliferative drug, indicating a further cycle of antimonials followed by a longer allopurinol administration (30mg/Kg daily per os). Two year follow-up showed no recurrence of either TVT or leishmaniosis.

IMMUNOHISTOCHEMICAL DETECTION OF CYCLIN E, CYCLIN D1 AND Ki-67 IN THE MAMMARY TUMORS IN THE CAT.

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The evaluation of cellular proliferation is fundamental in histopathological diagnosis of mammary tumours of the cat and it is also a prognostic factor. In this work, the immunohistochemical methods was used; besides markers of cellular proliferation today usually employed such as Ki-67, the use of cyclin E and cyclin D1 was testing.

From our laboratory files, 35 feline mammary tumours, classified according to standard diagnostic criteria by WHO (7 simple adenomas, 10 simple carcinomas solid-type, 8 simple carcinomas tubular-type and 10 complex carcinomas) were selected. For immunohistochemical investigations 4µm-sections were cut from archived paraffin blocks. After microwaves antigen retrieval, the slides were incubated with: rabbit Pab anti-cyclin D1, rabbit Pab anti-cyclin E (Neomarkers, USA) and mouse Mab anti-Ki-67 (DAKO, USA). The reaction was detected using an avidin-biotin peroxidase method. Higher cyclin D1, cyclin E and Ki-67 expressions were found in carcinomas than in benign tumors. The majority of the tumours had less cyclin E and D1 than Ki-67 positive cells indicating a conserved cell cycle specific expression of the protein. Significant correlations were obtain among the growth fraction, the percentage and intensity of cyclin E and D1 positive cells suggesting a relationship to tumour progression and to a poor prognosis.

MALIGNANT PERIPHERAL NERVE SHEATH TUMOR IN A DOG

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A 10-year-old, male, mixed-breed dog was examined at the Veterinary Hospital of the Faculty of Camerino. The subject showed a nodular, subcutaneous mass, 6 cm in diameter, in the thyroid region and also in left side of breast. Ecographic investigation revealed also a perirenal mass, adhering to the underlying tissue, and nodular lesions in lung and liver. Clinical conditions deteriorated rapidly and the animal was subjected to euthanasia. At necropsy, presence of nodular masses were confirmed in lung, kidney and liver. The neoplastic tissue appeared encapsulated, white-greyish in colour, with some reddish area of necrosis.

Microscopically, the neoplastic masses were characterized by a population of spindle-shaped cells; sometimes foci of epithelioid or mixoid appearance were observed. The spindle-shaped cells were arranged in fascicles, whorls and sheets with a variable amount of fibrillar collagenous stroma. Immunohistochemical labelling, performed with the ABC-complex method, gave positive staining to Vimentin, GFAP, S-100 and no reaction to CKs, NFs, NSE, EMA and Desmin.

The histopathological study and immunohistochemical results led to diagnosis of uncommon metastatic visceral malignant peripheral nerve sheath tumour as occur in human beings which often show a preexisting NF1.

HISTOPATHOLOGIC EVALUATION OF A NEW BIOCOMPATIBLE MATERIAL FOR TREATMENT OF VESICoureTERAL REFLUX IN DOGS

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Purpose: Endoscopic correction of vesicoureteral reflux is an alternative to open repair . For this goal the biocompatible and reliable injected material has been used . We investigated the pathologic effect of a new biocompatible for endoscopic correction of vesicoureteral reflux in dogs.

Materials and methods: In stage one , sixteen dogs was unroofing the ureters for creation of reflux bilaterally and in four groups with 3-5mm without suturing, 3-5mm via catheter guide and suturing with silk, 3-5 mm incision with catheter guide and suturing with nylon and finally 5-7 mm incision with catheter guide and suturing with nylon . Ten days after reflux creation all dogs of stage one (four groups) undergone cystography for reflux assessment..In the second stage for the evaluation of new biocompatible material nine dogs were randomly divided into three groups of three animals in each groups . In the first group for any three dogs endoscopic treatment was done by injecting new material for one side and the UROCOL for the other side . In the second group for any three dogs endoscopic treatment was done by injecting the new material for one side and water for the other side . In the third group for any three dogs endoscopic treatment was done by injecting the UROCOL for one side and water for the other side.

Results: Ten days after treatment second cystography for all groups was done . In which all 3 dogs of group one was treated by new material and UROCOL bilaterally . In animals of the group 2 unilaterally treated by new material but reflux was remained in the another side .In the animals of group 3 the same result was acquired by UROCOL as in group two . Three animals were followed up for three months and euthanized for migration of treated material to the different organs on histopathological studies.

Conclusion: With the result satisfactory results was acquired in all animals of group one but persistent reflux in water treated ureteral junction in group two and three.In long term animal studies needed to evaluate this material further before human clinical trials can be initiated. No local complication and other complications due to migration of new material in this study were observed. No distinct migration of new material articles were observed after submucosal injection of such implant in the dogs of stage two in different organs (kidney, spleen, bladder and ureters) in the histopathologic studies.

Key words: *Vesicoureteral reflux, cystography, bladder, dog.*

THE ASSOCIATION BETWEEN ANGIOGENESIS AND CELL PROLIFERATION IN CANINE ORAL MUCOSA

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Neoplastic cells, having an high proliferative rhythm and increased metabolic requirements, become able to induce the formation of new vessels, by angiogenesis. Angiogenesis has been proposed to have prognostic significance in numerous cancer. Therefore, a correlation between neoplastic proliferation and angiogenesis has been demonstrated in several tumoral types. The purpose of this study was to investigate the association between neovascularization and cell proliferation in 27 samples of canine gingivas, 2 normal and 25 pathologic; these one were classified, in according to Pindborg criteria, as medium dysplasia (10), severe dysplasia (7) and carcinomas (8). Proliferative cells were visualized by staining with Ki-67 antibody and blood vessels with von Willebrand factor (vWF) antibody.

A significant increase in vessels number were detected, with disease progression from normal oral mucosa, through moderate and severe dysplasia to carcinoma ($P=0,07$). Moreover, the increase of microvessel density was accompanied by a significant increase of cell proliferation ($P=0,0008$), suggesting that increased vascularity occurs in support of actively proliferating oral epithelial cells in order to permit growth.

In conclusion, there is a strict correlation between cell proliferation and angiogenesis in oral canine pathology ($r = 0,988$), that may provide an additional criterion in evaluating the intrinsic malignancy and growth potential of these lesions, and may have a potential application as diagnostic and prognostic indicators.

HISTOPATHOLOGICAL, TOXICOLOGICAL, MICROBIOLOGICAL AND PARASSITOLOGICAL INVESTIGATIONS IN MUSSELS (*MITILUS GALLOPROVINCIALIS*) FROM CESENATICO. PRELIMINARY DATA

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Mussels are filtering organisms, thus they feed on organic particles in water, so that they are naturally exposed to the risk of accumulating biological and chemical pollutants which could be present in waters. The amount and the kind of microorganisms present in the body and the intervallar fluid can change as function of the geographical distribution, of the season and of the physiological state of the mussel, which can influence the filtration efficience.

Histopathological investigations were focused on the observation of any evident and/or criptic lesion eventually correlated to microbiological, parassitological and toxicological results.

Production areas control was performed by monitoring some microbiological, toxicological and chemical parameters. Specifically, faecal pollution markers (total Coli, *E. coli* and *Salmonella* spp.), biotoxins (DSP, PSP, ASP) and some heavy metals (Pb, Hg, Cd) were quantified.

HELMINTH PARASITES INFECTIONS OF ALIMENTARY CANAL OF SHEEP AND GOAT IN SHAHREKORD, IRAN

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The aim of present study was determination of funa in alimentary canal helminthes Parasite of sheep and goat at Slaughter house in shahrekord, Iran. In order to this purpose 60 digestive system from oesophagus to rectum were examined. The worms found in the washed contents of alimentary canal were collected, counted and identified under the microscope after cleaving in lactophenol. Abomasum, small and large intestine were only organs that were infected with 9 species of nematodes and 3 species of cestudes. The found helminthes in goat and sheep were as follow in abomasum: *Haemonchus contortus* (6.7% and 1.7%), *Marshallagia marshalli* (8.4% and 6.7%), *Ostertagi occidentalis* (21.7% and 16.7%), *O. circumcincta* (0 and 1.7%) and *O.trifurcata* (6.7% and 0), in small intestine: *Nematodirus oiratianus* (5% and 5%), *Moniezia benedeni* (0 and 3.4%), *M.expansa* (11.7% and 0) and *Avitellina centripunctata* (5% and 6.7%), in large intestine: *Oesophagostomum venulosum* (0 and 1.7%), *Trichuris ovis* (13.4% and 23.4%) and *Skrjabinema ovis* (3.4% and 0) respectively. Sever infection with *Ostertagia* spp. and *haemonchus* spp. caused chronic abomasitis with mucous hyperplasia, metaplasia, infiltration of mixed inflammatory cells especially lymphocytes, leukocytes and hyperplastic gastritis respectively. Mucoheamorrhagic typhlocolitis was diagnosed in infection with *Trichuris* spp. In other cases there were no noticeable pathologic change.

PROLIFERATION ACTIVITY IN CANINE LYMPHOMAS

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Forty five lymphomas, 14 of T cell origin, 28 of B cell and 3 with nonB nonT phenotype, were included in this study. Tumors were classified according to the updated Kiel classification adapted to canine lymphomas by Fournel-Flery et al. Proliferation activity was identified immunohistochemically. The percentage of Ki67+ cells and mitotic index (MI) was estimated in each specimen.

Most of lymphomas (39 of 45) had high proliferation activity. Among them 27 cases expressed 50-70% Ki67+ cells, the highest Ki67 expression (>70% Ki67+ cells) was identified less frequently, in 12 cases. Moderate Ki67 expression (30-50% positive cells) was observed in 5 cases, only one tumor had low Ki67 expression (<30% positive cells). Lower percentage of Ki67+ cells was usually accompanied with lower MI. The mean MI values in discussed groups differed significantly. Mean MI value was also significantly higher in T cell than in B cell lymphomas. There were also differences between mean MI values in the lymphomas of different morphological subtypes, but in some of them high variations in the range of MI values were identified and wide overlaps of MI between individual cases from different subtypes were observed. Because of differences in the proliferation activity in single cases of the same subtype of lymphoma, the proliferation activity assessment may be helpful to choose appropriate scheme of treatment and should be commonly performed during routine histopathological diagnosis of canine lymphomas.

CORRELATION OF TELOMERASE ACTIVITY, DNA PLOIDY AND MITOTIC INDEXES IN CHOSEN MALIGNANT SKIN NEOPLASMS IN DOGS

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Introduction. The characteristic trait of malignant cells is their ability for unlimited divisions. Normal somatic cells can divide a certain times and then they are subject to ageing. An important role in the regulation of this process play telomeres - DNA segments situated at the end of chromosomes. With each cell division the telomeres are shortened by 20 - 200 pairs of bases. Cells obtain the possibility of unlimited multiplication through the activation of telomerase enzyme. Telomerase is a reversed transcriptase using its own RNA matrix for the synthesis of telomeres and maintaining their constant length. Telomerase activity was noted in normal tissues which had constant ability for renewal and also in 85% of malignant neoplasms in man. There are few reports on telomerase activity in dogs especially in the neoplasms of mammary gland and skin. The aim of the work was the investigation of correlation between proliferative activity, DNA ploidy and the telomerase activity in the chosen neoplasms of skin in dogs.

Materials and Methods: The research material comprised skin neoplasms obtained surgically from dogs. Paraffin sections were stained with H-E, by the Feuglen's method and, when necessary, by additional methods.

Results: Seven mastocytomas, 6 squamous cell carcinomas, 3 liposarcomas and 2 basal cell tumors with the determined telomerase activity were examined. No correlation between the telomerase activity and mitotic index was observed in any of the neoplasms. The correlation between hyperploidy with telomerase was observed in the liposarcomas and basal cell tumors, however, there was no correlation between ploidy with telomerase activity in the mastocytomas and squamous cell carcinomas. The correlation between proliferative activity determined by the PCNA or Ki67 expression and the telomerase activity was noted in the mammary gland neoplasms in dogs. The research is continued.

CHRONIC RENAL FAILURE OR JUVENILE RENAL DISEASE? IN TWO SPITZ PUPPIES

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Introduction: Two male offsprings born from same pair of Spitz dogs with 2 months of age referred to the Veterinary Clinic of Islamic Azad University Karaj Campus.

Materials and methods: Clinical examination, haematobiochemical tests, abdominal radiography, necropsy and histopathological exam were done.

Results: Clinical examination revealed abdominal distention, poor body condition, lethargy, anorexia, vomiting, polydipsia and weight loss. In CBC and biochemical tests: anemia, azotemia, hyperphosphatemia, isosthenuria, hypoalbuminemia, and proteinuria were mentioned. Lateral radiography showed loss of intra-abdominal contrast.

Necropsy findings included: pale, firm and shrunken kidneys, patchy of renal surface, thin cortex, fibrotic medulla of both kidneys with numerous small cysts (Polycystic kidney). Histopathological exam showed renal hypoplasia, cystic glomerular atrophy, presence of large numbers of immature (fetal) glomeruli, dilated tubules, infiltration of mononuclear cells in interstitial tissue and cortical interstitium contained segmental areas of fibrosis.

Discussion: Hypoplastic kidneys appear as miniature replicas of normal kidneys composed of reduced numbers of histologically normal nephrons. Juvenile nephropathy has been documented in many breeds but apparently has not been reported in the Spitz breed so far. These cases demonstrate that Juvenile renal disease in Spitz dogs may develop as a familial renal disease because both of them were born from the same pair. In many dogs a familial tendency is demonstrated, but the mode of inheritance has been determined with certainty in only a few dog breeds.

HISTOPATHOLOGICAL STUDY OF THE EFFECTS OF ACTHAND GnRH ON SHEEP ADRENAL GLAND

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Histopathological effects of intramuscular Tetracosactide (synthetic ACTH) and Gonadorelin (GnRH) on adrenal cortex of sheep were studied. Morphohistopathological studies demonstrated that, Tetracosactide causes cortical hyperplasia specifically in two inner layers (zona fasciculata and zona reticularis), in which light cells were involved. Electron microscopic study of this hyperplastic light cells, demonstrated the increase in the cytoplasmic organelles. However, it was revealed that, Gonadorelin, in turn, affects the dark cells of these layers and cause their abundance characteristically. Ultrastructural studies, confirmed this involvement via demonstrating the elevation of cytoplasmic organelles of these cells. Statistically analysis (T²- test) showed a significant thickening of the adrenal cortex after hormone administration, (P<0.01). The results suggest that the difference between light and dark cells in sheep, like cattle, is in their cytoplasmic organelles and is not in their cytoplasmic lipid contents. Thus, these cells unlike humans and dogs have not cyclic rotation, in resting and active stages. In conclusion the investigated cells are different not only in their structures but also in their functions. The function of light cells is related to production and secretion of glucocorticoids, while the function of dark cells is related to production and secretion of sex hormones.

Key words: *Adrenal gland, Sheep, ACTH, GnRH*

CHOLANGIOCELLULAR CARCINOMA IN A SHEEP: CASE REPORT

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Cholangiocellular carcinomas are malignant neoplasms of biliary epithelium that usually arise from the intrahepatic ducts. These neoplasms occur in all species. A large single mass or multiple nodules may be present within the liver. These neoplasms typically are firm, raised, often with a central depression, pale gray to tan, and unencapsulated. Microscopically, the tumors consist of acini lined by columnar cells. In some places cells line cyst-like spaces which may be filled with solid masses of neoplastic cells. Metastasis to extrahepatic sites is common.

On March 2005, a five-year-old ewe with clinical signs of unthriftiness, lethargy, cachexia, variable appetite, anemia and ascite along with unsuccessful previous treatment, was presented to the veterinary clinic of Tabriz, Islamic Azad University. Its total weight was 38 kg; temperature was not clinically abnormal, but heart rate and respiratory rate were slightly increased. The affected ewe was subjected to laparoscopy for exploratory diagnosis. In laparoscopy the liver was obviously tumoral. The case was presented to euthanasia and post mortem examination. At necropsy multiple firm, often umbilicated white nodules were scattered throughout the liver. Infrequently masses of coalescing small nodules were encountered. Inspection of other tissue and organs for detection of a possible metastasis was negative. In order to make a precise diagnosis, tissue samples were stained with H&E. On histologic examination, we saw characteristically, well-differentiated carcinoma in which neoplastic cells had been organized into a tubular or acinar arrangement and retained a resemblance to biliary epithelium. However, some acinar arrangement was detected among solid masses of neoplastic cells. Pathologic gross and microscopic features of the tumor were described and cholangiocellular carcinoma was diagnosed.

ACCIDENTAL SELF-INOCULATION WITH MYCOBACTERIUM PARATUBERCULOSIS VACCINE (ATTENUATED BACTERIA OF JOHNE'S DISEASE) BY A VETERINARIAN IN TEHRAN

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Introduction: Since 10 years ago some veterinarians in Iran have used Mycobacterium paratuberculosis vaccine to control paratuberculosis (Johne's disease) in cattle. The vaccine which has been used was live attenuated M.paratuberculosis bacteria.

Materials and methods: This is a case report of a veterinarian that became infected with a needle-stick exposure of live attenuated vaccine of M.paratuberculosis and location of inoculation was left thumb. Gradually his finger became inflamed and painful and started discharging exudates. The lesion did not respond well to common antibiotics, but oral administration of isoniazide and rifampin for approximately 2 months were effective. For accurate diagnosis, needle biopsy was prepared from infected tissue and then fixed in 10% buffered formalin solution. After processing, the tissue samples were sectioned with 7 micron thickness and stained with hematoxylin and eosin (HE), and special staining for acid-fast bacteria (Zeihl-Neelsen staining).

Results: Grossly tissue mass was gray-white, firm and widely attached to dermis and bone. Microscopic findings included tuberculoid granulomatous inflammation without any caseous necrosis. Inflammatory infiltrates into dermis were macrophages, lymphocytes, plasma cells and Langhans giant cells. The granuloma was surrounded by a connective tissue. No acid-fast bacteria in macrophages, giant cells and other inflammatory cells were seen in special staining (Zeihl-Neelsen).

Conclusion: Because this man was vaccinated against tuberculosis many years ago and had a cell mediated immunity against Mycobacterial organisms, he had a good resistance to similar organisms such as M.paratuberculosis. Therefore, just tuberculoid reaction was seen microscopically and lepromatous reaction was absent. This case revealed that human can be infected with M.paratuberculosis vaccine (bacterin or attenuated). For this reason during vaccination of animals healthy management programs must be considered.

SALIVARY DUCT CARCINOMA OF THE MANDIBULAR GLAND IN A BARDIGIANO PONY

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Salivary gland tumors are uncommon in horses, and almost exclusively represented by sporadic cases listed in equine tumor reviews.

A monolateral swelling in the right mandibular area in a fourteen-year-old intact male Bardigiano pony was sampled for cytology, and a presumptive diagnosis of low-grade carcinoma was made. A 14 cm roundish subcutaneous mass, firm and well-circumscribed, was surgically removed and identified as the rostral portion of the mandibular gland; cut section showed an encapsulated whitish-yellow glandular-like tissue, composed by multiple irregular lobules separated by fibrous stroma. Histopathology revealed nests and islands of moderately pleomorphic epithelial cells frequently arranged around small duct-like structures, and lined by myoepithelial cells. Several inflammatory cells (neutrophils) and large plump mononuclear cells with eosinophilic intracytoplasmic globules were frequently observed in interstitial and luminal spaces.

Tumor cells stained positively for cytokeratins AE1/AE3, 5/6, 14 and negatively for cytokeratin 19, vimentin, MAC-387, S-100, NSE, sm-actin, desmin and laminin; globular material within the plump cells was strongly PAS+ (with and without diastase), PTAH+, NSE+, vimentin+, and negatively to cytokeratins, MAC-387, S-100, actin, desmin, and laminin. These findings, along with transmission electron microscope observations, suggested the diagnosis of salivary gland carcinoma arising from the nonkeratinized squamous epithelium of the terminal portion of the excretory duct.

