PATHOLOGY IN NOWADAYS

UWM

Olsztyn – Poland
2004

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PATHOLOGY IN NOWADAYS

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OF WARMIA AND MAZURY IN OLSZTYN

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OLSZTYN – POLAND
15 – 18 SEPTEMBER 2004

Congresses organized jointly by:

European Society of Veterinary Pathology
and European Society of Veterinary Clinical Pathology
in cooperation with
Polish Society of Pathologists
and Small Animal Veterinary Association

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OFFICIAL CONGRESSES WEBSITE

http://www.uwm.edu.pl/esvp

WELCOME MESSAGE

Dear Colleagues and Friends,

On behalf of the Organizing and Scientific Committee of the 22nd Annual Meeting of the European Society of Veterinary Pathology and of the Faculty of Veterinary Medicine, University of Warmia and Mazury, it is a great pleasure and honour to welcome all of you in Olsztyn, Poland.

Our meeting will be held at the University of Warmia and Mazury in Olsztyn, in significant academic and cultural centre of North-Eastern Poland. We are convinced that both, our academic village (counting over 40000 students, 1700 scientists, situated near two lakes) and 650 years old Olsztyn (with castle of the XIV century, where Nicolaus Copernicus lived and worked) will appeal to you very much. All the participants will have the chance to know the beauty of Olsztyn and Poland.

Let me introduce you to the 22nd Annual Meeting of the European Society of Veterinary Pathology. Traditionally ESVP Congress is held every year. The Olsztyn Meeting will be the first joint congress of ESVP and European Society of Veterinary Clinical Pathology. In that way, 22nd Congress of ESVP and 6th Congress of ESVCP will offer a unique platform for the exchange of information and experience as well as presentations and discussions about the last achievements in our research. It should enhance international scientific cooperation and elaborate applications for daily practice. It will provide a comprehensive update of the state-of-the-art in all relevant disciplines contributed to pathomorphology, morphology and clinical pathology. Plenary lectures (6), lectures (29), parallel sessions (with 51 oral presentations) and posters (179) will cover the subjects of histopathology, ultrastructural pathology, molecular pathology, pathology of genetically engineered animals, toxicological pathology, experimental pathology, techniques in pathology and morphological sciences, clinical pathology and toxicological clinical pathology. Programme also includes two workshops in the form of Mystery Slide Seminar on Reproductive Tract Pathology and Case Reviews.

The organizers of the Congresses would like to thank all those who have contributed to the organization of these events, particularly to the board of European Society of Veterinary Pathology, European Society of Veterinary Clinical Pathology, Polish Society of Pathologists and Polish Small Animal Veterinary Association. We are also grateful for help of all our sponsors and trade exhibitors. We also thank to all Congress Delegates for coming to Olsztyn and for taking part in the creation of these events for the first time in North-Eastern Europe.

We believe that 22nd Congress of ESVP and 6th Congress of ESVCP will be held at as high level as those of previous years and that they will be "good harvest of the research field" and also provide you with the opportunity to stimulate your own investigations.

We wish you a pleasant and fruitful staying in Olsztyn.

Prof. Dr. hab. Józef Szarek
President of the Organizing and Scientific Committee

EUROPEAN SOCIETY OF VETERINARY PATHOLOGY

The contemporary image of veterinary pathology

Veterinary pathology, due to its nature, holds an honourable place among the veterinary sciences. Its historical role, i.e. post-mortem diagnosis of morphological lesions and finding causes of death, is still important; however, biopsy pathology has caught on and spread. It involves intravital morphological examination of organs, tissues and cell obtained with bioptic techniques. Immunopathology nowadays enables often a comprehensive and precise diagnosis of tumours and determination of degree of their malignancy, supported during recent years also by molecular biology methods. It became evident that precise pathomorphological results obtained from macroscopic, histological and subcellular analyses not only enriches scientific knowledge but also becomes a tool for a veterinary surgeon who treats patients and saves lives.

ESVP founding history

ESVP originates from the "Arbeitsgemeinschaft für Veterinärpathologien", an organisation founded in 1950 in the Federal Republic of Germany. In the 1950s, this organisation brought together veterinary pathologists from the Federal Republic of Germany, Switzerland, Austria, Holland, Belgium and Italy. After adding the word "Europäische" to its name in the late 60s, it was transformed into the "Europäische Gesellschaft für Veterinärpathologie" in the 70s – still having a German name. Back then, German, English or French were spoken at the board meetings and scientific congresses. Membership and the range of the association activities expanded in time and in 1979s English was made the official language for communication within the society which then got the present name, European Society of Veterinary Pathology (ESVP).