ULTRASTRUCTURAL STUDY OF PANCREAS IN MICE INFECTED BY FOOT AND MOUTH DISEASE VIRUS

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Foot-and-mouth disease (FMD) is a severe, clinically acute, vesicular disease of cloven-hoofed animals including domesticated ruminants and pigs and more than 70 wildlife species. The aetiological agent, foot-and-mouth disease virus (FMDV), is classified within the Aphthovirus genus as a member of the Picornaviridae family. Seven distinct serotypes of FMDV, with indistinguishable clinical effects, have been defined, namely types O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1 mountain gazelles. Synchronous diabetes mellitus and FMDV in cattle and mountain gazelle is reported.

This study was performed to obtain FMD virus effects on pancreas of infectious animals. For this purpose, 14 male mice Balb/Cj and 14 female mice Balb/Cj in two groups were selected. In each group, 10 mice were as experimental animals and the rest were control animals. Twelve of each group (10 mice of experimental group and 2 mice of control group) were treated by cyclophosphamide (an immunosuppressive drug). Ten mice of experimental group were inoculated by FMD virus strain O1 intraperitoneally. The serum glucose measured in all mice before and after virus inoculation up to 10 days. After 10 days, necropsy was carried out on all mice. Two samples of each pancreas were taken and referred to pathological laboratory for light and electron microscopic studies. The results showed that in male group, there are significant differences among glucose values of second day to before inoculation and seventh day to ninth day, and tenth day respectively. In female group, there are significant differences in glucose values of first day to before inoculation, first day to second and third day of inoculation and finally second day to third, fifth and eighth day of inoculation ($P < 0.05$).

Histopathological investigation on langerhans and exocrine region showed cellular vacuolation, apoptosis in beta cells and acinar cells and degranulation of beta cells. It is concluded that FMD virus is able to produce direct damage of beta cells, through of apoptosis.

A MIXED EPITHELIAL AND STROMAL TUMOR OF THE KIDNEY IN A RINGTAIL LEMUR (*LEMUR CATTALINA* SP.)

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Primary renal tumors are rarely reported neoplasms in nonhuman primates. We describe here a mixed epithelial and stromal tumor of the kidney in a 14.5-year-old female Ringtail Lemur (*Lemur catta* sp.). The well demarcated, solid and cystic mass of 2 cm in diameter was located in the pelvis of the left kidney. Histologically, it consisted of both epithelial and mesenchymal components. The neoplastic mesenchymal cells were arranged in a solid pattern around cysts lined by a well differentiated epithelium. Neither the mesenchymal nor the epithelial parts showed significant nuclear atypia. Mitotic figures were not observed. Immunohistochemically the spindle cells were positive for vimentin and actin; the epithelial cells for cytokeratins 14 and 22. To our knowledge only one related case (designated at the time as adenoleiomyofibromatous hamartoma) has been reported in a female Ringtail Lemur of a similar age. In human pathology this tumour is considered to be benign and affects predominantly perimenopausal women. This tumour could moreover express oestrogen and progesterone receptors. However, neither oestrogen nor progesterone receptors could be identified in the kidneys of the present Ringtail Lemur so that a hormonal mechanism of pathogenesis could not be demonstrated in this case.

A CASE OF FIBROSARCOMA WITH TYPICAL FEATURES OF POST-INJECTION SARCOMA IN SITE OF MICROCHIP IMPLANT IN A DOG

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A case of fibrosarcoma developed in a dog at the site of microchip implant is reported. A 9-year-old, male, French bulldog was examined for a subcutaneous mass, located at the site of microchip implant. The cytological examination of the mass highlighted a single population of large spindle cells in swirling bundles, suggestive of a malignant mesenchymal neoplasm. The mass was surgically removed with the microchip that was attached. Histologically, the mass was confirmed as a high grade infiltrative fibrosarcoma, with multifocal necrosis and peripheral lymphoid aggregates. By immunohistochemistry, the sample was investigated for vimentin (V9), smooth muscle actin (SMA), CD3, CD79 and CD18. All the neoplastic cells were positive for vimentin. Furthermore, scattered cells at the periphery of the lesion were also positive for SMA, highlighting a myofibroblastic phenotype. The lymphoid cells were positive for both CD18 and CD3. Based on histology and immunohistochemistry a diagnosis of fibrosarcoma with typical features of post-injection sarcomas was made. Feline post-vaccinal sarcoma is a well known pathological entity. Recently, fibrosarcomas at site of injections have also been reported in dogs and ferrets. Furthermore, neoplastic growth in site of microchip implant in dog and in laboratory mice and rats has been described.

POLYSTOTIC LYPHOMA IN A YOUNG DOG: CLINICAL AND PATHOLOGICAL FINDINGS

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An unusual clinical presentation of a primary bone lymphoma in a dog is reported. A twenty-month-old intact female Golden Retriever was presented for progressive paraparesis and dysorexia. By clinical examination, dyspnoea and prescapular lymphadenopathy were observed. Complete blood count and serum biochemistry profile highlighted pancytopenia and hypercalcemia, respectively. Radiographically and by computed tomography, ventral fusion of the lumbar and thoracic vertebrae by new bony tissue deposition was evident. By fine needle aspiration, neoplastic lymphoid cells were observed in lymph nodes and bone marrow. The dog was euthanized at the owner's request, due to the poor prognosis. Histologically, vertebral and esophageal lesions, bone, liver, bone marrow, kidney and lymph nodes were diffusely infiltrated by neoplastic, monomorphic lymphoid cells, with scant cytoplasm and round hyperchromatic nuclei. A diagnosis of polyostotic lymphoma with spondylosis was made.

Lymphoma primary affecting bone is an uncommon diagnosis in the dog. Polyostotic lymphoma with osteolytic lesions affecting multiple bones has been described in young dogs as well as in children. However, the clinical presentation of this case has never been described before.

RENAL DYSPLASIA IN SLAUGHTERED ADULT JAPANESE BLACK CATTLE

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Claudin-16 (CL-16) deficiency is hereditary disorder characterized by renal dysfunction in the Japanese black cattle. Kidneys from 13 cases with renal dysplasia in slaughtered Japanese black cattle with or without *CL-16* deficiency were examined.

Seven cases were diagnosed as homozygote for *CL-16* deficiency, two were heterozygote and 4 cases were normal by genotypic examination. Seven cases showed growth retardation and emaciation but 4 cases showed no significant clinical signs irrelevant to each gene type. Histopathologically, streaky-focal lesions of dysplasia were scattered from the cortex to outer zone of medulla. The area of dysplastic lesions was varied among the individual cases. Dysplastic changes were consisted of small immature renal tubules, small-sized and irregularly-shaped tubules with thickening of basement membrane, increased interstitial tissue with high nuclear density of mesenchymal cells, lymphocytic infiltration in interstitium, small-sized immature glomeruli and decreased number of glomeruli *etc*. The degree and extent of these changes were varied in each case and they were not related to the genotype of *CL-16* deficiency. However, there were obviously larger number of renal tubules with thickened basement membrane in 4 cases of 6 homozygotic animals for *CL-16* deficiency and the small immature tubules was apparently decreased in these cases.

ESCHERICHIA COLI O157:H7 IN CATTLE: TISSUE TROPISM AND PATHOLOGICAL RESPONSES

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Abstract

Cattle are considered to be the primary reservoir host for the human enteric pathogen *E. coli* O157:H7. Infection may be transmitted to man thorough contaminated food, water or by direct animal contact. Therefore, to protect human health any potential control measures have to eliminate *E. coli* O157:H7 from the food chain and the environment and must reduce the prevalence of the organism in cattle.

This study examined the tissue tropism of *E. coli* O157:H7 at necropsy in 21 experimentally infected calves. Traditionally, infection in the bovine host is clinically inapparent and *E. coli* O157:H7 is considered an innocuous commensal.

In this work we present evidence of mucosal attachment and pathological changes in rectal tissue detected by histochemistry, immunohistochemistry and electron microscopy.

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EXPRESSION OF DIFFERENT NITRIC OXIDE SYNTHASE ISOFORMS IN PIGS INFECTED WITH AUJESZKY'S DISEASE VIRUS.

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The infection with neurotropic viruses is one of the stimuli that induce the expression of nitric oxide synthase (NOS) in the central nervous system. However, the role of nitric oxide (NO) generated by this enzyme is controversial as NO has been demonstrated to be both neuroprotective and neurotoxic. In this study we investigated the expression of both constitutive and inducible isoforms of NOS in brains of pigs infected with Aujeszky's disease virus (ADV). To address this issue we used brain stem from pigs infected intranasally with ADV, and euthanized at 12, 24, 48, and 72 h postinoculation (hpi). The NOS isoforms were identified with anti-inducible NOS (iNOS), anti-universal NOS (uNOS), and anti-endothelial NOS (eNOS) antibodies. The eNOS was localized in endothelial cells at all postinoculation times studied, but also in some neurons at both 48 and 72 hpi. The iNOS was expressed among infiltrating immune cells and neurons; they were present in higher numbers as the time postinoculation increased. A significant increase of uNOS expression was mainly observed in neurons at higher postinoculation times. These results indicate that a direct correlation exists between the extent of the infection and the appearance of lesions with the expression of different NOS isoforms in the brain stem of pigs infected with ADV.

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ROSENTHAL FIBRE ENCEPHALOPATHY IN A DOG RESEMBLING ALEXANDER'S DISEASE IN HOMANS

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A 4-month-old male Bernese mountain dog presented with a three-week history of progressive tetraparesis, tremors particularly of the hind limbs and depressed mentation. The dog had previously been treated with corticosteroids, but the condition deteriorated rapidly. At the request of the owner, the dog was euthanatized and a complete necropsy was performed. On gross examination of the brain there was a moderate enlargement of the lateral ventricles. Histological changes consisted of a marked proliferation of astrocytes with abnormally large cell bodies, and of numerous eosinophilic, round or elongated bodies distributed throughout the central nervous system, but most pronounced in the brain stem and midbrain. These deposits were identified as Rosenthal fibres, as they were strongly immunoreactive with antibodies to GFAP and ubiquitin, but not to vimentin; at the ultrastructural level, they consisted of electron-dense amorphous masses located within the processes of astrocytes, most frequently in the perivascular astrocyte feet, and they were tightly associated with dense aggregates of glial filaments. The histological and immunohistochemical findings of this canine case were consistent with the published neuropathological descriptions of Alexander's disease in human beings, a rare condition caused by dominant mutations in the GFAP gene.

UROLITHIASIS IN TWO SWEDISH HORSES - CASE REPORT

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Introduction: The presence of calcium carbonate crystals in equine urine is regarded as normal. Considering this, it is surprising that calcium carbonate urolithiasis is rare in horse. Here we report on two cases.

Material and Methods: Two horses, a 12-year-old thoroughbred and an 18-month-old Swedish warmblood, both geldings were submitted to necropsy and further examinations. The thoroughbred had previously a history of polydipsia, polyuria, loss of appetite and weight loss. The Swedish warmblood was found dead with a short history of polydipsia and slight depression.

Results: Gross pathology revealed severe distension and thickening of renal pelvis and ureters bilaterally, containing high number of calculi in various sizes and semi-solid sedimented sludge in both horses.

No obstruction was observed in the thoroughbred but a bilateral obstructive ureterolithiasis was found in the Swedish warmblood. In the Swedish warmblood the kidneys were irregular and firm with chronic interstitial nephritis. In the thoroughbred the right kidney was replaced by a thick walled cyst with no demonstrable kidney tissue and the left was enlarged with a dilated pelvis and a chronic interstitial nephritis.

The chemical analysis showed calcium carbonate as major component of the calculi in both horses.

Since the aetiology is unknown, studies are in progress to elucidate the aetiology of these cases.

EFFECT OF ALLIUM SATIVUM INGESTION ON BLOOD AND LIVER LEAD CONCENTRATIONS AND HISTOPATHOLOGICAL FINDINGS IN SUBCLINICALLY LEAD POISONED GOATS

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The therapeutic potential of *Allium sativum* (garlic) was evaluated in experimental subclinical lead poisoning in goats. Eight goats divided into two groups were used. Goats of group A received oral doses of 80 mg/kg body weight lead acetate for 5 days. Goats of group B received concurrent lead acetate orally at the rate of 80 mg/kg body weight and dried garlic powder orally at the rate of 45 gm daily/animal for 5 days. At the end of the experiment, goats were slaughtered. Mean blood and liver lead concentrations in goats of group A were significantly ($p < 0.05$) higher than the mean blood and liver lead concentrations in goats of group B. Acid-fast intranuclear inclusion bodies were found in the hepatic cord cells and in the cells of the proximal convoluted tubules of the kidney of the goats of group A but were not found in the liver and kidney of the goats of group B.

BIOCHEMICAL STUDIES ON NATURAL THEILERIA ANNULATA INFECTION IN CROSSBRED CATTLE

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The biochemical parameters were studied in adult crossbred cattle naturally infected with *Theileria annulata* in the Kazeroon city of Fars Province, Iran. Twenty clinical cases of tropical theileriosis were studied, together with 20 clinically healthy crossbred cattle. Cattle clinically infected with *T. annulata* had significantly ($p < 0.05$) lower serum magnesium, copper, iron and zinc concentrations and significantly ($p < 0.05$) higher serum Ast, Alt and Alp activities than the healthy cattle.

ACUTE TOXOPLASMOSIS IN KOREAN SQUIRRELS (*TANIAS SIBERICUS*).

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A group of four korean squirrels (*Tanias sibericus*) were sent to the Faculty of Veterinary Medicine at Cordoba University, due to the animals had dead two days after arriving to a pets shop, developing nervous symptoms (hiperexcitability mainly) and dyspnea.

After the necropsy, which only made evident pulmonary congestion and splenomegaly, samples of all the organs were taken, and they were placed in 10% neutral buffered formalin and processed routinely for the histopathologic tests.

The histopathologic study developed a non purulent encephalitis, an interstitial pneumonia and a necrotic hepatitis, and also evidence of parasite cysts. Additionally, in one of the animals was observed in the hepatic parenchyma nematodes and bioperculated eggs, reason for which was diagnosed as *Capillaria hepatica*.

For the identification of the parasitic cysts responsible of the process, the samples were refined in osmium tetroxide and processed for the ultrastructural study, which identified the parasite structures as cysts and taquizoites of *Toxoplasma gondii*, diagnosis that was confirmed by an immunohistochemical study.

The severity of the clinical sings of the animals lead us to the suspicion that the infection of the squirrels was caused for the pollution of the water with oocysts from a feline in quarantine.

ANAURYSM OF VENTRAL CAVA VEIN IN BORDEAUX DOGGE

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A two year-old Bordeaux dogge developed a sudden spontaneous hind limb paralysis. Radiographic and MRI scanning studies showed a spontaneous fracture of the vertebral body of T10, which also displayed an incomplete development as a hemivertebrae. Compression and severe injury of the spinal cord was diagnosed and the animal was euthanized.

At necropsy, the radiological findings were confirmed, and a fusiform dilatation, 10 cm long x 4-5 cm in diameter, of the ventral cava vein was observed. The wall of the vessel, at this level, showed a marked thickening, firmness and rough luminal surface. Histopathological examination showed that the increased thickness of the wall was due to infiltration of dense connective tissue, being the longitudinal and transversal elastic fibres fragmented, disrupted and grouped in irregular bundles. The existence of a similar dense connective tissue infiltrate in other anatomical regions is suggestive of a possible disorder of connective tissue of congenital or hereditary origin.

PARASITIC GASTRITIS BY *SIMONDSIA PARADOXA* IN WILD BOAR.

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A 30 kg wild boar was sent to the Faculty of Veterinary Medicine at Cordoba University, due to the existence of enteric problems together to, sometimes, dyspnea in a farm from the South of Spain.

In the necropsy we observed a pneumonic process, hemorrhages in linfonodes and in the renal cortex, hyperemic splenomegaly, local difteroid enteritis and an enlargement of the mucosa of the fundus of the stomach with evidence in the surface of nematodes. The cut of the gastric mucosa, showed multiple nodes with 6-8 mm of diametre and necrotic centre. Samples of all the organs were processed for the histopathologic and microbiologic studies.

The case was diagnosed as a parasite gastritis by nematodes in wich the female was inside the mucosa, with a dilatation of the posterior region and developing an extensive necrotic area, rounded for an intense inflammatory reaction, being able to affect the muscle layer. In the other organs, there were lesions compatibles with a salmonelosis infection, that was later confirmed by the microbiologic study, being identified the agent as *Salmonella cholerasuis*. The morfologic characteristics and its placement in the gastric mucosa, allowed us to identify the parasite as *Simondsia paradoxa*.

AMPHIBIOCYSTIDIUM LIKE PROTOZOANS CAUSING GENERALIZED INFECTION IN A LABORATORY COLONY OF ALPINE NEWTS

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Amphibiocystidium sp. is a recently created genus of the class Mesomycetozoa that includes known pathogens such as *Rhinosporidium* sp. and *Ichthyophonus* sp. It has been described in frogs and newts causing cystic skin lesions but rarely spreading to internal organs and causing serious illness. Therefore *Amphibiocystidium* sp. is considered to be of low pathogenicity. We report herein the identification of an *Amphibiocystidium* like protozoan associated with generalized disease and mortality in a laboratory colony of alpine newts (*Triturus alpestris*). The severity and distribution of lesions were unusual compared to the lesions that have been previously attributed to *Amphibiocystidium* sp. Histopathological examination of six individuals revealed lesions with numerous protozoans affecting the skin, skeletal muscle, liver, and spleen. Skin lesions consisted of cutaneous hyperplasia and mild hyperkeratosis with ballooning degeneration and necrosis of keratinocytes in association with intracellular protozoans. Tissue necrosis and histiocytic infiltrates with intracellular protozoans were present in the skeletal muscle, liver and spleen. Furthermore, capillaries in various organs were plugged with macrophages containing intracellular protozoa.

**APPLICATION OF PROSTATE ANALYSIS METHODS
(IMMUNO- AND HISTOCHEMISTRY) ON BARTOLINI'S GLANDS
FROM MEET BOVINE TO CONTROL ILLEGAL TREATMENT**

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In Italy, exogenous sex hormones alone or mixed with other xenobiotic substances are illegal, but widely used for anabolic purposes during the fattening period. Female meet bovine are generally rare in practice and difficult to evaluate due to the physiological cycling presence of endogenous estrogenic hormones. The aim of this work is to apply the same analytical methods used to evaluate treatment lesions in prostate, to identify in female rather than in male indirect markers of illegal administration of sex hormones. The animal were derived from practice and consisted of 70 female meet bovine of variable age. On the samples, different staining (HE, Alcyan Blu PAS), lectin histochemistry (BSI-B4 and SBA) and cytokeratin immunohistochemistry were carried out. Here the results are reported with comparison of the different methods.

“NEW” LESIONS ON THE PROSTATE OF MEET BOVINE IN ITALY

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In Italy, exogenous sex hormones alone or mixed with other xenobiotic substances are illegal, but widely used for anabolic purposes during the fattening period. In the last more than five years, several research groups are working in Italy on identify indirect markers of illegal administration of sex hormones. After several experiments done and with the experience derived from the control of practice, several lesions, alone ore mixed together, are now thought to be directly correlated to administration of exogenous substances. However, in the last year, different lesions and particularly different mixed features are seen on the histological slices. In this work, "new" lesions and picture are presents in HE staining, differential staining and lectins histochemistry and compared with the "classical" well-know lesion.

ADRENAL CORTICAL MICROSCOPIC LESIONS IN DOGS WITH KIDNEYS' DISEASES

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Introduction: The aim of this work was to find out the correlation between the degree in kidneys' and adrenals' pathological changes. There are few data concerning the problem of the reactive cellular changes in the adrenal cortex caused by the progressing functional disturbances in the dog kidneys.

Material and methods: Fifty dogs, 5 ages' groups from 3 months to 22 years old, of both sexes were examined post mortem using pathomorphological methods as follow: HE, AB/PAS reaction, silver impregnation method according to Grimelius, van Gieson and von Kossa and immunocytochemical methods for vimentin and antichromogranin A

Results: The results of the research revealed the progression in the proliferation of zona glomerulosa cells with adenoma formation, as well as in zona fasciculata cells. In the few cases the proliferation/neoplasia in the adrenal medulla was observed.

The pathological changes in kidneys were increasing along with the dogs' age. The glomerular lesions were dominated, and were followed by tubular cells alteration. The changes observed in the expression of vimentin were significant for the degree in kidneys' alteration. In the advanced disease cases the decrease of vimentin expression in glomeruli and kidneys' blood vessels' walls were noted, with the simultaneous appearing of vimentin expression in tubular cells.

Conclusion: Results of this work indicated without any doubt, that there is the close correlation in the degree of the kidneys and adrenal pathological changes. Adrenals changes may be interpreted, as the compensatory reaction to kidneys' functional disturbances. Moreover, the very remarkable were changes in kidneys' vimentin expression observed in the presented work.

DEVELOPMENTAL FAILURES IN EUROPEAN BISON (*BISON BONASUS*) FROM BIALOWIEŻA PRIMEVAL FOREST

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Introduction: At the beginning of the XXth century the restitution of the European bison population in Poland was performed. The bison of the Lowland line originated from the 7 parents. The contemporary Białowieża bison's line possesses 5 ancestors' genomes only. The high inbred index characterizing this animal species promotes the appearance of genetic and inherited developmental failures. The increase of genetic variability in the contemporary population is observed. It is the result of the "bottleneck" in the number of animals in the population.

Material and methods: The post mortem macroscopic examination of European bison culled during 1993-2004 years in Białowieża Primeval Forest were performed. The animals were of both sexes, from 3 months to 24 years of age.

Results: The post mortem examination revealed the following anatomopathological changes: epididymal cysts (60,3%), kidney cysts in bison up to 1 year old (10,4%), cryptorchism (1,3%), developmental failures in the gall bladder (6,9%), lack of the one uterus horn (3,3%), the septum in the vagina (3,3%), kidney hypoplasia (0,2%) and dermal cyst (0,2%). The very interesting findings were the presence of the so-called males' uterus in the majority of examined males observed.

Conclusion: The developmental failures observed in European bison are numerous compared to other mammals. These changes are especially often found in the epididymis and the gall bladder.

EOSINOPHILIC POLYPOID CYSTITIS WITH SARCOMATOUS PROLIFERATION IN A DOG

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Eosinophilic polypoid cystitis is a rare disease characterized by development of polypoid mass with prominent infiltration of eosinophils and thought to be a variant of polypoid cystitis. The lesions were nonneoplastic, but differential diagnosis to true neoplasm should be needed in the case with abundant mesenchymal component.

A 5-year-old, spayed, female Shihtzu-dog was presented for hematuria and was detected two small polypoid masses in urinary bladder. The polypoid masses, 25X15X10 and 12X12X10 mm in size, protruded into the lumen. Histologically, the masses were located in mucosal or submucosa layer without invasion to the muscular layer. They were composed of fibrous tissue covered by slightly hyperplastic epithelium with partially ulceration. Inflammatory cells, predominantly eosinophils, were infiltrated among the fibrous tissue. Fibrous tissue of one polyp consisted of spindle, round and pleomorphic cells with single or multiple atypical nuclei and abundant cytoplasm. These atypical cells were confirmed as fibroblasts, SMA-positive myofibroblasts and Mac387-positive macrophages. Mitotic figure was rarely seen, and MIB-1 index was 1%.

These findings suggested that these masses did not have malignant character despite of morphological feature, because they consisted of wide variety of cells and low growth activity without any invasive potential.

IMPORTANCE OF BOVINE TUBERCULOSIS IN BURDUR IN SOUTHERN TURKEY: EPIDEMIOLOGICAL, PATHOLOGICAL AND ECONOMICAL STUDY

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Burdur is the most important dairy cattle breeding and milk production area of Turkey and Burdur Slaughterhouse is one of the most biggest slaughterhouse in southern part of the country. No scientific information is available on the prevalence of tuberculosis (TB) in cattle in Burdur. A survey was, therefore, undertaken to determine the prevalence of generalized TB infection in cattle in this distinct. The objective of this study is to implement a disease monitoring system in Burdur Slaughterhouse to estimate annually prevalence of generalized TB in cattle slaughtered and inspected in 2003-2004. Prevalence of bovine TB was investigated in Burdur slaughterhouse from January 2003 to December 2004. All of the affected animals were Holstein breed and the prevalence of the generalized TB in all slaughtered cattle was 0.38 % in this period. Economical value of the condemned meat and organs were calculated for establishment of economical losses. Although to the strict eradication program, bovine TB still important health issue among cattle and remains one of the greatest threats to cattle and human health in the southern Turkey. Veterinarians and cattle producers in this region are encouraged to develop and work on herd plans aimed at controlling and eradicating TB.

OBSERVATIONS OF ASPERGILLOSIS IN THE BRAINS OF TURKEY POULTS USING DIFFERENT HISTOPATHOLOGICAL STAINING TECHNIQUES

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Different staining methods were evaluated for studying aspergillosis of the central nervous system (CNS). The pathological changes and fungal elements in cerebrum and cerebellum of 17 turkey poults with aspergillosis were examined and described. Tissue sections were stained with hematoxylin-eosin (HE), Kluver-Barrera's and Grocott's methods, and periodic acid-Schiff (PAS). Focal granulomatous reactions with central necrosis were observed in the HE stained slides. Fungal hyphae were easily demonstrated using Grocott's method and PAS. These two methods, however, were not suitable for describing detailed histopathological changes. The Kluver-Barrera method was used to demonstrate the neural tissue reaction. Neurons were found to be sensitive to aspergillosis, in contrast to glial cells that showed fewer pathological changes. The fungal elements were clearly visible with the Kluver-Barrera method, resulting in better information about the interactions of neural tissue, the inflammatory response, and the fungus. The use of the Kluver-Barrera method for this purpose has not been documented previously.

**ABNORMALITIES OF THE UBIQUITIN-PROTEASOME SYSTEM (UPS)
IN SPORADIC INCLUSION-BODY MYOSITIS (S-IBM) MUSCLE FIBERS:
A HUMAN MODEL OF AN UNRECOGNIZED AMBULATION DISORDER OF
ELDERLY CANINES AND FELINES?**

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Elderly dogs and cats often have difficulties walking and the causes are various. Probably existing in veterinary medicine but not yet diagnosed is the equivalent of human muscle disease s-IBM, which has interesting morphologic parallels to Alzheimer disease brain. s-IBM, the most common progressive muscle disease of older persons leads to severe disability, and is of unknown etiology. Light-microscopic features of s-IBM muscle biopsies include: 1) vacuolated muscle fibers; 2) various degrees of mononuclear-cell inflammation; and 3) accumulation of intra-muscle-fiber multi-protein aggregates, indicating ineffective protein degradation and misfolding (in Askanas and Engel, 2003, 2005). Several of the accumulated proteins, including amyloid- β , phosphorylated tau and α -synuclein, have a propensity to unfold. E3 UB-ligases, play an important role in preparing proteins for proteasomal degradation by transferring UB to their targeted proteins. The current study was to investigate whether E3 ligase parkin, participates in the ubiquitin-proteasome system (UPS) in s-IBM muscle fibers. We studied parkin by immunofluorescence and immunoblots using three well-characterized polyclonal and monoclonal antibodies in 10 s-IBM muscle biopsies, and 25 control normal and diseased muscle biopsies. By immunoblots, parkin's expression was significantly increased in s-IBM as compared to controls. By immunofluorescence, aggregates of strong parkin-immunoreactivity, co-localizing with α -synuclein were present only in IBM fibers. These novel studies 1) indicate that parkin plays a role in the UPS function in human muscle, and 2) provide more evidence of UPS abnormalities in s-IBM. In aged dogs and cats having difficulty walking, it would be interesting to study whether similar abnormalities occur.

POLYMYOSITIS IN CANINE LEISHMANIASIS: CHARACTERIZATION OF CELLULAR INFILTRATES AND EXPRESSION OF MHC CLASS I AND II ON MUSCLE FIBERS

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Polymyositis (PM) is an autoimmune inflammatory muscle disease of unknown cause. PM occur in dogs in several infectious disease including *Toxoplasma gondii*, *Neospora caninum*, *Ehrlichia canis* and *Hepatozoon canis*. However muscle disease during Leishmania infections have been documented less well. So we have investigated distribution and types of cellular infiltrates and the expression of MHC class I and II on muscle fibers in 15 dogs with a parasitologically and serologically established diagnosis of leishmaniosis. Biopsy samples from the biceps femoris were examined with routine stains and immunohistochemical analyses using monoclonal antibodies against canine leucocytes antigens, IGg and MHC class I and II. The presence and the amount of leishmania DNA in the muscle tissues was also evaluated by a PCR analysis.

The pathological features of the muscle were characterized by necrosis, regeneration, fibrosis and infiltration of mononuclear inflammatory cells consisting of lymphocytes, plasma cells and histiocytes. The phenotype of cellular infiltrations was immunohistochemically defined and a score of immunoreactivity was established. More than 50% of muscle fibers showed MHC class I expression on the sarcolemma. Less percentage of muscle fibers were positive for MHC class II. The mechanism of induction of class I and II antigen expression in inflammatory myopathy is not known yet. They can be considered like a target for cytotoxic T cells and may thus have a role in muscle fibre damage in inflammatory myopathies. Our results indicate that in PM during leishmaniosis muscle fibers synthesize and express HLA-DR molecules and may contribute to the inflammatory responses together with lymphocytes.

PLACENTAL ABNORMALITIES IN OVINE SOMATIC CELL CLONES: A LIGHT AND ELECTRON MICROSCOPIC INVESTIGATION

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The aim of the study is to provide, for the first time in literature, a morphological description of the ovine placental abnormalities after somatic cell nuclear transfer.

Three cloned lambs were delivered stillborn at term and placental degeneration was observed. Tissue samples from cloned and control ovine placentae were collected and processed for histopathological, immunohistochemical (Factor VIII, vimentin, desmin, alpha-SMA, calponin) and TEM examination.

Histologically, in the cloned placentae were observed: reduced villous vascularization, flat or cuboidal trophoblastic cells or the absence of epithelial layer covering the villous surface, lack of binucleate cells. The immunohistochemical examination revealed a reduced or absent expression of factor VIII in endothelial cells and calponin in vessels wall. At the ultrastructural level, thickening and lamination of trophoblastic basement membrane were noted, associated with the deposition of collagen fibrils. Moreover, the number of duplications around some of the capillary basal lamina was found to be increased.

These results strongly suggest that a downregulation of maternofetal exchanges and an abnormal regulation of differentiation and growth of trophoblastic cells could be occurred, both of which responsible of death and immaturity of the epithelial layer. This immaturity seems to involve also the vascular endothelial and smooth muscle cells.

IMMUNOHISTOCHEMISTRY AND TRAP-ELISA COMPARATIVE EVALUATION OF TELOMERASE IN FELINE MAMMARY TISSUES

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Telomerase represents a marker for neoplastic tissues, employed as a diagnostic and prognostic tool for use in clinical medicine and in planning new therapies. The aim was to detect telomerase in 13 mammary samples and 2 positive control testes comparing two different methods: immunohistochemistry (to assess the presence of the catalytic subunit by anti-h-TERT MoAb, clone 44F12, Novocastra, diluted 1:50) and TRAP ELISA (a PCR based protocol which utilizes a colorimetric detection method). With TRAP, a cut off limit of 0.2 ΔA was considered discriminant between the 6 negative and 9 positive cases. Immunohistochemistry revealed a specific reaction mainly in nucleoli, at a lesser extent in nuclei and rarely in the cytoplasm of epithelial cells. The stroma showed nucleolar positivity only in two cases. Cut off between positive and negative cases was considered 20% of positive epithelial cells. Pearson correlation between TRAP and immunohistochemistry was significant only when nucleolar+nuclear (9 positive and 6 negative) stain was compared ($R=0.69$, $P=0.009$), and not when cytoplasmic stain was considered alone ($R=0.31$, $P=0.3$) or together with nucleolar+nuclear ($R=0.53$, $P=0.59$) stain. On the basis of these results only immunohistochemical nucleolar plus nuclear stain correlates with the results of TRAP allowing the former to be a method alternative to the latter.

ACCUMULATION OF OXIDATIVE DAMAGE PRODUCTS IS ASSOCIATED WITH ALZHEIMER-LIKE PATHOLOGY IN THE FELINE AGED BRAIN

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Introduction: Oxidative damage, especially the free radicals, may also play a critical role in the development of neuropathology in the age-associated neurodegenerative disease, Alzheimer's disease. In the present study we investigated the presence of oxidative damage products and the association of these products with the pathogenic mechanism of Alzheimer-like pathology in the feline aged brains.

Materials and Methods: Brains of 14 cats of different ages (varied from 7.5 to 21 years old) were investigated. Paraffin sections were stained with haematoxylin and eosin (HE) and alkaline Congo red while for immunohistological investigations both monoclonal antibodies (against β -amyloid and 4-hydroxynonenal) and polyclonal antibodies (against GFAP, A β PP, A β 40, A β 42, A β 43, SAP, lysozyme) were used performing the ABC method.

Results: A β immunostaining labeled two types of A β deposits in the neuropil representing senile plaques. Accumulation of 4-hydroxy-nonenal material, an oxidative damage product, was detected in neurons, macrophages and plaques. Perivascular cells representing mononuclear phagocytes were also positive with the anti-HNE antibody.

Conclusions: The present study confirms that free radical injury is present in amyloid deposits and it appears that they may participate in the pathogenic mechanism of Alzheimer-like lesions especially in the early stages of amyloidosis in the aged feline brain.

MALIGNANT FIBROUS HISTIOCYTOMA (MFH) IN DOGS: PRELIMINARY STUDY OF SOME HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES ACCORDING TO THE WORLD HEALTH ORGANIZATION (WHO) CLASSIFICATION OF 2002

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The objective of this study is to correlate clinical, histopathological and immunohistochemical data of canine MFH, defining criteria of diagnosis according to the WHO Classification. Samples of two canine MFHs were evaluated through histological preparations utilizing H&E and immunohistochemistry for CD18 and vimentin. Histologically, the neoplastic cells were arranged in sheets and streams, supported by fine fibrous stroma. Two populations, with high mitotic and apoptotic rate, were observed: one consisting of medium sized round to polygonal cells, with moderate amount of eosinophilic, granular cytoplasm, large nuclei and prominent nucleoli; and a second population consisting of stellate to spindled cells with small amount of eosinophilic cytoplasm and oval nuclei. Many, variable in shape and distribution, giant cells were present. Immunohistochemically, both cases were CD18+ and vimentin+ demonstrating histiocytic origin and stromal lineage of the neoplastic cells. Clinical data, indicated localization of the tumour in the lateral rib cage (first case) and involvement of the kidney and liver (second case). The diagnosis of MFH requires careful histological evaluation, immunohistochemical support, as well as correlation with clinical data. Further updating of the WHO Classification is required to better define the characteristic features of canine MFH to enable accurate diagnosis and, thus, effective treatment.

THREE CASES OF CANINE ANAPLASTIC LARGE CELL LYMPHOMA (ALCL): DESCRIPTION AND PRELIMINARY EVALUATION OF SOME NEW DATA

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ALCL, well known in humans, has not yet been well described in dogs. The objectives of this study were to define criteria for diagnosis and to give a preliminary evaluation of this canine entity. Skin samples of 3 canine ALCLs were evaluated through histological preparations utilizing H&E and immunohistochemistry for CD3, CD79 and CD18. Results of these examinations were correlated with available clinical data. The histo-and-cytopathology of all ALCLs showed: cohesive pattern of growth, deep dermis infiltration, neoplastic perivascular cuffs, necrosis, high mitotic and apoptotic rate; large, pleomorphic, horseshoe-kidney-shaped like nuclei, multinucleation, prominent nucleoli, peripheralized chromatin, abundant cytoplasm. Immunohistochemically, all were CD3+CD79 -CD18-, demonstrating T-cell lineage. Clinical data, indicated, in two cases, tumour localization in the skin (primary cutaneous type) and, in one case, systemic involvement (primary systemic type). From two cases already published in the World Health Organization Classification (WHO) of 2002 (p.132, figures 71-73 and pp.46, 129-130, figures 67-69) and elsewhere a confusion in the understanding of canine ALCL emerges. Thus, more specific studies need to be performed and further updating of the veterinary WHO system is required to better define the characteristic features of canine ALCL to permit more accurate diagnosis and, thus, effective treatment.

TWO CASES OF CANINE MYCOSIS FUNGOIDES (MF): PRELIMINARY RESULTS REGARDING THE PRESENCE OF RETINOID RECEPTORS ON THE NEOPLASTIC CELLS

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Retinoids influence cell differentiation, proliferation and apoptosis. In human and veterinary medicine, retinoids have been shown to be effective in the treatment of MF when retinoid receptors are present. The objective of this study is to determine the presence of retinoid receptors on the neoplastic cells, since their prevalence may have therapeutic efficacy and favourable prognosis in Canine MF. Skin samples of two cases of canine cutaneous lymphoma treated with H&E confirmed the diagnosis for MF. Immunohistochemistry for CD3 and CD79, in both cases, resulted CD3+CD79 -, confirming the T-cell lineage of the neoplastic cells. Then immunohistochemistry for retinoid acid receptors (RARs) and retinoid X receptors (RXRs) isomers was performed. Both cases were RAR +, RXR +, RXR +, RAR -, RXR -; one case was RAR +. The presence of RARs and RXRs suggests that, as in humans, the use of specific ligands (last generation of retinoids/bexarotene), may be effective in the treatment of MF in dogs. Therefore, more immunohistochemical studies, together with concurrent clinical trials are required to identify the prevalence of retinoid receptors in Canine MF. In their presence, an effective response to treatment with retinoids may open new horizons in the tumour battlefield of veterinary oncology.

DIFFERENT HORMONAL TREATMENTS OF ESTRUS SYNCHRONIZATION IN EWES: EFFECTS ON SEROLOGICAL LEVELS OF OESTRADIOL AND PROGESTERONE AND THEIR RECEPTORS IN THE PREIMPLANTATIONAL UTERUS.

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The objective of this study was to investigate the effect of estrus synchronization by prostaglandin analogues (cloprostenol) comparing it to the classic treatment with progestagens that has the disadvantage of residue deposits. The preovulatory follicular dynamics, estradiol and progesterone secretion, and the uterine expression of their receptors (ER , PR) in six different uterine compartments were evaluated. Two experimental groups of 20 adult Manchega breed pregnant ewes each were used, collecting embryos on days 4 and 7. The quality of embryos was also studied. Lower plasma estradiol concentration due to poorer quality of the preovulatory follicles detected in ewes treated with progestagens downregulates ER expression in target cells, and this could explain the weaker ER immunostaining detected in the uterus of this group. Ewes treated with cloprostenol showed higher ER levels of expression on day 4 in all the uterine cell compartments compared to day 7, because of the high serological levels of oestradiol close to estrus. In general, the average amount of staining for PR expression was lower in the different compartments in sheep treated with progestagens, demonstrating that progesterone downregulates its own receptor. A higher PR immunoeexpression in the uterine stroma ($p < 0.01$) of the group treated with cloprostenol would facilitate the stromal cell production of various growth factors sensitive to progesterone that regulate essential functions in the implantation period. The higher embryo viability along with these results makes the use of cloprostenol an adequate method for estrus synchronization in sheep.

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ROLE OF THE AETHIOLOGIC AGENT IN THE TYPE AND LOCATION OF LESIONS IN THE EARLY PHASES OF RUMINANT PARATUBERCULOSIS

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Animals infected by *Mycobacterium avium* subsp. *paratuberculosis* (Map) show a variety of lesions, with differences among individuals and species. Among the factors explaining such differences, the type of Map strain should be considered.

Six groups of 1-month-old lambs were infected orally with different strains of Map: Group 1: a bovine strain, grown in laboratory media, showing a genetic pattern type A; group 2: a bovine strain, grown in the laboratory, with type E pattern; group 3: an ovine strain, directly purified from the intestinal mucosa of a clinical case; group 4: an ovine strain, directly obtained from a clinical case ileal mucosa; group 5: the same strain than in group 4 after culturing in laboratory media; group 6: uninfected group. Three calves 1-month old were also infected with the same strain than in group 4, as species control. All animals were euthanased at 150 dpi. Location and morphology of the lesions were evaluated. The number of granulomas and the area occupied by lesion in the lymph nodes were measured.

Differences were observed in the type and location of lesions. Those caused by bovine strains, regardless the type, appeared mostly in the lymph nodes and show a high number of giant cells. In group 3, lesions appeared in the intestine. Differences in the severity of lesions were also detected among groups. The type of Map strain influences the location and type of paratuberculosis lesions.

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EPIDERMOLISIS BULLOSA IN ASSAF LAMBS

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The main pathological findings observed in a 4-days-old lamb, consistent with epidermolysis bullosa, are described. In a 2-3% of the lambs born in the last seasons in an Assaf flock, appeared, between 6h and 4 days after birth, areas of redness of the coronary band of the palms. Finally, exungulation of the hooves takes place. The skin of the axillary folds, nozzle or lips appeared blistered and eroded, as well as the tongue and oral mucosa. The lamb has serious limitations in the movements and difficulty in feeding. Microscopically, subepidermal splitting with an intact epidermis in the blister roof was observed, in different degrees, in freshly fractioned skin and in natural blisters from all tissues examined. Inflammation was minimal and found only in long-standing blisters. Similar lesions were found in samples from lips, oral mucosa, tongue, esophagus or anus. Ultrastructural studies confirmed the subepidermal location of blisters. Lesions were consistent with epidermolysis bullosa, a hereditary skin disorder characterized by insufficient coherent along the dermo-epidermal basement membrana zone. It has been described previously in Suffolk, South Dorset Down or Swiss Huyeses Alpenschaf. In the latter, it has been demonstrated the absence of collagen VII in the hemidesmosomes, and compared to epidermolysis bullosa simples of humans.

MOLECURAR AND PHENOTYPIC CHARACTERIZATION OF LISTERIA MONOCYTOGENES STRAINS ISOLATED FROM HUMAN AND RUMINANT CLINICAL CASES AND FROM FOOD PRODUCTION IN NORTHERN ITALY

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To elucidate the relationship of *Listeria monocytogenes* (L.m.) strains involved in ruminant listeriosis, human disease and food contamination, we applied serotyping and automated ribotyping to 20 animal, 11 human and to 20 food strains isolated in Northern Italy since 1998; furthermore we evaluated the distribution pattern of the neuropathological lesions and the immunopositivity for L. m. antigens in ruminants CNSs. Serotypes 4b, 1/2a, and 1/2b were found to be the most represented ones, while ribotyping, that offers a more accurate subtyping scheme, identified 3 major ribogroups (with Dupont Identification Number 1038, 1039, 1042) including human, feed and animal isolates.

The neuropathological examination showed that light microscopic changes in ruminants CNSs were represented by moderate to severe meningoencephalitis in the brainstem, with mild to strong immunolabelling in all cases, independently from the involved strain.

Our data indicate that a variety of L.m. ribotypes, which have been shown to be associated with human listeriosis cases and outbreaks all over the world, were commonly present in ruminant cases in our study.

The finding of two common clusters within humans, feed and animals support the hypothesis that ruminants represent a reservoir for human L. m. infections.

THE ROLE OF CYCLOOXYGENASA-2 IN MEDIATING TUMOR ANGIOGENESIS IN FELINE AND CANINE INVASIVE CARCINOMES

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Cyclooxygenase-2 (COX-2) is an inducible enzyme linked to tumor growth and angiogenesis and its expression occurs in a wide range of inflammatory, preneoplastic, and neoplastic conditions in humans, including colon and breast carcinomas. The aim of this study was to evaluate the role of COX-2 as a mediator of angiogenesis in feline and canine invasive carcinomas and its prognostic value. COX-2 expression was assessed by immunohistochemistry in neoplastic samples and in healthy mammary glands, and related to several clinicopathological parameters, estrogen and progesterone receptor status (ER and PR), MIB-1, HER-2, microvessel density (MVD), vascular endothelial growth factor (VEGF) and overall survival (OS). In both species, COX-2 immunoreactivity was not observed in healthy tissues, while 96% of feline and 100% of canine invasive carcinomas scored positive. In queens COX-2 over-expression was significantly correlated to ER-negative receptor status ($P=0.040$), to increased PR expression ($P=0.039$), and increased angiogenesis assessed by VEGF expression ($P=0.002$). In bitches an increased COX-2 expression was significantly correlated to HER-2 overexpression ($P=0.013$) and to tumor dedifferentiation ($P=0.03$). In both species increased levels of COX-2 were correlated to poorer prognosis ($P=0.03$ in dogs and 0.002 in cats). COX-2 is expressed in mammary tissues during tumorigenesis and its expression is associated with a poorer prognosis in bitches and queens. The correlation of COX-2 expression and angiogenesis provides support for a potential role of COX-2 inhibitors for the prevention or the treatment of feline invasive carcinomas via their anti-angiogenic properties. In the canine species, moreover, COX-2 may be important for mediating HER-2-induced mammary tumours.

keywords: *COX-2, angiogenesis, mammary tumors, dog, cat, prognosis.*

TEACHING VETERINARY PATHOLOGY IN THE 21ST CENTURY - APPLICATION OF E-LEARNING MODULES

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In recent years new learning strategies have been introduced into programmes at all educational levels including veterinary medicine. It is generally accepted that interactive, student-driven learning increases longterm memory and the ability of students to apply the learned content to their professional life. The development of e-learning tools has opened up a multitude of possibilities to complement traditional learning strategies with interactive modules that increase learning efficiency markedly. As pathology (general, and organ pathology) traditionally is heavily image dependent and didactically best taught in a highly interactive manner, it is an ideal subject for e-learning.

New curricula of Veterinary Medicine being developed in several places now demand an even more active student participation, with at least 20% of their study time devoted to individual study. First attempts are made to apply online examinations based on e-learning modules used in teaching and learning veterinary pathology. To meet these needs, and those of advanced students studying for European Board qualifications (European College of Veterinary Pathology, ECVP), we aim to take a significant step forward and build on the strengths of an existing e-learning tool at the University of Zürich, the Doit platform, to develop a new integrated online learning platform in veterinary pathology. This Pathology Platform will be composed of three major areas: lecture accompanying modules, a veterinary pathology trainer for student self study and an expert forum for communication. The platform will build on and incorporate existing e-learning modules (CD, online programmes) which exist at the Vetsuisse Faculty of Berne and Zurich. Additional e-learning modules will be developed. Integrated will be links to virtual histopathology slides based at the Institute of Veterinary Pathology in Zurich (scanscope), a picture database (digital asset management, CantoCumulus) other platforms, such as OLAT and Pathobasiliensis (Human pathology). This is an ambitious project, which will break new ground in the teaching of veterinary pathology. To this end we have assembled a highly qualified team of experts, committed to its successful implementation.

This application of e-learning modules will serve not only local or national students of veterinary medicine but will allow in the near future to form a pan-european learning network for veterinary students and veterinary pathologists. In addition students of Animal Sciences (ETHZ) will profit from the e-learning modules and based on this cooperation a common Master`s programm will be initiated.

DETECTION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN OVINE INTESTINE: CORRELATION BETWEEN HISTOLOGICAL LESIONS MYCOBACTERIAL STAINING AND IMMUNOHISTOCHEMICAL FINDINGS

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Aim of the study was to investigate the histopathological lesions and the sensitivity of Ziehl-Neelsen staining (ZN) and immunohistochemistry (IHC) to detect *Mycobacterium avium* subsp. paratuberculosis (MAP) in different tracts of small intestine of ELISA positive sheep.

Paraffin embedded sections of distal ileum and of proximal and distal jejunum of 14 paratuberculosis affected sheep were stained with haematoxylin-eosin and ZN staining and tested with IHC (PoAb anti-MAP).

Following the simpler classification suggested by Corpa (2000) in goats, the histological lesions observed were "diffuse multibacillary" in 4 sheep, "diffuse lymphocytic" in 7 sheep, and "diffuse mixed" in 1 sheep, similarly in the three intestinal tracts. In 2 sheep, differences in features were observed between proximal jejunum sections ("diffuse mixed") and distal jejunum-ileum sections ("diffuse multibacillary"). ZN staining demonstrated mycobacteria in 8 animals (4 multibacillary, 1 lymphocytic, and 3 mixed lesions), while IHC demonstrated MAP antigen in 13 sheep (4 multibacillary, 6 lymphocytic, and 3 mixed lesions).

IHC is more sensitive than ZN staining for MAP detection, mainly when few mycobacteria are present such as in "diffuse lymphocytic" lesions. The avidin-biotin amplification system does increase the sensitivity of immunoperoxidase diagnostic system, and it is preferable rather than ZN mainly in paucibacillary infections.

MORPHOLOGIC AND ELECTROPHYSIOLOGIC CHARACTERIZATION OF DIABETIC NEUROPATHY IN MICE

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Diabetic neuropathy (DN) is one of the most common complications of Diabetes Mellitus, progressively affecting the distal peripheral sensory and motor nerves as well as the autonomic nervous system. Nerve conduction deficits and loss of myelinated nerve fibres are the most distinct morphological abnormalities found in human diabetic nerves.

After 16 weeks of streptozotocin diabetes induction, physical examination, electrophysiological assessment, and biochemical, histological, and immunohistochemical analysis of nerves were performed in diabetic and nondiabetic animals.

Diabetic animals demonstrated features of hyperglycemia, including polydipsia and polyuria. In addition, decreased nerve conduction velocity was detected in diabetic mice. Histological studies of footpads revealed significantly reduced numbers of epidermal fibers, whereas no differences were observed in sweat gland-associated autonomic axons. Myelinated fiber density and number in tibial (predominantly motor axons) and sural (sensory and some autonomic axons) fascicles were not altered by diabetes.

Our findings indicate that electrophysiological assessment and immunohistochemical analysis have potential to serve as a model system for investigations of functional and morphological changes in a mouse model of human diabetic neuropathy.

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NEURONAL INTRANUCLEAR INCLUSION DISEASE IN A HORSE

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Neuronal intranuclear inclusion disease (NIID) is a slow progressive fatal neurodegenerative disease which is characterised by the presence of eosinophilic inclusion bodies within nuclei of nervous cells in the central and peripheral nervous systems. Neuronal loss is also present in specific brain areas such as cerebrum, cerebellum pons and spinal cord. The actual pathogenesis is unknown but an association between inclusion bodies and the ubiquitin-proteasome complex has been suggested. Up to date only a few cases have been reported and exclusively in human neurology. NIID is reported in a 16-year-old Pure Spanish breed female horse suffering from progressive ataxia and motor deficiencies. The neuropathological study revealed NIIs throughout the central nervous system, although mainly in the brain stem and spinal cord. This distribution did not correlate with neuron loss, which was marked in the hippocampus and moderate in the neocortex. As in humans, NIIs in the horse were hyaline autofluorescent inclusions composed, ultrastructurally, of non-membrane-bound aggregates of filaments and fine granules. Immunohistochemically NIIs were stained with anti-ubiquitin and anti-clusterin antibodies. In addition, NIIs were immunoreactive to antibodies raised against subunits of the 19S and PA28, but not of the 20S, components of the proteasome. These observations indicate similarities between NIID in humans and horses, and suggest that clusterin and abnormal ubiquitin-proteasomal expression participate in NII formation.

IMMUNOHISTOCHEMICAL STUDY OF NEURAL TISSUE SAMPLES OBTAINED FROM SLAUGHTERHOUSE.

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Aim of this retrospective study was to describe histopathologic and immunohistochemical characteristics of rare tumor cases of suspected neural origin found in slaughtered cattle. The study has been performed on 15 cases collected between 2002 and 2004. The data on precise location of pathological changes were available in only two cases (tumors attached to peripheral nerves at the base of heart and in the intercostal space). Routine H&E as well as Van Gieson's staining as a control for the presence of connective tissue has been performed. Histopathological examination revealed lack of Antoni A and Antoni B patterns typical for human cases of neurogenic tumors. Immunohistochemical study was performed using antibodies against glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE), S-100 protein and vimentin. All 15 cases showed positive reactivity for S-100 protein. 13 cases were positive for GFAP and 12 cases for NSE. Presence of vimentin was confirmed in 8 cases. Negative staining for vimentin in 7 cases can be considered as a result of overfixation rather (archival material) than lack of antigen. Altogether the obtained results confirmed the neural origin of the observed tumors.

EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) AND ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) IN CANINE MAMMARY TUMOURS.

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Nitric oxide (NO) is an inorganic free radical synthesized by nitric oxide synthases (NOS), a family of isoenzymes comprising nNOS and eNOS, constitutively expressed by neuronal and endothelial cells respectively, and iNOS whose synthesis is induced by cytokines, in many biological processes and pathologic events, such as neoplasias. High NOS activity has been observed in many human tumour types and it has often correlated with neoplastic growth and metastatic potential. In this study, iNOS and eNOS expression was evaluated by immunohistochemistry in a series of canine mammary tumours and correlated with stage and grade. In addition a colocalization of both enzymes was performed by double immunofluorescence staining observed at confocal laser microscope. In normal breast, iNOS immunolabelling was observed in macrophages and eNOS was expressed by vascular endothelial cells while few epithelial cells expressed both enzymes in benign tumours. In malignant tumours, the iNOS and eNOS expression increased in well differentiated tumours, in which neoplastic cells exhibited a strong immunolabelling often restricted to the luminal pole, while in less differentiated ones (grade 3 and stage 2/3 carcinomas) it decreased again and neoplastic cells showed a weak positivity diffused in the cytoplasm. iNOS and eNOS resulted colocalized in neoplastic cells. Our data seem to demonstrate that NOS expression changes during tumour progression, suggesting an involvement mostly in the early stages of mammary carcinogenesis.

HISTOPATHOLOGICAL EVALUATION OF CANINE FEMORAL HEAD ARTICULAR CARTILAGE FOLLOWING RESURFACING USING OVINE MEMBRANOUS FETAL SKULL

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Due to the weight bearing properties of the hip joint and the fact that this joint is consistently under stress of daily activities, the articular cartilage is destroyed in many disease conditions resulting in severe lameness. In this respect, biological resurfacing of the femoral head using periosteal grafts, chondrocytes of articular cartilage and osteochondral grafts have been recommended and in this study membranous skull bone of ovine fetus was evaluated. Twelve male and female dogs of at least one year of age were used in this study. Ovine fetuses were collected from Tabriz abattoir. The animals were randomly assigned to three groups of 4 animals per group. In group I, only the articular cartilage was removed. In group II after removal of articular cartilage to the level of subchondral bone, the resurfacing was done using ovine fetal skull. In group III, procedure as in group II was done but the hip joint was restricted using an ehmer sling. All animals were cared for 60 days and macroscopic and microscopic evaluations were made after this time. The articular surface in groups II and III appeared smooth macroscopically in comparison to group I which shows that the graft has healed. In group I, because the articular cartilage was partially damaged, the newly formed cartilage was incomplete. In groups II and III the mesenchymal fetal skull model resulted in better stimulation of subchondral bone and better cartilage growth. In group II the cartilage was hyaline but without perichondral tissue but in group III scattered cartilage tissue with perichondral tissue was observed without any real resemblance to hyaline cartilage. Considering the result of this study, it seems reasonable to recommend the application of membranous bone of ovine fetal skull to resurface the damaged articular cartilage of hip joint.

PATHOHISTOLOGICAL AND IMMUNOLOGICAL CHARACTERISTICS OF ULCERATIVE COLITIS CAUSED BY TNBS IN RATS

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Introduction: Ulcerative colitis caused by 2,4,6-trinitrobenzenesulfonic acid (TNBS) acid includes many aspects associated with the pathogenesis of inflammatory diseases, such as provoking an inflammation with mucosal damage and activation of the immune system. It can also be used as an experimental model in the testing of pharmacological molecules or agents used in the therapy of the inflammatory bowel diseases (IBD). The aim of the study was to establish a correlation between the pathohistological, immunological and clinical parameters of inflammation during the development of this model.

Material and Methods: Male Wistar rats (230-250g, 12-15 weeks old) were used in the experiment. After 24 hours starvation, in anesthetized rats, 0.5ml TNBS in 50% ethanol (150 mg/kg body-weight) were administered rectally, 8 cm proximally from the anus. The induction and development of inflammation were monitored periodically during 3 weeks. Rats (in groups of 5) were sacrificed every day during the first week and the 8th, 14th and 21st day of administration (dpa). The development of inflammation was evaluated in respect to the clinical activity (quantified by loss on weight, consistency of feces and rectal bleeding), macroscopic and pathohistological changes, colon weight/body-weight ratio, myeloperoxidase activity (MPO) and distribution of CD3 T lymphocytes and CD79 B lymphocytes. In control experiments, rats treated with 0.9% saline alone, were used.

Results and Discussion: Macroscopic and histological changes of the colon the 1st dpa included appearance of local hyperemia, ulcers smaller than 1 cm and mild inflammatory infiltrate. In the period of the most intensive inflammatory activity i.e. 6th dpa, marked thickening of the intestinal wall associated with hemorrhages, severe cellular infiltration and ulcers that exceed 2 cm were seen. In the same period, the damage score was the highest, 15 ± 2 vs. 5 ± 1 on the 1st dpa and 0 for the control group. From the 8th to the 21st dpa, the damage score decreased, so the 21st dpa, it was 3 ± 1 . During the study, the colon weight/total body weight ratio increased from 13 ± 1 mg/mg, the 1st dpa, to 30 ± 6 mg/mg, the 6th dpa, while on the 21st dpa the ratio decreased to 11 ± 1 mg/mg. The activity of MPO correlated with these observations entirely, so the lowest activity on the 1st dpa (41.23 ± 11.65 U/g) and the maximum activity on the 6th dpa ($578.48 \pm 10,576$ U/g) was observed, while the activity on the 21st dpa was $63.92 \pm 11,9$ U/g. Activity of MPO within the control group during the research was 34.09 ± 4.09 U/g, while the colon

weight/total body weight ratio was 8.5 ± 0.5 mg/mg. CD3 T lymphocytes and CD79 B lymphocytes within the control group are mainly visible along the epithelia, from lamina epithelialis and diffuse in lamina propria of the colon. With the development of inflammation their number significantly decreased and in the areas covered with ulcers, as a result of necrotic and apoptotic changes, they cannot be observed.

In conclusion, the TNBS model appears to show high correlation between pathohistological, immunological and clinical features of the inflammation in IBD.

CUTANEOUS LARGE B CELL LYMPHOMA: MICROSCOPICAL, IMMUNOHISTOCHEMICAL AND MOLECULAR CHARACTERIZATION IN THREE CATS

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Feline cutaneous epitheliotropic lymphomas (mycosis fungoides) and non-epitheliotropic lymphomas (NECL) are usually of T cell origin. Dermal B cell lymphomas are uncommon in most species including humans. Three male castrated cats (1 Devon rex, 1 Siamese, 1 Persian), age range 9-15, presented with multiple truncal dermal nodules. Biopsies were processed for histology and immunohistochemistry (anti-CD3 and anti-CD79a). Clonality was evaluated on DNA extracted from paraffin embedded material. PCR was performed with primers that span the V-D-J junction of the immunoglobulin heavy chain (IgH). PCR products (native and denatured) were visualized on 10% TBE polyacrilamide gels. The tumors were located in the deep dermis and skeletal muscles and were composed of large B cells (CD79a +, CD3-) with round nuclei and prominent nucleoli. Neoplastic cells were associated with mature reactive T cells (2/3) or with B cells organized in pseudofollicles (1/3). Angiotropism and angiodestruction (2/3) were present. Clonality was confirmed in 2/3 cases. The case demonstrating no amplification had the most undifferentiated morphology. Two cats died three months after diagnosis, one was lost to follow up. These three cases are the first large B cell NECLs reported in cats.

HEMOTHORAX DUE TO VASCULAR NECROSIS OF THE VENA CAVA CAUDALIS IN AN ABORTED FOAL INFECTED WITH EQUINE HERPESVIRUS 1

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Herpesviruses are known to cause abortion in domestic animals. Equine herpes virus type 1 (EHV-1) is the most important viral cause of equine abortion.

An aborted foal was presented. Gross pathology revealed no specific EHV lesions. The thoracic cavity was filled with several liters of blood and blood clots. In the *Vena cava caudalis* near the diaphragm, there was a necrotic area in the venal wall, which leaked blood. Polymerase chain reaction for EHV-1 on several organs was positive, as well as the seroneutralisation test on the mare's serum. Histopathology revealed moderate necrotizing inflammatory reactions in several organs associated with (peri)vasculitis. No intranuclear inclusions of EHV-1 were observed. The examination of the vena caval wall revealed severe necrosis of the tunica media and mixed inflammatory reaction, haemorrhages and clots.

EHV-1 is known to cause vascular necrosis of small blood vessels in equines. However, this case showed vascular necrosis of a larger blood vessel, i.e. the *Vena cava caudalis*, resulting in haemothorax, associated with an EHV-1 infection. The presence of EHV-1 infected cells in the vena cava could not be demonstrated, but it is strongly suspected to be the causative agent of the vascular necrosis.

HEAT SHOCK PROTEINS EXPRESSION IN CANINE MALIGNANT MAMMARY TUMOURS

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We investigated immunohistochemical (IHC) expression of Hsp27, Hsp72, Hsp73 and Hsp90 and their role on overall survival (OS) in 3 normal canine mammary gland (NMG) and 30 mammary tumours (MT) (stages: in situ 10, locally invasive 10, with intravascular emboli 10). Tissues were immunostained using a streptavidin-biotin-peroxidase technique. A semiquantitative (absent, low, intermediate, high) IHC assessment was compared between NMG and MT and among stages by Chi square test. Influence on OS was tested by Log Rank Test. In MT, a significant increase of Hsp27 ($P<0.01$), Hsp72 ($P<0.05$) and Hsp90 ($P<0.01$) and a significant Hsp73 reduction ($P<0.01$) were observed. Intermediate-high Hsp27 expression was observed only in invasive stages ($P<0.01$), particularly in cells with squamous metaplasia and infiltrating elements. Hsp72 and Hsp73 immunoreactivity was absent to high in all stages, though intermediate-high pattern was mainly detected in invasive stages, where Hsp72 expression was often associated to tumour necrosis or inflammation. Hsp90 immunolabelling was high in all stages and, like Hsp73, it was strong also in lymphatic emboli. Only Hsp27 was significantly associated to poor prognosis ($P<0.01$). We conclude that Hsp27, Hsp72 and Hsp90 could be involved in carcinogenesis of canine mammary gland, while only Hsp27 could be indicative of poor outcome.

IMMUNOHISTOCHEMICAL DETECTION OF HSP 73 AND HSP 72 IN KIDNEY OF PIGS WITH CHRONIC, PRRSV RELATED, INTERSTITIAL NEPHRITIS: PRELIMINARY RESULTS

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Heat Shock Proteins (HSPs) are phylogenetically highly conserved proteins, classified by molecular weight and involved in stress related intracellular pathways. Many HSPs are constitutive (HSP 73) while others (HSP 72) are induced by stress. A number of in vitro studies were recently performed concerning the HSPs role in inflammation, but little is known regarding HSPs tissue expression during inflammation. We investigate expression of constitutive and inducible forms of HSP 70 (HSP 73 and 72 respectively) in kidneys with interstitial nephritis of PRRSV and PCV2 positive pigs. Sections of kidneys from 38 pigs were immunohistochemically stained with two monoclonal antibodies (MAbs): the former recognizing both HSP 73/72, the second specific for HSP 72. Sections of unaffected kidneys were used as negative controls. Moderate intracytoplasmic expression of HSP 72 in tubular epithelium was present in control kidneys. MAb HSP72/73 stained also small numbers of glomerular cells. Kidney with interstitial nephritis showed an increased HSP 72 staining intensity with a positive correlation with severity of lesions. Staining intensity could be explained with the double activity of T cell recruitment and cellular chaperone performed by HSP 72 during inflammation. Further investigations regarding the role of HSPs in these disease will be performed.

EUROPEAN SOCIETY OF VETERINARY PATHOLOGISTS - NAPLES 7-10/09

PAPILLOMAVIRUS-ASSOCIATED URINARY BLADDER TUMOURS IN CATTLE:

THE ROLE OF THE BLOOD-STREAM

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Bovine Papillomaviruses (BPVs) are species-specific, double-stranded DNA viruses responsible for cutaneous and mucosal neoplastic lesions. In particular, Bovine papillomavirus type 2 (BPV-2) belongs to the genus delta-papillomavirus, species 4, the biological properties of which are characterized by inducing fibropapillomas in cattle and sarcoids in equine species as well. It is well known that BPV-2 in the presence of environmental cofactors such as bracken fern may lead to cancers. The BPV-2 infection has been associated with urinary bladder tumours in cattle suffering from a clinical syndrome known as chronic enzootic hematuria.

We investigated the presence of BPV-2 in peripheral blood to gain insights into mechanism(s) of infection in target organs.

We examined 120 tumours and peripheral blood samples collected from cattle clinically suffering from chronic enzootic hematuria caused by urinary bladder cancers. PCR amplification of viral sequences using consensus primers was utilized to detect the presence of papillomavirus. To determine papillomavirus genotype, the amplicons were sequenced. The presence of BPV-2 in peripheral blood was found in 75 of 120 samples of blood (~63%).

In conclusion our data indicate that BPV-2 might be transported to urinary bladder tissue by the blood-stream, thus playing a crucial role in the cancerogenesis of bladder tumours.

A CASE STUDY: CEREBELLAR LEPTOMENINGEAL CARCINOMATOSIS IN A DOG

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Meningeal carcinomatosis is well recognized in human medicine as a diffuse, focal or multifocal brain and spinal cord leptomeningeal infiltration of neoplastic cells, deriving from a solid tumour. In veterinary medicine it remains an uncommon neoplastic lesion in dogs and cats.

An old German Shepard dog was presented with a ten-day history of seizures, rapidly progressed to stupor. Brain CT scan revealed an irregular cerebellar meningeal contrast enhancement. Physical examination showed a mammary gland mass. Given the poor prognosis, the owners requested euthanasia. Immunohistochemistry tests were done using antibodies against vimentin and pancytocheratins.

At necropsy, nodular masses were observed in the mammary gland, lungs, tracheobronchial lymph nodes and adrenals. Cerebellar leptomeninges were affected by diffuse blood effusion. Histology showed a solid anaplastic mammary tumor, characterized by round to oval, occasionally multinucleated, epithelial cells. Neoplastic emboli were observed in the mammary vessels. The tumor has spread to the lungs, tracheobronchial lymph nodes and adrenals. Cerebellar leptomeninges were diffusely infiltrated by the neoplastic cells focally involving the molecular layer.

An anaplastic solid mammary carcinoma and a diffuse cerebellar meningeal carcinomatosis secondary to hematogenous dissemination were diagnosed. CT scan suggesting vascular disorder failed to reveal a diffuse meningeal neoplastic infiltration.

**EVIDENCE OF ANTI-GLIADINE AND ANTI-TRANSGLUTAMINASE ANTIBODIES
IN SERUM OF DOGS WITH LYMPHOPLASMACYTIC ENTERITIS:
CORRELATION BETWEEN SEROLOGICAL DATA AND PATHOLOGY.**

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Lymphoplasmacytic enteritis (LPE) of dog is a pathological condition of unclear aetiology and pathogenesis. Affected animals show various gastric and enteric clinical signs as vomitus, malabsorption and diarrhoea. Generally, moderate to severe mononuclear cells infiltration is observed in small intestinal bioptic samples of affected dogs, similar to those observed in celiac patients. To evaluate the correlation between this pathological condition and a possible gluten sensitization, the serum of 49 dogs with histological diagnosis of LPE, performed by biopsy, and 10 histologically negative dogs were assayed by an immunoblotting method for anti-gliadine antibodies (AGAbs) and for anti-transglutaminase antibodies (tTG-Abs). No AGAbs and tTG-Abs were detected in sera belonging of controls dogs, while symptomatic and asymptomatic dogs, selected on the basis of positive histology, showed a constant positivity (49/49; 100%) for AGAbs and an high prevalence (37/49; 75%) of tTG-Abs. High correlation ($p < 0.05$) was observed between symptomatic dogs with severe histological form of LPE and serological presence of tTG-Abs. A constant presence of IgG1 AGAbs in sera of LPE affected dogs indicates a possible gluten sensitization while IgG1 tTG-Abs are associated with symptomatic disease and are correlated with a severe mucosal destruction. These data suggest that in dogs tTG-Abs could reflect inflammatory events associated with epithelial destruction.

UP-REGULATION OF E-CADHERIN, α -CATENIN AND CD29 IN THE ILEAL EPITHELIUM OF SHEEP WITH PARATUBERCULOSIS.

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Changes of the intestinal mucosal barrier are considered to play a role in the pathogenesis of inflammatory bowel disease (IBD). In this optic the E-cadherin-catenin complex is important for the maintenance of epithelial architecture. We studied its expression in sheep with paratuberculosis at different stages and in controls. Immunohistochemical stainings for E-cadherin, α -catenin, and β 1-integrin (CD29) were performed. In chronic-active inflammation, there was up-regulation of the complex. There was a statistically significant correlation between the expression of E-cadherin, α -catenin, β 1-integrin and disease activity. Additionally, RT-PCR demonstrated up-regulation in TNF- α and IL-1 β messengers expression in tissues from infected sheep. These findings indicate that altered expression of E-cadherin, alpha-catenin, and β 1 integrin occurs during active inflammatory bowel disease. Since structural or functional perturbation in any of the molecules of the E-cadherin-catenin complex results in loss of intercellular adhesion, the preexistent epithelium may benefit from up-regulation to try to maintain its normal architecture under inflammatory conditions. CD29 up-regulation confirms the central role of this integrin in promoting migration of T lymphocytes to areas of inflammation and adhesion between T cells and APCs.

UROPLAKIN EXPRESSION IN THE UROTHELIAL TUMORS OF COWS

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Urinary bladder tumours are very rare in cattle accounting for 0.01% of all bovine malignancies. These tumours are commonly encountered in cows that have grazed on pastures rich in fern (*Pteridium* spp.); the fern contains toxic principles, and prolonged ingestion of which is responsible for urothelial cell transformation. It is believed that bovine papillomavirus type-2 (BPV-2) also plays an important role in the bladder carcinogenesis.

Several immunohistochemical markers have been used to study the differentiation pattern of urothelial cell tumours of the urinary bladder. Uroplakins (UPs) are major specific urothelial differentiation products of mammalian urothelium. They are mainly produced in cells of the superficial layer of transitional cell epithelium, and they consist of five components: the UPIa, UPIb, UPII, UPIIIa, and UPIIIb.

It has been suggested that the ability of urothelium to terminally differentiate is progressively lost during neoplastic transformation. UPs may therefore be a useful biomarker of assessing the degree of differentiation in bladder carcinogenesis.

The aim of this study is to evaluate UPs immunoreactivity in 39 papillomavirus-associated spontaneous urothelial tumours of the bovine urinary bladder.

TOWARDS A NOVEL CORONAVIRUS-BASED VECTOR VACCINE: STUDIES ON BIOSAFETY IN PIGLETS

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Aim of this project is to study the biosafety of recombinant expression systems based on the Transmissible Gastroenteritis Coronavirus (r-TGEV) to establish an oral vaccine inducing a mucosal and systemic immune response. Four r-TGEVs include either various cloning sites or a deletion of gene 7. To study the tropism and the virulence, 3-days-old piglets were infected with 10⁸ PFU of each of the rTGEVs. Mortality was between 25% (r-TGEV-Δ7) and 87.5%. Histopathology showed atrophy and fusion of intestinal villi and submucosal edema with a mild bronchointerstitial pneumonia. Plaque assay revealed preferentially intestinal tropism of the vectors with higher replication efficiency in the small intestine (10⁷ PFU/ml) compared to lower titers in the lungs (10⁴ PFU/ml). TGEV-antigen was present in enterocytes of the jejunum and ileum including small numbers of M-cells. Electron microscopy demonstrated replication stages of r-TGEV in the enterocytes.

These experiments show that the rTGEV-Δ7-vector is a promising candidate for further vaccine development.

IMMUNOHISTOCHEMICAL DETECTION OF CORONAVIRUS, CRYPTOSPORIDIUM PARVUM AND GIARDIA INTESTINALIS IN NEONATAL DIARRHOEA IN LAMBS AND KIDS

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In this study, clinical, parasitological, macroscopical, histopathological and immunohistochemical examinations were performed on 19 kids and 11 lambs (30 animals) with neonatal diarrhoea to detect the presence of Coronavirus, *Cryptosporidium parvum* and *Giardia intestinalis* as etiological agents causing illness. Clinically severe dehydration, yellowish-green to brown coloured diarrhoea and death were observed. Mortality rates were approximately 10-30% in the examined flocks. In the present study the most common agent was *C. parvum* and was diagnosed in twenty animals as a single causative agent, whereas *G. intestinalis* was found in five out of thirty animals. These two protozoa were detected together in four animals upon faeces examination. Fifteen out of twenty-four cases of *C. parvum* and three out of eleven cases of *G. intestinalis* were also confirmed histopathologically. Following immunohistochemical examination, all cryptosporidiosis cases were confirmed by positive immunostaining of intestinal sections. Two additional Giardiasis cases which had given negative results upon parasitological and histopathological examinations were diagnosed by means of immunohistochemical examination. Coronavirus was detected immunohistochemically in one kid with neonatal enteritis. Following diagnosis, herds were treated with Trimethoprim + Sufodoxine (Animar Injectable – Roche) and multivitamin complexes. Intravenous and intramuscular administration of these drugs was effective for both treatment and prevention.

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PATHOLOGIC INTESTINAL EFFECTS OF CADMIUM IN BROILER CHICKEN

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During a study of toxicity effects of various concentrations of Cadmium in broiler chicken, some pathological injury was demonstrated in intestine. In this study ,288 chicken were randomly divided into 4 groups and each group were further divided into 3 replicates. The replicates were randomly distributed in the 12 batteries. All of four groups received water and feed al-libitum, but two groups of them also received 5, 50 and 100ppm cadmium in their feed . After 7,14,28 and 49 days ,necropsy was done to collect the immunologic tissues. During the physical examination, many macroscopic hematopoietic locals were seen in deudenum serous and mucosa in group, feed 100 ppm cadmium for 49 days. In microscopic studies of these sites, it was revealed that the hematopoietic sites in parin of submocusa changed the crypts and villi and hematopoietic foci were seen all between intestine glands compared to other groups. The cause of this injury will be follow up later.

PATHOLOGICAL CHARACTERIZATION OF A MODERATELY VIRULENT AFRICAN SWINE VIRUS (UGANDA 2003H) INFECTION

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In May 2003 an outbreak of African Swine Fever (ASF) was reported in Uganda, in the Hoima district (Eastern Uganda) and was spread very fast across the country. Here we describe the characterization of the Ug03H, the isolate of ASF virus (ASFV) associated this epizootic in Uganda. Although we don't have information regarding the virulence of this isolate, this outbreak resulted in the death and slaughter of more than one hundred thousand pigs. 2 pigs were experimentally inoculated by intramuscular route with a high dose of ASFV isolate Ug03H at BSL-3 animal facilities area at CISA-INIA, and one pig was used as contact animal. Animals developed pyrexia from 3-5 dpi and no typical clinical signs related to acute ASF such as cyanosis or anorexia were observed. Animals were painlessly killed at 9-12 dpi. At necropsy, a severe haemorrhagic enteritis was observed together with haemorrhagic lymphadenitis in renal, mesenteric and gastrohepatic lymph nodes. Interestingly, no macroscopic changes were observed in the spleen neither in the kidney. Histopathological study revealed a moderate lymphoid depletion in spleen and lymph nodes, septal oedema in the lungs and haemorrhages together with the presence of ASFV in these organs observed by immunohistochemistry and PCR. With these results we conclude that this isolate of ASFV produce a subacute form of the disease, rarely observed in african isolates, and further studies are being carried out to elucidate a possible relationship of these findings with possible mutations in the genome regarding to other african ASFV isolates.

STUDY OF ACUTE PHASE PROTEINS IN PIGS EXPERIMENTALLY INFECTED WITH AFRICAN SWINE FEVER AND CLASSICAL SWINE FEVER VIRUS

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The aim of our study was to test the serum levels of the acute phase proteins (haptoglobin (HPT), serum amyloid A (SAA) and C-reactive protein (CRP)) in pigs experimentally inoculated with African swine fever (ASF) and classical swine fever (CSF) viruses.

Four Largewhite x Landrace pigs were each inoculated intramuscularly with the virulent CSF isolate "Alfort 187". Others four pigs were inoculated with ASF Spain-70 isolate. Pre-inoculation blood samples were taken from all pigs to obtain baseline values. Blood samples from inoculated animals against CSF were taken from 1 to 24 days post-inoculation (dpi), and from 1 to 6 dpi from pigs inoculated against ASF.

During CSF, serum concentrations of SAA were higher than observed in ASF, although SAA serum concentration peaked in earlier dates (4 dpi) than in CSF. Two-phase behaviour exhibited by SAA protein in CSF was not observed in ASF, perhaps due to the less duration of the experiment. However, the kinetic and serum levels showed by CRP and HPT proteins in the course in both diseases were very similar, giving emphasis to the low serum concentrations of HPT. Cytokines induce the synthesis of APP by hepatocytes. For this reason, the increased presence of cytokines described during CSF and ASF, might play an important role in the kinetic of the acute phase proteins.

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DETECTION OF IMMUNE SYSTEM CELLS IN PARAFFIN WAX-EMBEDDED BOVINE TISSUES

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The aim of our study was to describe a method (fixation, paraffin wax-embedding and immunolabelling) for the demonstration of several immune system cell epitopes (CD4, CD8, WC1 and MCH II antigens) in bovine lymphoid organs (spleen, thymus, ileum and lymph nodes) collected at necropsy from four healthy calves of 4-6 months old. Samples were fixed in 10% buffered formalin solution, Bouin's solution and zinc salts fixative. The avidin-biotin peroxidase method in combination with different antigen unmasking techniques were used.

Zinc salts and Bouin's solution were the best fixative for immunohistochemical detection of T-cells subpopulations (CD4, CD8 and WC1) and cells expressing MCH II antigens respectively. pH 3.2 sodium citrate solution for CD4, CD8 and WC1, and pH 6 for MCH II warmed up in stove to 37°C were the most suitable unmasking antigen methods. Lymphocytes immunolabelled against CD4 and CD8 were located mainly in the cortex of thymus, lymphoid follicles (mantle zone) of spleen and lymph nodes, as well as in periarterial lymphoid sheaths of spleen and Peyer's patches of ileum. Moreover, immunolabelled cells were observed occasionally in the medulla of thymus, inside of the lymphoid follicles in spleen and lymph nodes and in the interfollicular areas. Marked lymphocytes against WC1 were located in peripheral areas of lymphoid structures, as well as in the cortex and medulla of thymus. Monocytes-macrophages and dendritic cells immunolabelled against MCH II were observed in different lymphoid organs, specially inside and in the mantle zone of lymphoid follicles.

This work was supported financially by grants from MCYT (PB98-1033; AGL 2003-252) and from Seneca Foundation (Región de Murcia)

FUNCTIONAL PROPERTIES OF ALPHA1 ACID GLYCOPROTEIN ON BOVINE CD14+ CELLS

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α 1-acid glycoprotein (AGP) is a lipocalin, a subfamily of proteins that combine immunomodulatory activities and binding and transport of small hydrophobic molecules. AGP is expressed mainly by hepatocytes in response to the systemic reaction that follows tissue damage caused by inflammation, infection or trauma. AGP is a minor acute phase protein in bovines, and its concentration is increased during chronic inflammatory conditions. The present study investigates whether AGP can modulate some functional properties of magnetic sorted CD14+ cells (monocytes). The experimental design include the treatment of bovine monocytes with AGP purified from bovine plasma. The two functional parameters that were considered were the rate of staurosporin-induced apoptosis and the LPS-induced adherence on monocytes to plastic surfaces. The treatment of the cells with a concentration of 1.5 mg/ml increased apoptosis rate up to 50% while physiological concentration had no effect.

Adhesion properties of monocytes were profoundly influenced by the treatment with bAGP. Acute phase concentrations of bAGP reduced the adherence of the cells, this effect being increased when the cells-adhesion is induced by LPS-treatment.

Our results suggest that the increase of the rate of apoptosis and the decrease of the adherence of monocytes may represent one of the several immunomodulatory pathways that AGP may activate to reduce the potential harmful effects of an un-controlled inflammatory reaction.

SCIATIC NEURITIS IN GOATS BY ALFATRIM 24% INJECTION

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Introduction: During injecting into a nerve can cause neuropathy, neuritis and scarring. Most commonly the sciatic & radial nerves are the subjects of injury due to improper injection techniques. The sciatic nerve is damaged by deep intramuscular injections. Temporary lameness in goats is common after injections with irritant substances because of the relatively small masses of muscle in the hind leg, particularly in the gluteal region. Lameness, sometimes permanent, is a common sequela to intramuscular injection.

Materials and methods: 40 cases of sheep and goats about 3 years old, had received Alfatrime 24% , 1cc per 20 kgBW in the route of intramuscular each 12 hours for 3 days.

Results: Clinical signs included lameness, loss of function in the hind limbs with loss of skin sensation on the lateral surface on the tibial region, hock & elbow. The feet were dragged with each step. In some of cases after 5 minutes, clinical findings were appeared. In hematological examination the rate of mature neutrophil was 61 to 73%. In CSF level of glucose was 59 mg/dl and total protein was 1 gr/dl.

Macroscopic lesions were swelling of sciatic nerve stem, submeningeal hemorrhage, fibrinopurulent inflammation & necrosis. In microscopic lesions neuronal fibers were normal (axon & myelin sheath), but there were fibrinous lymphoplasmacytic perineuritis, subepineuronal hemorrhage. Some of cases showed recovery 4 days after receiving Ibuprofen.

Discussion: The combination of trimethoprim & sulfamethoxazole secures a synergistic action against most gram positive & gram negative bacteria including penicillinase producing ones. *Leptospira*, *Pseudomonas*, *Erysipelothrix* & *Mycobacterium* are not very sensitive. These two components affect the synthesis of purine & nucleic acids of the bacteria in a different way resulting in a double blockade. Alfatrime 24% can be used in primary & secondary bacterial diseases; Respiratory, GI tract, genital & urinary infections, mastitis, wound infections, eye & ear infections in cattle, calves, pigs, dogs & cats.

Trauma is the usual cause of peripheral injuries leading to paralysis. While injury may occur to all the peripheral nerves, the most common peripheral nerve dysfunction in goats is damaged to the sciatic nerve from mechanical injury caused by poor intramuscular injection techniques or the introduction of irritating medications on or near the nerve. The sciatic nerve & its terminal branches the tibial & peroneal nerves are the most

commonly injured peripheral nerves of the hind limb.

In this report, adjuvant or drug might be so the cause of producing these kinds of lesion. Infiltration of lymphocyte& plasmacyte could be the result of immunologic reaction& purulent myositis may be the sign of secondary bacterial infection. The severity of the damage varies with the drug& dose administered. Certain drugs have greater potential for causing neuronal damage. Extreme care should be taken with intramuscular injections as permanent lameness can result because the goats have relatively small muscle masses in the hind legs, particularly in the gluteal region. Temporary lameness is common after injections of irritant substances. The volume of the drug administered at one side should never be more than 5 ml, using a needle of 18 g or less& 25_38 mm (1_1.5 inches) in length in adult goats. Smaller amounts& shorter needles should be used in kids. It is always safer to use the subcutaneous route than give the intramuscular injections& this route should be adapted where ever possible.

HISTOPATHOLOGICAL AND SEROLOGICAL STUDY ON MAEDI DISEASE IN SHEEP

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Summary

In this study, one of the most important of "ovine slow viral infections" which called "Maedi disease" was studied. Maedi, is a fatal, progressive pneumonia of mature sheep. During the study, pathologic samples of ovine lungs from 170 sheep (>1 year old) with their serum samples were collected in the industrial abattoir. At first histopathological and then serological

studies on the lungs and serum samples were performed by using an indirect ELISA test. Histopathological study on lung and lymph node tissues showed smooth muscle hyperplasia (SMH) of alveolar walls, lymphofollicular hyperplasia (LFH), and interstitial pneumonia (IP). Degree of involvement of each lung sample was estimated from mild, moderate and severe degrees.

Results of histopathological study showed 45 cases (26.5%) with moderate degree and 15 cases (8.8%) with severe degree of involvement with maedi like lesions that included 60 cases (35.3%) of the whole ovine lung samples. Results of serological study showed 34 cases (20.0%) with positive serums of the whole ovine serum samples.

Histopathological study on 15 cases (8.8%) of pulmonary lesions were similar to the lesions which previously described for maedi disease and serological results confirmed them abundantly. However, there are some pathogens that can cause nearly pathological lesions to maedi disease in ovine lung. This study showed that the pathogen causing maedi disease (maedi-visna virus) can be one of the pathogens causing chronic to subacute lymphoid interstitial pneumonia in Iran. Pearson chi-square and kappa tests on correlating histopathological and serological results were performed and discussed.

RELIABILITY OF NOVEL CANINE-SPECIFIC PUBLIC DATABASE SEQUENCE SETS

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The dog genome sequence release in July 2004 and the subsequent genome-wide computational prediction of protein coding sequences (GNOMON) are powerful tools for scientific applications. We present an analysis of cloned cDNAs involved in the intrinsic pathway of apoptosis, including a comparative analysis of genomic organization, to evaluate the reliability of dog genome and GenBank sequences.

We obtained complete coding sequences (cds) using RT-PCR on MDCK cell mRNA. Amplicons were cloned in a GST-tagged expression vector and sequenced.

Novel data retrieved so far include the complete cds of Bcl-w, Bak, Bad, Caspase-9, and Omi/Htr2A. The predicted Bcl-w and Caspase-9 sequences differed from our clones at the N-terminus by an additional sequence stretch and by a major incongruity, respectively. We further cloned the cds of p53, Bcl-XL, Bax, Survivin, and Caspase-3, which are already described. The dog genome sequence was instrumental for resolving differences between our sequences and those available in GenBank. A large portion of the C-terminus of Bax was found not to be covered by the dog genome sequences.

In conclusion, public domain data provide a large amount of reliable sequence information. However, they are incomplete and can contain errors, thus critical appraisal is recommended in every single case.

MALIGNANT OCULAR MEDULLOEPITHELIOMA IN A LLAMA

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Introduction: An adult female llama presented with a mass within the right globe. After enucleation, the llama developed masses within the right orbit and mandible and bilateral enlarged mandibular- and retropharyngeal- lymph nodes. Euthanasia was elected 30 months after initial diagnosis.

Material and methods: The carcass was submitted for necropsy. The enucleated globe and samples of affected organs and additional tissues were processed for microscopic examination. Electron-microscopy and immuno-histochemistry were performed on tissue specimens of the intraocular mass and affected lungs and liver.

Results: Post mortem examination revealed presence of masses within right orbit, mandible, lungs and liver and multiple lymph nodes. The primary intraocular mass and the secondary masses were composed of neoplastic undifferentiated neuroepithelial cells, which formed tubules, nests and scattered Flexner-Wintersteiner and Homer-Wright rosettes. Neoplastic cells were connected by desmosome-like junctions and contained intracytoplasmic organelles and basal bodies. Tumour cells were vimentin, nestin and microtubule associated protein 1B positive.

Conclusion: Findings were consistent with intraocular malignant medulloepithelioma with metastasis in lymph nodes, lungs and liver. Ocular medulloepitheliomas are derived from primitive neuroectoderm and are classified as benign or malignant teratoid or nonteratoid medulloepitheliomas. Medulloepitheliomas are uncommon ocular tumors in all animal species and rarely reported in llamas.

FIRST DESCRIPTION AND IMMUNOHISTOCHEMICAL STUDY OF MAST CELL IN CANINE MAMMARY GLAND TUMOUR.

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Several researches in human beings have demonstrated that mast cells accumulate in the stroma surrounding mammary adenocarcinoma, and molecules they secrete could benefit the tumor or act as a decoy and inhibit metastases. Mast cells have traditionally been evidenced by using metachromatic stains. However, two types of human mast cells have been described on the basis of the differences in their neutral protease composition: MCT cells, which contain tryptase only, and MCTC cells, which contain both tryptase and chymase. In the present study we investigated the presence of mast cells in canine benign and malignant breast lesions by staining them histochemically for metachromasia and immunohistochemically for tryptase and chymase. Mast cells were then counted morphometrically, and the results were statistically analysed. Our findings demonstrate, for the first time in dog, that MCs are a cell population well represented in mammary gland lesions and that their number could correlate with tumor malignancy. Moreover, on the basis of our findings, we speculate about the role of MCs products in tumour growth and progression.

AIDS AS A CELL CYCLE DISEASE: CLINICAL AND EXPERIMENTAL STUDIES ON SIV INFECTED PRIMATES

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Human AIDS is characterized by progressive loss of CD4(+) T cells and functional abnormalities of the surviving lymphocytes (bystander T cells) including loss of proper cell cycle control. Circulating T cells from HIV-infected patients display a marked discrepancy between a metabolic profile typical of G(0) and a pattern of expression of phase-dependent proteins that indicates a more-advanced position within the cell cycle. This discrepancy is enhanced by in vitro activation with ConA and ultimately results in a marked increase of apoptotic events. In this work, to better characterize the molecular features of this cell-cycle perturbations, we performed a detailed analysis of (i) the posttranslational regulation of nucleolin, a key structural protein in the nucleolus, (ii) intracellular concentration of cyclin B1 and related p34cdc2 kinase ; (iii) immune activation markers usually utilized in clinical pathology for the follow-up of HIV infection. We demonstrate here that p34cdc2 kinase activity; cyclin B1 expression and nucleolin fragmentation are increased only in pathogenic model of primate SIV infection (rhesus macaque). This is an indication that cell cycle disease is part of pathogenic mechanism of death of bystander T cells during lentiviral infection.

A CASE REPORT OF LIPOSARCOMA IN CAT

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A (7-year-old) mixed short-hair male cat was referred to Small Animal Hospital, Faculty of Veterinary Medicine, University of Tehran .On the basis of history, a mass with relatively fast growth was seen from 1-month before referring date. Clinical observations revealed a large subcutaneous mass at the right inguinal region. The mass was oval-shaped with a soft consistency that resembled to consistency of fat tissues.

Hematology examination was carried out. Radiography of thorax, abdominal and pelvic cavity showed a subcutaneous mass with fat consistency, without calcification and metastasis. Ultrasonography of right inguinal region, abdominal cavity and associated lymph nodes indicated an echoic circumscribed subcutaneous mass with an expansive growth and without foci of hemorrhages and necrosis. Surgically the mass completely was removed, and referred to department of pathology.

Macroscopically, the mass was yellowish in color with a fat consistency but there were foci that consistency of them were hard, resembled to connective tissue. There were no necrosis and hemorrhages. It seemed the mass was circumscribed but there was no obvious capsule surrounding the mass. Histopathologic examination revealed pleomorphic neoplastic cells with oval nuclei and single prominent nucleoli. Almost all neoplastic cells had a vacuole in the cytoplasm. The vacuoles had different size and special staining showed these were vacuoles of fat. There were foci of tumoral cells that were hypercellular. In these foci, the nuclei of cells were dark and elongated without obvious vaculation. Stroma of the tumor was scanty and there were no evidence of metastasis of tumor cells in blood vessels or lymphatics.

On the basis of clinical and radiological findings, biological behavior and histological characteristics, the mass was diagnosed as a relatively well - differentiated Liposarcoma.

HEPATOCELLULAR CARCINOMA IN SHEEP (A CASE REPORT)

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An adult (4-5-year-old) native male sheep (breed of mehraban) slaughtered at the Hamedan abattoir in January of 2004. Although the animal showed no symptoms that indicated liver involvement on the basis of history, but during the pre-slaughter physical examination, hepatomegaly caused slightly abdominal enlargement and a palpable mass in the cranial abdomen was evident.

Macroscopically, the liver was large, with numerous small, round discrete foci, a few millimeters in diameter to large circumscribed masses that were greater than 3 centimeters in diameter and involved throughout of the liver. These foci were tan in color. Grossly, there was no metastasis to other organs or implantation on the serosal membranes.

Microscopically, the neoplastic cells were often poorly differentiated and pleomorphic. The cells arranged in solid sheets with no apparent pattern. The nuclei of neoplastic cells were hyperchromatic and variable in both size and shape. Nuclear to cytoplasmic ratio (N/C) was obviously increased from 1/6 to 1/4 in normal hepatocytes to 1/1 or 2/1 in poorly differentiated cells. Mitotic figures were common and mitotic index was 5 to 6 mitoses in a microscopic field of 400 magnifications. The mitotic figures were often atypic and abnormal. Individual tumor giant cells were found in among of poorly differentiated cells. The neoplastic cells masses compressed adjacent liver parenchyma, but were no evidence of neoplastic cells invasion to blood vessels or lymphatics. The nonneoplastic liver parenchyma was histologically normal and there were no evidence of cirrhosis. Congestion and mild chronic cholangitis was also present.

MYOTROPIC AVIAN LEUKOSIS VIRUS (ALV) INFECTION IN A CHICKEN

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The study describes the morphological features of a highly productive myotropic avian leukosis virus infection in a chicken. A 3 months old, female backyard chicken in poor body condition was submitted for necropsy and specimens of selected organs were collected and processed for routine histologic examination. Tissue sections were examined immunohistochemically for detection of ALV group specific antigen p27 and samples of heart, intestine and ovary were examined by transmission electron microscopy. Distention of the body cavity, ascites, hepatic fibrosis and cardiomegaly were the main gross lesions observed. Histologically, the most striking pathologic change was the presence of oval, cytoplasmic basophilic inclusions in myocardial fibers in association with oedema and haemorrhages. ALV p27 immunostaining revealed a massive and diffuse presence of virus antigen in cardiac myofibers, in smooth muscle fibers of most of the organs examined, and in rare, scattered pancreatic and ovarian theca cells. At electron microscopic examination, myocardial cytoplasmic inclusions consisted of clusters of 50-60 nm round particles similar to A-type retrovirus particles with interspersed electron-dense granules. Moreover, numerous C-type particles were found in intercellular spaces of ALV p27 positive tissues. These results confirm previously reported data indicating that occasionally ALV is highly myotropic.

ICHTHYOSIS IN CHIANTINA CATTLE

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The study describes pathological changes, cytogenetical and genealogical data of three cases of ichthyosis observed in Chianina newborn calves. Three affected calves, sons of the same sire, were examined for gross lesions and skin specimens from different body regions were processed for histologic and ultrastructural examination. For cytogenetical analysis, mitotic chromosomes of the affected calves and their relatives were prepared for RBA-banding. Genealogical data were collected from ANABIC database and microsatellite genotyping was performed. The body of affected animals was diffusely covered with thick, scaly, horny skin, which was fissured into plates by deep clefts. The fissures, wide and reddened, were arranged according the normal skin foldings. Eclabium and ectropion were present. Intense and diffuse hyperkeratosis was detected by histological examination. The stratum corneum was thicker than normal as a consequence of a massive lamellar orthokeratosis that involved skin and hair follicle infundibula. Ultrastructurally, lamellar bodies were not detected in affected calves keratinocytes. Any abnormality was detected in chromosomes of affected calves. Pedigree data strongly suggested an autosomal recessive single trait Mendelian inheritance for the disease. In conclusion, clinico-pathological findings observed in these calves are consistent with a form of ichthyosis fetalis with an autosomal recessive inheritance

PATHOLOGICAL STUDIES ON SHEEP ENDOMETRITIS

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The study was carried out on 106 non-gravid uteri of slaughtered ewes in Basatein slaughter house. Blood and uterin tissue samples were collected and used for serological, bacteriological and histopathological examinations.

Blood samples were collected at time of slaughter into dry and clean tubes and kept at room temperature. Serum samples were harvested and stored at (-20 C) till used. The bacterial isolates from the non-gravid uteri were Staph. aureus (9 cases), Strept. pyogen (14 cases), Corynebacterium pyogen (17 cases), Salmonella spp. (5cases), Campylobacter fetus (4 cases), Mycotic isolates (17cases) and anthracoides (18 cases).This isolates were detected either as single or mixed infection.

Serological examination of the collected serum samples revealed that 58 out of the 106 examined cases reacted positively for Toxoplasma gondii (31 cases) using Latex, Salmonella spp.(21 cases) using Vidal test, Brucella spp. (3 cases) using Rose Bengal plate agglutination test and confirmed with Rivanol test and by using compliment fixation test , Chlamidia psittaci antibodies were detected in 3 cases.

Tissue samples from the uteri of slaughtered ewes were collected and fixed in 10 % formalin. The fixed specimens were washed , dehydrated and embedded in paraffin. Sections at 4-5 micron thickness were prepared and stained.

Endometritis was detected in 48.1% of the examined cases, which divided into: acute lymphocytic endometritis (33 case) , acute suppurative endometritis (6 cases) , chronic suppurative endometritis (7 cases) and granulomatous endometritis (5 cases) . Two cases of the granulomatous endometritis showed spherical yeast-like fungus invading the epithelial covering. Toxoplasma gondii antibodies were detected in 31 case of endometritis with percentage of 29.24% , salmonella spp. antibodies were detected in 21 case.

PATHOMORPHOLOGICAL FINDINGS OF SUBINVOLUTION OF PLACENTAL SITES (SIPS) IN THE BITCH

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Uterine samples of six bitches with persistent bloody vaginal discharge that occurred at different times after parturition were examined.

On gross examination, the placental sites appeared enlarged and irregularly thickened. The luminal surface was rough, grey to brown, with foci of haemorrhages. The interplacental endometrium was inconspicuous.

The most remarkable histological findings were large amounts of eosinophilic collagen masses in the subepithelial layer and numerous intact large polynuclear cells with foamy cytoplasm (so-called "trophoblast-like cells") lying within these masses and underneath. Furthermore, multifocal haemorrhages and haemosiderophages were recognised. The endometrial glands in the basal layer were moderately to severely dilated and filled with mucus. The periglandular stroma showed numerous mononuclear cells accompanied by a marked fibrosis.

The diagnosis SIPS was based on the course of disease and the characteristic pathomorphological findings, which differ from normal postpartum involution of the uterus.

MEMBRANE ATTACK COMPLEX DO NOT CAUSE THE DIFFERENT INFECTIVITY OF *TRICHINELLA SPIRALIS* AND *T. NATIVA* IN RATS

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Trichinella spiralis and *T. nativa* show clearly different infectivity to rats. We infected complement factor C6 deficient (C6-), and control (C6+) rats with *T. spiralis* and *T. nativa* to compare the effect of membrane attack complex (MAC) on these parasites *in vivo*. The 2000 larvae infection dose yielded with *T. spiralis* 652 larvae per gram (lpg) in C6- group and 608 lpg in C6+ group, but with *T. nativa* only 1.05 lpg in C6- group, and 1.87 lpg in C6+ group. The difference between the trichinella species was evident, but the infection intensity was not affected by the complement factor C6 deficiency. For *in vitro* observation, newborn larvae (NBL) were incubated in C6 deficient and control rat serum for 24h, with no changes in viability. There was no notable influence of the presence or absence of C6 on either of the *Trichinella* species *in vivo* or *in vitro*. We concluded, that both *T. spiralis* and *T. nativa* can control the effect of complement in rats.

DETERMINING INTERLEUKIN IL-6 LEVELS IN SERA OF SHEEP INFECTED WITH BOVINE LEUKEMIA VIRUS (BLV)

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Experimental data suggest that proinflammatory cytokines such interleukin 6 (IL-6) are very important in the pathogenesis of viral infections and neoplasma development. The study compared the production of this cytokine in 10 sheep experimentally infected with leukemia cow lymphocytes and in a healthy control group. Flow cytometry analysis with the use of monoclonal antibodies and conjugates was performed for lymphocyte phenotypes determination. Proviral DNA in lymphocytes and tumor cells was determined in PCR and nested-PCR. The presence of anti-BLV antibodies in the sera was detected by ELISA test. Expression of BLV glycoprotein gp51 and bcl-2 presence in lymphocytes and tumor cells in inner organs were detected with the same method. Interleukin 6 was determined by the serological ELISA test. The commercial diagnostic kit was used. There was tendency for higher IL-6 levels in the experimental animals compared with the control group. A negative correlation between the production of IL-6 and tumor development was found in animals with high lymphocytosis. IL-6 levels in the sera were much lower and rapidly decreased in the terminal stage of the disease. Tumors developed much earlier in these animals than in those with high concentrations of IL-6 in their sera. In conclusion, the correlation of the levels of the mentioned above cytokine having parameters of tumor development suggest that interleukin IL-6 is associated with neoplastic changes in BLV infected animals.

IL-6 has been found to play a central role in defence mechanisms, the immune response and acute phase reactions. Future studies on the regulation of IL-6 gene expression and the mechanisms of IL-6 action through its receptor, and development of inhibitors for IL-6 action, would provide information on the molecular mechanisms of diseases and the development a new diagnostic methods.

IMMUNOHISTOCHEMICAL DETECTION OF LEPTOSPIRES IN FORMALIN-FIXED AND PARAFFIN-EMBEDDED TISSUE SAMPLES OF ABORTED EQUINE FETUSES AND FETAL MEMBRANES

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Although leptospirosis is a well-known cause of abortion in veterinary medicine, there is a need for improved techniques for examination of fetal tissues. Nineteen-six cases of equine abortion from 57 Hungarian farms that occurred between 1998 and 2000 were investigated for the presence of leptospires by immunohistochemistry, silver staining and serology of the aborted mare. *Leptospira* induced abortion was found in 3 cases (3.1 %) from 3 (5.3 %) different farms by all of these diagnostic techniques. The two antisera (i, commercial available hyperimmune rabbit multivalent leptospiral antiserum; ii, polyclonal rabbit antibody produced against the leptospiral outer membrane protein LipL32) used for immunohistochemistry were found to be equally sensitive for detection of leptospires. These methods demonstrated leptospires in 19/21 (90.5%) tissue samples compared to silver staining which technique was positive only in 8/21 (38.1%) tissue samples. The attempt to identify of *Leptospira* serovars involved in the infection was also made with immunohistochemical method by using the serial dilutions of serovar-specific polyclonal reference sera produced for microscopic agglutination test. These sera showed strong cross-reactions with this test and so it was not possible to identify the *Leptospira* serovars responsible for abortion. These studies demonstrate the utility of immunohistochemistry in the diagnosis of leptospirosis as a cause of equine abortion.

INTESTINAL ULTRASTRUCTURE IN SHEEP WITH PARATUBERCULOSIS

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Bioptic samples of distal portion of ileum from 14 necroscopied sheep serologically and microbiologically infected by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and 4 control sheep were investigated by transmission electron microscopy. Characteristic changes were observed in the mucosa of all infected sheep, most typically an infiltration of numerous macrophages and eosinophils into the propria. Eosinophil granules showing Major Basic Protein lost, cytotoxic tissue changes and degranulating mast cells were present in tissues from MAP-infected sheep. Macrophages displayed large lysosomes with dense, irregularly shaped, myelin-like figures. Similar inclusions were also found in the surface epithelial cells of ileal mucosa. Severe alterations were observed in dilated sub-serosal and mucosal lacteals in which only closed intercellular junctions were observed, strictly associated with bilayered basal-laminae, strong perilacteal collagen deposition and heavy accumulation of protein rich lymph at the abluminal surface. MAP infection was constantly confirmed by evidencing of gold particles-labeled bacteria into macrophages by using an immunogold technique. In our opinion, intraepithelial and intramacrophagic storage of non-degradable microbial components as lipoprotein catabolites could be responsible for the initiation and propagation of a chronic inflammatory process.

EXPERIMENTAL HISTOPATHOLOGIC STUDY OF THE LESIONS INDUCED BY SEROTYPE 4/91(793/B) INFECTIOUS BRONCHITIS VIRUS

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Introduction: Infectious bronchitis continues to be a cause of serious economic problems for the poultry industry and the constant emergence of new serotypes of the virus, and their wide and variable tropism for tissues challenge vaccine strategies. Recently, a serotype infectious bronchitis virus (4/91, 793/B) has been reported in the Iran (Momayes et al., 2001) associated with serious economic losses. The aim of the study was threefold. Firstly, to describe the histopathologic changes of experimental infection of this serotype in 20 day-old specific pathogen-free (SPF) chicks, Secondly, to compare the effect of route of infection, and thirdly, to revealed the tissue tropism and infective virus content in the different tissues that was examined with the sites of virus replication.

Materials and methods: The study was conducted to determine the type, frequency and severity of gross, histopathologic changes, effect of route of infection and also, tissue tropism of serotype 4/91(793/B) infectious bronchitis virus. 20 day-old specific pathogen free chicks were inoculated intra-tracheally and intra-orally with 4/91 serotype and lesions studied histopathologically at interval up to 10 days post-inoculation (pi).

Results: The mortality was 7.5% in the IBV-inoculated groups but not in the controls one. Grossly, a small amount of clear mucus and slight congestion were present in the lumen of the trachea, also congestion observed in the lungs. Swollen and pale kidneys due to IBV infection were slightly severe. At the early stage of infection, the histopathological changes in the lungs, kidneys, intestine and other organs, were similar in both groups. So, the route of infection with IBV may affect the incidence of disease is not supported. At the late stage of infection, the changes of kidneys were more severe and characterized by nephritis with formation of lymphoplasmacytic nodules in kidneys and other feature of infection with this serotype on intestine was enteritis of both IBV-inoculated groups.

Conclusion: The serotype 4/91(793/B) isolated in Iran, had a broad tissue distribution that included: respiratory, digestive and urinary tract tissues. The histopathological changes had reported here were characterized by prominent lesions in the trachea and kidneys. Replication of IBV in the respiratory tissues causes characteristic changes. In the present study, the frequent finding of histopathological changes was in the epithelium of trachea and also in kidneys, intestine...

**THEM IMMUNOHISTOCHEMISTRY STUDY OF LESIONS DUE
TO AVIAN INFECTIOUS BRONCHITIS (SEROTYPE 4/91)
ON DIFFERENT TISSUES IN SPF CHICKS**

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Introduction: Infectious bronchitis, a viral disease of great economic importance to the poultry industry, affects the respiratory, urinary and reproductive systems of chickens. While IB is considered primarily a disease of respiratory system, different IBV strains may show variable tissue tropisms. Chong & Apostolov (1982) demonstrated with using immunofluorescence, that viral antigen was mainly distributed in many organs and virus replication was also confirmed ultrastructurally by Condron & Marshall (1986).

Materials and methods: A serotype of IBV was isolated from broiler commercial poultry flocks ,inoculated intra-tracheally and intra-orally into 20-day-old specific pathogen free(SPF)chicks [two infected groups(30),two control groups(30)].The lesions in trachea ,lung ,kidney and intestine was examined immunohistochemically at intervals up to 5 days post-inoculation(pi).

Results: Grossly, a small amount of clear mucus and slight congestion were present in the lumen of the trachea, also congestion observed in the lungs .The kidneys were pale and slight enlarged. The immunohistochemical method revealed that the changes in kidneys and other tissues were similar in both groups (intra-tracheal &oral).Viral antigens in infected cells of different organs diagnosed not only in intact epithelial cells, but also observed in degenerated and desquamated cells. Also, viral antigens appeared prior to the development of lesions and were detected in the cytoplasm of epithelial cells by 3 days post-inoculation .In the most cases, antigen-positive cells of organs were degenerated and desquamated.

Conclusion:This study revealed serotype 4/91 isolated in Iran (Momayes et al.,2001), introduced lesions on different tissues, especially on kidney .On the other hand, the serotype has a greater affinity and positive-tropism for the kidney than to other tissues.

FIBROTIC MYOPATHY OF THE SARTORIUS MUSCLE IN A DOG

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Introduction: Fibrotic myopathy is an acquired, progressive, chronic, degenerative disorder, with an unclear etiopathogenesis. It is associated with contracture and fibrosis, mainly of the gracilis or semitendinosus and sporadically of sartorius or other muscles in dogs.

Material and methods: A 5-year-old, male Irish Setter dog was presented for the evaluation of mild left hind-limb lameness of 2-month duration. After a thorough clinical and laboratory examination, diagnosis of sartorius muscle fibrosis was made. A complete surgical resection of the affected muscle was performed. Serial tissue samples were fixed in 10% NB-formalin and processed for histological and immunohistochemical evaluation.

Results: Histopathology of H-E, Masson trichrome, van Gieson and reticulin sections revealed increased proliferation of fibrous tissue, which replaced most of the striated muscle mass. The presence of bundles with abundant amount of collagen, areas with increased vascularisation and with spindle cell hypercellularity were characteristic. Degeneration of peripheral myofibers and mild mononuclear cell inflammation were less seen. Immunohistochemistry showed that most of fibrous tissue strongly expressed calponin and vimentin, was weakly positive for smooth muscle actin and negative for sarcomeric actin. More over, some peripheral areas were positive for myoglobin and desmin.

Conclusions: Possible trauma with subsequent inflammation and progressive massive fibrosis are considered as etiopathogenetic factors, whereas the myofibroblastic cell proliferation seems to play the major role in cellular content of prominent fibrosis

LARYNGEAL RHABDOMYOMA OF THE VOCAL FOLD IN A DOG

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Introduction: Although canine laryngeal tumors are rare, rhabdomyoma has been well documented in few publications. Some of these neoplasms had previously been mistaken as oncocyomas.

Material and methods: A 9-year-old, female German shepherd dog was presented with a history of loss of bark. During laryngoscopy, a localized mass on the left vocal cord was noticed. Endoscopic biopsy and cytology were performed.

Results: Imprint cytology indicated clusters of large ovoid or polygonal cells with abundant cytoplasm and uniform, mainly peripherally located nuclei. Histologically, the tumor consisted of medium sized to large polyhedral cells with distinct cell borders and abundant granular eosinophilic cytoplasm. Some cells resembled oncocytes or rhabdomyoblasts, other tumor cells were spindle or small and poorly differentiated. Mild focal inflammatory infiltrations with PMNs, plasma cells and macrophages were seen. Immunohistochemistry revealed strong staining for myoglobin and vimentin and focal staining for desmin. Tumor cells were weakly positive for muscle-specific actin and moreover, negative for pankeratin, S-100, GFAP and collagen- IV.

Conclusions: Laryngeal rhabdomyoma has to be differentiated from other rare tumors and immunohistochemistry is usefull. Reviewing tumor type, incidence and sites of occurrence in the larynx, only one case concerning "a granular cell tumor arising from vocal cord" has been found. To our knowledge, this is the first report of a rhabdomyoma localized on the vocal fold in the dog.

WSAVA LIVER DISEASES AND PATHOLOGY STANDARDIZATION RESEARCH GROUP

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AIMS OF LIVER HISTOLOGY STANDARDIZATION

To obtain worldwide standardization in histological evaluation of liver diseases of dogs and cats

THE LIVER STUDY GROUP

Internationally recognized specialists in veterinary clinical hepatology and hepatopathology, and one renowned human liver pathologists.

MATERIALS AND METHODOLOGY

Between 2001 and 2004 seven meetings were held. Before each meeting microscopic slides representing the spectrum of liver diseases in dogs and cats was sent to participating pathologists. During the meetings the complete group discussed and compared the slides with respect to the literature. They presented a proposal for the classification of the liver lesions as well as the description and name of the specific lesions at various specialist meetings (ECVIM,ACVIM,ACVP). After taking the comments into account, a final consensus report was formulated and photographs selected.

THE MONOGRAPH

The monograph provides: unified classification and nomenclature of liver diseases in dogs and cats, well-defined histological diagnostic criteria illustrated by more than 190 color photographs, and definition of chronicity stages and grades of diseases. Also, the relation with clinical and laboratory findings including ultrasonography, are given as well as recommendations regarding sample size and staining techniques. The presented poster shows an outline of the monograph including examples of diseases and photographs.

GLUCOCORTICOID INJECTION SITE ALOPECIA IN DOGS

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Subcutaneous injection of Glucocorticoids can cause injection site alopecia. The aim of this study was to clarify the clinical & histopathological changes. A single dose of 5mg/kg Methylprednisolon acetate subcutaneously was injected.

12 dogs of terrier cross breed, male and female with bodyweight between 5-6 kg and 1-2 years old were prepared. All of them were checking up and no sign of problem and symptoms were found. Ivermectin 0.3 mg/kg (two injections every 15 days) and Cephalexin 50 mg/kg were used for them and after a week, they were injected a single dose of 25mg Methylprednisolon acetate subcutaneously, dorsal part of the body near the neck.

All of them were shown focal alopecia after 45-60 days of injection. The sites of alopecia were round (3-4 cm in diameter), smooth, without erythma and itching and there wasn't any seborrhea on it. Biopsies were taken from the area and histopathologic findings were infiltration with macrophages and lymphocytes, dermal fibrosis and follicle dilated with keratin.

8-YEAR FOLLOW-UP OF RECURRENT PENILE PAPILLOMATOSIS AND CONVERSION TO SQUAMOUS CELL CARCINOMA IN A HORSE

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Genital papillomatosis in humans and several animal species including the horse is considered to be a pre-malignant lesion in the development of squamous cell carcinomas. This report describes the clinical and histopathological long-term evolution of a papilloma on the penis of a horse. A 13-year old mixed-breed gelding, was, over a period of 6 years, repeatedly presented with recurrent penile warts that were histopathologically confirmed as squamous papillomas. Immunohistochemistry did not reveal the presence of papillomavirus antigen. Eight years after the first papilloma was diagnosed and two years after the last papilloma had been removed, the horse (21 years old) was presented with a large coarse multinodular swelling of the penis and prepuce necessitating euthanasia. At necropsy, an ulcerated, infiltrating multinodular mass of the penis and prepuce was observed. The superficial and deep inguinal lymph nodes were enlarged and on cut section, multiple friable white bulging areas were present. Histopathologic examination of the genital mass and regional lymph nodes revealed a squamous cell carcinoma with metastasis to superficial and deep inguinal lymph nodes. No papillomavirus antigen could be immunohistochemically detected. This detailed follow-up indicates that conversion from a benign to a malignant lesion occurred late in the course of disease.

A CANINE CUTANEOUS EPITHELIO TROPIC LYMPHOMA WITH CEREBRAL AND OTHER METASTASES

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Epitheliotropic lymphoma in dogs is an uncommon cutaneous neoplasm. In general, epidermotropic malignant T-lymphocytes stay localised in the skin. However, sporadically, distant metastases may occur especially in animals with a prolonged clinical course. Brain localisations of malignant T-lymphocytes associated with epitheliotropic lymphoma have only been described once by Czasch et al. (2000) in a dog with mucocutaneous lesions.

This report describes brain localisation and metastases to heart and lung parenchyma secondary to a primary cutaneous epitheliotropic lymphoma associated with generalized skin disease. An 11-year-old Yorkshire Terrier, was presented with generalized alopecia, erythema and scaling of the skin of relatively sudden onset. A biopsy showed the presence of malignant CD3-positive T-lymphocytes within the epidermis and in the follicular epithelium. One month after the onset of cutaneous lesions, the dog died after a series of severe epileptic attacks. At necropsy, besides the generalized skin lesions, a firm white nodule was found in the temporal lobe of the right cerebral hemisphere. Both lungs contained multifocal white nodules and in the myocardium, white streaks were observed. Histopathologic examination revealed the presence of large amounts of malignant CD3-positive T-lymphocytes. The chronology and typical histological appearance of the lesions suggest metastasis of a cutaneous epitheliotropic lymphosarcoma.

MORPHOLOGICAL AND PROLIFERATIVE ASSESSMENT OF 30 FELINE INTESTINAL T-CELL LYMPHOMAS

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The current WHO classification of animal lymphoma is derived from the REAL schemes and allows to typify 75% of cases with routine histopathology while immunophenotyping is mandatory only for the remaining cases. Intestinal T-cell lymphoma (ITCL) is a category characterized by darkly stained, small-to-medium T-cells previously described as medium-to-large cells. The microscopic observation of 30 CD3+ feline ITCL shows that cell morphology can actually vary from small lymphocytes to large immunoblasts and the essential factors are represented by peculiar topography, epitheliotropism and frequent neoangiogenesis. Immunohistochemistry proves huge differences in proliferation activity, ranging from 0.2 to 83% of MIB1+ cells, and suggests to rearrange the ITCL category, that does not perfectly fit to atypical alimentary feline T-cell lymphomas, creating a further new subclass for them, as similar critical points have been solved in human medicine. Further steps in the improvement of lymphoma classification could be the challenge of histologic subtypes with respective biological behaviour.

ACUTE INTOXIKATION OF FATTENING PIGS WITH SELENIUM

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Pigs from two different farms in Austria were submitted for post mortem diagnosis. Clinically the animals showed elevated body temperature up to 41.0 °C and lesions of the claws. The morbidity was close to 100 percent. Both farms had feed from the same producer. The clinical suspect was an infection with swine vesicular disease with the differential diagnosis selenium intoxication.

Gross lesions were limited to the changes of the skin, haemorrhagic plaques under the horn of the claws and a marked swelling of different subcutaneous lymphnodes .

In the grey substance of spinal cord bilateral symmetric malacia with degeneration of nerve cells and circumscribed acute bleedings were found by microscopy.

High levels of selenium were detected in serum and tissues of the pigs. No virus was present. The way of the intoxication and the differential diagnosis will be discussed.

NEURONES UNDER STRESS: RESPONSES AND DEATH OF NEURONES TO PRIONS

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It has been proposed that the cellular PrP protein may be protective against oxidative stress. To determine whether this PrPC protein is upregulated in response to non-prion neuronal injury in vivo, brains of human cerebral infarct and murine middle cerebral artery occlusion were examined by immunocytochemistry and in situ hybridisation. PrPC was upregulated in both human and mouse ischaemic lesions. The volume of infarct lesion was significantly increased in PrP null mice suggesting that PrPC is stimulated as part of the generic neuroprotective response to injury. To investigate whether the misfolded PrPSc of prions induced further cellular stress, cell trafficking of synthetic prion peptides in neurones was examined in vitro using confocal microscopy and cell fractionation. A range of drugs was used to alter the surface distribution of PrPC and examine the effect on prion peptide trafficking and neurotoxicity. The cycling of pathogenic prion peptide through the Golgi and ER was strongly associated with stress-induced ER molecules; diverting the peptide away from the recycling pathway abrogated its toxic effects. Microarray analysis of scrapie-infected mouse brain also showed upregulation of stress-induced molecules before the onset of clinical disease. We conclude that infection of neurones by prions induces a stress response that includes upregulation of PrPC - the same molecule that is required for prion propagation. Interaction of PrPSc molecules with PrPC then induces further ER stress as PrPC undergoes its normal cell trafficking through the Golgi and ER, and leads to neuronal dysfunction and degeneration.

POSSIBLE MECHANISMS FOR TOXIC RENAL TUBULAR NECROSIS CAUSED BY *NARTHECIUM OSSIFRAGUM*

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Narthecium ossifragum, a perennial herb of the lily family, causes toxic renal tubular necrosis in several ruminant species. Nevertheless clinical signs of disease have only been diagnosed in cattle and moose (*Alces alces*). Aqueous extract from *N. ossifragum* causes similar lesions, and 3-methoxy-2(5H)-furanone has been suggested to be the nephrotoxic principle of *N. ossifragum*. Thus, aqueous extracts are ideal for in vitro studies of the nephrotoxic mechanisms involved, using LLC-PK1-cells grown as monolayers and in 3-D cultures as spheroids performing active water transport. When monolayer cultures were exposed to increasing amounts of extract, an increased concentration dependent cell death was observed. Similarly, reduced neutral red uptake and 3H-thymidine uptake (DNA synthesis) was observed. There was increased apoptotic activity with increasing concentration of 3M2F. When confluent monolayer cultures grown on Millipore filters were exposed, increased diffusion of inulin was observed. Similarly, multicellular spheroids stopped water transport, showed degenerative changes and collapsed.

These studies indicate junctional damage as well as reduced cellular endocytosis and (also) DNA synthesis as possible mechanisms for the acute tubular necrosis observed in ruminant species.

A FIRST CASE REPORT OF A MENINGIOMA IN A DOLPHIN (*DELPHINUS DELPHIS*)

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An intracranial tumor was found at necropsy in a grounded wild common dolphin. A gray- to tan-colored mass of 7 x 5 x 4 cm in diameter, lobulated and firm with a meningeous attachment was located at the cerebellopontine angle. The severe compression of the right cerebellum and the right hemisphere observed at the post-mortem examination could be the cause of an abnormal behavior of the dolphin leading to the stranding and the subsequent death of the animal. Microscopically, the tumor was composed of small interlacing sheaths and lobules of polygonal to elongate neoplastic cells surrounded by thin collagenous septae. The neoplastic cells strongly expressed vimentin and S-100 protein but were negative for Glial Fibrillary Acidic Protein. Tumor cells immunolabelling was in agreement with the histopathological diagnosis of a grade I meningioma (World Health Organisation classification). To the best of our knowledge, meningioma has never been described before, either in the dolphin or in other cetacean species.

TRANSMISSIBILITY OF AMYLOID DERIVED FROM CHEETAHS (*ACINONYX JUBATUS*)

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Our work on amyloidosis in captive cheetahs has shown a positive correlation between the incidence and grade of amyloid and the number of cheetahs housed per facility. Recent reports have shown that AA amyloid derived from cattle, mice and humans is highly transmissible. We conducted a study on the transmissibility of amyloid derived from cheetahs.

AIM: To clarify the transmissibility and pathogenicity of cheetah-derived amyloid.

MATERIAL & METHOD: Inflammation, which is known to enhance amyloid deposition, was stimulated in 61 ICR mice by hypodermic injection of 10% casein and Freund's complete adjuvant (0.3ml/mouse). Liver tissue from two cheetahs with hepatic amyloidosis and crude amyloid fibril from mice in which amyloid transmission has been demonstrated were prepared and inoculated as follows. Group 1(30 mice): Cheetah liver emulsion. Group 2(6 mice): Cheetah crude amyloid fibril solution, extracted by Pars' method. Group 3(12 mice): Cheetah purified amyloid fibril solution, prepared by NaCl precipitation. Group 4(6 mice): Mouse crude amyloid fibril solution, extracted by Pars' method. Group 5(7 mice): Non-inoculated control group, inflammation stimulation only. Inoculation was by oral administration or intraperitoneal injection in Group 1 and by intraperitoneal injection in Groups 2, 3 and 4. Histopathological analysis of all organs from the mice was carried

ied out at from 2 to 120 days after inoculation.

RESULTS: In mice inoculated with cheetah material, no amyloid deposition was found in Groups 1 and 2, but in Group 3, amyloid was observed in the spleen of 58% (7/12 mice), and also in the liver of 3 mice and the thymus of 2 mice. In Group 4, inoculated with mouse material, amyloid was found in the spleen of all mice (6/6). No amyloid deposition was seen in Group 5.

CONCLUSION: Our study showed that cheetah amyloid is transmissible, in the presence of deposition-enhancing inflammation, but to a lesser degree than amyloid derived from cattle, mice or humans.

HETEROGENEITY OF MACROPHAGE POPULATIONS AND ORIGIN OF MYOFIBROBLASTS APPEARING IN CISPLATIN-INDUCED RAT RENAL INTERSTITIAL FIBROSIS

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Macrophages and myofibroblasts play important roles in renal interstitial fibrosis. To know macrophage types and origin of myofibroblasts, we investigated immunophenotypes of these cells appearing in cisplatin-induced rat renal fibrosis models. In the acute model by a single injection, macrophages labeled by ED1 (exudate macrophages), ED2 (resident macrophages) or ED3 (activated resident macrophages) showed the highest number on day 4 or 7, and thereafter, the numbers were gradually decreased up to day 16; the number of macrophages labeled by OX6 (rat MHC II-recognizing antibody) was increased on day 7 and remained elevated up to day 16. Myofibroblasts show various cytoskeletons (vimentin, alpha-smooth muscle actin (SMA), desmin) during the development. In the chronic model by repeated injections, both renal epithelia and interstitial cells showed a strong positive reaction to SMA and vimentin, supporting epithelial-myofibroblastic transition (EMT); desmin was expressed in interstitial cells, but not in renal epithelia. Additionally, in vitro exposure of TGF-beta1, a fibrogenic factor, to porcine renal epithelial cells (LLC-PK1), rat renal interstitial fibroblastic cells (NRK-49F), and rat pluripotential mesenchymal cells (MT-9) increased selectively the SMA-positive cell number. These findings indicate heterogeneity of macrophage populations and origin of myofibroblasts in renal fibrosis.

$\gamma\delta$ T CELLS REGULATE THE EARLY INFLAMMATORY RESPONSE TO BACTERIAL INFECTION IN THE RESPIRATORY TRACT

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$\gamma\delta$ T lymphocytes, although comprising a small percentage of the pulmonary leukocyte population, have a critical role in regulating the inflammatory response to bacterial infection. This role is investigated using a well established murine infection model where the inflammatory response and underlying cytokine and chemokine signalling are compared between mice with targeted disruption of their T-cell receptor δ -chain gene [$\gamma\delta$ knockout (ko) mice] and wildtype (wt) controls. Following aerosol challenge with *Bordetella pertussis* more severe pulmonary inflammation with increased influxes of neutrophils is observed in ko mice, suggesting $\gamma\delta$ T lymphocytes normally prevent such exaggerated, potentially harmful, responses. Large-scale gene expression array analysis (Affymetrix) and quantitative RT-PCR of mRNA expression in the lungs of the two mice strains at 6 days post-infection revealed up-regulation of mRNA encoding chemokines CXCL-5, -7 and -10 in addition to CCL3 and IFN- γ in ko relative to wt mice. Up-regulation of genes expressing IL-1 β , CCR9, Igh-1 α , 4 and 6 occurred in wt animals relative to kos. These data suggest that the presence of $\gamma\delta$ T lymphocytes prevents early, excessive neutrophil-mediated pulmonary inflammation through the down-regulation of a number of key chemokines associated with neutrophil migration and activation.

RESISTANCE TO ANTIBIOTICS EXHIBITED BY GRAM-NEGATIVE RODS ISOLATED DURING THE WINTERING PERIOD OF CARP (*CYPRINUS CARPIO L.*) IN POST-COOLING WATER

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Studies were carried out to identify and determine the resistance to antibiotics exhibited by potentially pathogenic bacteria isolated from water, mucous, skin surface and alimentary tract of fish. The isolates were measured for resistance to 12 antibiotics most commonly used in the breeding and growing fish. The resistance was measured with the application of diffusion rings on a Mueller–Hinton medium. Twenty bacterial strains were isolated and identified based on the phenotype traits: *Aeromonas hydrophila* (13), *Shewanella putrefaciens* (4), *Aeromonas salmonicida* (2) *Aeromonas sobria* (1) and *Pseudomonas putida* (1). About 67% of the strains isolated from the alimentary tract and 78% of the strains isolated from the mucous exhibited resistance to more than one antibiotic. All the strains isolated from water showed resistance to 4 to 8 antibiotics. The largest number of different bacteria was resistant to oxacilin (18), less strains were resistant to ampicilin (15), vancomycin (13), trimethoprim (5) and individual strains were resistant to neomycin, streptomycin, oxytetracyclin, kanamycin, erythromycin, gentamicin

**PATHOMORPHOLOGICAL PATTERN OF THE INTERNAL ORGANS OF
YELLOW-NECKED MOUSE (*APODEMUS FLAVICOLLIS*, MELCHIOR 1834)
LIVING NEAR PESTICIDE TOMB IN WARLITY IN THE YEARS 2002 - 2004**

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The research was carried out in 2002 - 2004 on 120 yellow-necked mice caught in the forest in 3 zones near pesticide tomb (PT) and in the control zone 4 km from the PT. The highest number of morphological changes was noted in the liver. They were fewer in the kidneys. The highest intensification of the lesions was noted in the animals that lived closer to PT (zone 1 and 2). The biggest was the distance from the source of xenobiotics the smallest was the number of lesions. The changes observed concerned small areas of the organ examined and they were found near the blood vessels. They had appearance of the limited deviations from the regular structures. Retrogressive changes sometimes included several hepatocytes in the whole section. Parenchymatous degeneration was dominant among the lesions enumerated. Electron microscopic examination showed mitochondria with obliterated pattern of the cristas and sometimes their oedema in hepatocytes. Myelin-like structures were also noted in cytoplasm. Lysosomes and phagosomes were also observed. Extension of RER canals and sporadic RER vesicles transformation were present.

The research was financed with the grant 3 P04 G019 22.

**EFFECT OF THE PESTICIDE TOMB IN WARLITY ON THE
PATHOMORPHOLOGICAL PATTERN OF THE INTERNAL ORGANS OF THE
BLACK-STRIPED FIELD MOUSE (*APODEMUS AGRARIUS*, PALLAS 1771)
NEAR PESTICIDE TOMB IN WARLITY IN THE YEARS 2002 - 2004**

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Black-striped field mice were caught in 2002 - 2004, 10 in number, from each zone nearby the pesticide tomb (PT) and from the control zone every year (total: 120 animals). Morphological changes in liver and kidneys were noted more frequently in animals that lived closer to the PT than in animals that originated from distant region. They were mainly retrogressive changes. Circulation disturbances and progressive changes were relatively fewer. Moreover, destructive and also adaptive changes were noted in hepatocytes in electron microscope. Regarding more numerous (number statistically significant) morphological lesions in mice from the zones 1 and 2 in comparison with mice from the zone 3 and control zone it can be stated that pesticide tomb had the influence on their creation.

The research was financed with the grant 3 P04 G019 22.

**PATHOMORPHOLOGICAL PATTERN OF THE INTERNAL ORGANS
AND LEVEL OF DDT AND ITS METABOLITES IN CARP
(CYPRINUS CARPIO LINNAEUS, 1758) BRED IN POND NEARBY PESTICIDE
TOMB IN WARLITY IN THE YEARS 2002 - 2004**

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In Poland in the 70's different plant protection chemicals, including DDT, were intensively eliminated by the creation of 330 pesticide tombs. Some of them became dangerous for the environment.

The investigations were carried out in 2002 - 2004 on 30 carps from the pond nearby the pesticide tomb (PT) and 30 carps (85 - 100 g) from the control pond (C). DDT and its metabolites were analysed in the periintestinal and muscle fat (method of Amarowicz et al.). The gills, hepatopancreas and kidneys were examined microscopically (HE stain, PAS method according to McManus) and hepatopancreas was examined ultrastructurally. Mean content of DDT and its metabolites in the fat of carp is shown in the table:

Origin of carp	Year of study	DDE	DDD	DDT		Origin of carp	Year of study	DDE	DDD	DDT	ΣDDT
pond nearby pesticide tomb	2002	0.010	0.004	0.008	0.023	control pond	2002	0.005	0.002	0.001	0.008
	2003	0.044	0.004	0.022	0.070		2003	0.022	0.002	0.008	0.033
	2004	0.018	0.003	0.012	0.033		2004	0.009	0.005	0.005	0.019

Microscopically, the most frequent changes noted in carp from PT pond were: in gills - focal proliferation and vacuolisation of epithelial cells; in hepatopancreas and kidneys - parenchymatous degeneration and ultrastructurally - increased number of mitochondria and lysosomes, presence of myelin-like structures, proliferation of RER dilatation of ER cisterns and reduction of glycogen content in hepatocytes.

These investigations have shown that the level and number of intensity of morphological lesions was higher in carp living nearby the pesticide tomb in comparison with carp from the pond which was over 2 km from the source of DDT.

The research was financed with the grant 3 P04 G019 22.

THE ULTRASTRUCTURAL EFFECTS OF THE SUPEROVULATORY DRUGS PMSG/HCG ON THE MOUSE ENDOMETRIAL BASAL MEMBRANE.

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Studies and experiences have recently shown that the rate of successful implantation in stimulatory cycles is less than normal cycles. On the other hand, it is also shown that the endometrial basement membrane has a main role in implantation.

The ultrastructure of the basal membrane was compared in endometrial samples taken from 12 normally cycling mouse and from 12 superovulated with PMSG (10 IU/animal) and hCG (10 IU/animal). Animals were killed by cervical dislocation at the time of implantation (120 h after hCG injection). Endometrial biopsies were obtained from female mouse which were under superovulation treatment (N= 12) and those mouse which never given any drugs (N=12).The specimens were processed for electron microscopic studies. Qualitative(based on electron density) and quantitative thickness of basement membrane studies were carried out on the electron micrographs.The data have been compared using statistical methods.

Qualitative findings has shown that basement membrane has more electron density in case group. Also qualitative results have shown that it has more thickness. It has also shown that in case group, volume fraction of nucleus, RER, mitochondrion, glycogen to cell and euchromatin to nucleus had significantly difference. These results (based on present study findings) suggest that ovulation drugs have a negative effects on endometrial tissues at the time of implantation which may lead to low implantation rate.

GASTROINTESTINAL NEMATODE INFECTION IN GOATS: HISTOPATHOLOGY AND IMMUNOHISTOCHEMICAL FINDINGS

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The Authors describe the histopathological changes in the abomasum, small and large intestine, and mesenteric lymph nodes of goats naturally infected by gastrointestinal nematodes. Tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned and stained with haematoxylin and eosin, Periodic Acid Schiff and Alcian Blue PH 2.5. A kit with peroxidase-conjugated streptavidin and a mixture of biotinylated goat anti-rabbit and anti-mouse immunoglobulins was used for immunohistochemistry. A panel of monoclonal antibodies against Myeloid/Histiocyte antigen, CD 79 - B cell, and CD 3 - T cell were utilized for evaluating inflammatory cells. In all cases, we observed chronic inflammatory gastric and intestinal disease characterized mostly by intense infiltrates of lymphocytic cells in the lamina propria of villi, between hypertrophic crypts and sometimes in the submucosa. The immunohistochemical technique revealed the presence of CD3+ and CD79+ lymphocytes. Other inflammatory cells such as eosinophils, mast cells, plasma cells, macrophages and globular leucocytes were evident.

The villi appeared clubbed or atrophic and occasionally fused. The surface epithelium was lost or appeared low columnar to cuboidal. A severe dilatation of lymphatic vessels and a marked increase of mucinous secretion were present. Acid mucines were found in the abomasum and small intestine. The mesenteric lymph nodes showed hyperplasia, particularly of CD79+ B cells and plasma cells in all infected goats.

ESVCP

CYTOLOGY AND HISTOLOGY CORRELATION IN INFLAMMATION AND INFECTION

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Cytopathology, both in humans and veterinary medicine, is mainly used to diagnose neoplastic lesions. However, utilizing abrasive, exfoliative, and fine-needle aspiration techniques to diagnose infectious organisms by cytopathologic means is common practice.

In air-dried cytology samples, infectious organisms are more readily visible than in histology. This is due to the evidence that in cytologic preparations cells and other component of the smear are viewed well-spread on slide ("fried-egg appearance").

Although some organisms cannot be definitively classified on cytologic preparations, morphologic clues that can guide the pathologist toward rendering a diagnosis helpful to clinicians are often present. In addition, some fungi and parasites rarely identified in cytopathology in the past are more frequently found in cytologic specimens today.

This presentation focuses on cytopathology of some organisms found in Europe. Special attention is paid to the morphologic findings on routine Romanowsky-type-stained cytologic preparations and readily available special stains. Moreover, is discussed the role of cytopathology in the study of the clinical characteristics and evolution of some infectious diseases (e.g. feline leprosy).

EQUINE RESPIRATORY CYTOLOGY AND HISTOLOGY CORRELATIONS

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The equine respiratory system is particularly challenging for antemortem evaluation by cytology and histology. The respiratory system is a tubular system with dynamic interactions between the lumen, its epithelial lining and the interstitium. The lung parenchyma is subjected to a variety of environmental insults, infectious agents and changes in transmural pressure during exercise that may affect the features we observe clinically and in cytologic and histology specimens. The contents of the respiratory lumina may differ significantly from those of the interstitium of the lung and the contents of the lumen may differ according to the level of the respiratory tree and the function of the mucociliary apparatus.

Two methods of respiratory collection are commonly used, but collection, preparation and examination techniques are not be standardized across all organizations, laboratories and teaching institutions. This makes comparison of data from different institutions and different collectors difficult, at best, or impossible in many instances.

The transtracheal washing, performed by penetration of the midtrachea has fallen into disuse because more convenient and less invasive means for tracheal washing collection are available by endoscopic collection. Endoscopic collection has the added advantage of visual inspection of the respiratory system during the collection process. The endoscopic tracheal washing is well tolerated by the patient and owner, causes little coughing or distress and usually does not require sedation. Theoretically, the tracheal washing has the potential to collect material from a large area of the pulmonary tree, as well as material from the tracheal carina or pool just anterior to the bifurcation of the large airways, where there is usually an anatomic depression in the trachea near the thoracic inlet. Care should be taken to attempt to collect material from a distance along the trachea and not confined to the tracheal carina where aged material may accumulate. Disadvantages of the tracheal washing technique have been reported to include a predisposition to accumulation of neutrophils and columnar epithelial cells compared to bronchoalveolar lavage and decreased likelihood of representation of cells from the interstitium. Advantages of this technique include ease of collection, potential to represent a large area of the lung and large body of literature with regard to interpretation of this type of specimen. It depends on the mucociliary apparatus and/or coughing to move material to the upper portion of the respiratory system where it is available for collection by washing....

CYTOLOGICAL BASIS FOR THE KIEL AND WHO CLASSIFICATIONS OF CANINE LYMPHOMAS, WITH EMPHASIS ON PARTICULAR ENTITIES AS A CRUCIAL STEP FOR PROGNOSIS.

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The new Classifications of lymphomas are based on morphology, immunophenotype, postulated normal counterpart in the immune system and correlation with clinical data. As a matter of fact, the goal is to define disease entities having clinical relevance and that can be recognized by pathologists.

In that way, a recent paper in the Veterinary Journal (F.Ponce et al, 2004) demonstrates the clear correlation between canine lymphoma morphological subtypes and prognosis during chemotherapy.

On a morphological point of view, the interest of a cytohistological classification of NHLs appears obvious as the examination at the cellular level is now an essential step to make the diagnosis of the various types of NHLs either in cytology or in histology.

In our experience, there appears to be a good correlation between morphology and phenotype allowing the pathologists to suspect the T-phenotype by cytohistologic analysis in most cases.

However, whatever the reliability of the morphological diagnosis, the precise determination of the immunophenotype by immunolabelling is the unquestionable method to establish the immunophenotype especially for difficult cases.

® The reliability of the diagnosis of NHLs depends:

- first, from a practical point of view, on the quality of the methodology, either in cytology or in histology;
- second, from a theoretical point of view, on:
 - the precise knowledge of the histological organization, cytological aspect and groupings of normal lymphoid cells;
 - the clear understanding of the lymphoid differentiation (successive morphological stages and immunological markers);
 - the rules of lymphomatous transformation.

® The morphological diagnosis is based on:

1. The architectural pattern

- diffuse or follicular organization;

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