The members of the ESVP

- Currently, the ESVP has more than 500 members from different countries, including such distant countries as Australia, South Africa, Japan and India.
- Central European nationalities predominate among the members.
- There are currently only two members from Poland: Prof. Dr. Józef Szarek, Ph.D., habil. (member since 1985) – szarek@uwm.edu.pl and Dr. Małgorzata Sobczak-Filipiak, Ph.D. (member since 2002) – filipiak@alpha.sggw.waw.pl
- Honorary membership in the ESVP was granted to 18 members who were distinguished for their particular scientific contributions and professional work as well as for their outstanding support of the society.
- New members are accepted during ESVP annual meetings based on the submitted documents i.e.:
  1. scientific biography of a candidate member,
  2. two written recommendations prepared by two members of the ESVP.
- Detailed information on the requirements for candidate members is available at the Internet address: http.//lang.bris.ac.uk/eurovet/esvp.htm
- Candidate member profiles are presented during plenary sessions of annual meetings of ESVP. Candidates are accepted after successful voting. Twenty one new

members were accepted during the 21st Annual Meeting of ESVP on 11 September 2003 in Dublin. The annual membership fee since 2002 has been Euro 75.

The ESVP authorities

Current board of the ESVP:

- President – Prof. Dr. Manfred Reinacher (Giessen, Germany, manfred.reinacher@vetmed.uni-giessen.de),
- Honorary Secretary – Prof. Dr. Cinzia Benazzi (Bologna, Italy, benazzi@vet.unibo.it),
- Honorary Treasurer – Dr. Klaus Krauser (Illinois, USA, klaus.krauser@abbott.com).

European College of Veterinary Pathology (ECVP) and Summer School activities

The ESVP pays great attention to extending the knowledge of veterinary pathology and keeping a high professional standard. Therefore, an European College of Veterinary Pathologists (ECVP) was founded in 1995. Both, ESVP and ECVP are now organizing summer school classes for the education of young veterinary pathologists. In August 2003, the first classes of a summer school were held in Nantes (France). Fifty five participants from England, Belgium, Finland, France, Spain, The Netherlands, Montenegro, Portugal, Germany, Serbia, Switzerland, Sweden and Italy took part. They were mainly scientific staff of veterinary faculties and some employees of state diagnostic laboratories and the pharmaceutical industry. The curriculum focused on, among others, liver and skin pathology, carcinogenesis, cell pathology, electronic microscopy and bird pathology.

The next summer school will be held in Padova (Italy). In 2004, about 70 participants were present. The curriculum includes pathology of the alimentary and respiratory tracts, nervous system and skeletal muscles as well as inflammations and pathologies in animals kept in zoological gardens.

Information on training by ESVP/ECVP is available via Internet: http://bris.ac.uk/pathandmicro/eurovet/eurovet.htm and from Prof. Dr. Anja Kipar, akipar@liverpool.ac.uk.

Professional training within veterinary pathology is organised by the Educational Committee, on which until now Cinzia Benazzi, Massimo Castagnara, Philip Detilleux, Pierre Duprat, Thomas Hodge, Anja Kipar, Manfred Reinacher, Eugenio Scanziani, John Vanderberghe and Monique Wyers have served. The main task of the committee' is to organize the summer school and other preparation courses for for the ECVP board exam.

Prof. Dr. Maja M. Suter (Bern, Switzerland, maja.suter@itpa.unibe.ch) currently is president of the ECVP. The ECVP membership principles were established during the 12th ESVP Congress in Mondovia (Italy) in 1994. Requirements for the ECVP member candidates are:

1. appropriate scientific output in the field of veterinary pathomorphology, experience in conducting classes in the field,
2. successful results at an examination before an appropriate commission.

Currently, about 250 members belong to the ECVP.

Annual meetings of the ESVP

Each year the ESVP organizes congresses in September, which are attended by 200-400 people. These meetings are of special importance due to the considerable role that veterinary pathomorphology plays in the progress of medicine and public health protection.

In Amsterdam in 2000, the board of the ESVP decided to hold the 22nd congress in Olsztyn, Poland. This is felt as an honour by both, the University of Warmia and Mazury in Olsztyn and Poland. Thus, this will be the first ESVP annual meeting to be held in the central or Eastern Europe. This choice of a venue is to serve the wide popularisation of veterinary and clinical pathology among veterinary doctors (scientific staff and practitioners) mainly in Poland and neighbouring countries.

The ESVP Congress in Olsztyn will be preceded (on 14-15 September 2004) by a preparatory course developed by Prof. Dr. Alun Williams (England, alunwilliams@rvc.ac.uk) within the European Branch of the DVM Charles Louis Davis Foundation, USA.

The 22nd ESVP congress will be held on 15-18 September 2004 at the University of Warmia and Mazury in Olsztyn. The honorary patrons will include the President of the Republic of Poland Aleksander Kwaśniewski and the Minister for European Integration, Prof. Dr. Danuta Hübner. The congress is organised by the ESVP and the European Society of Veterinary Clinical Pathology (ESVCP, Prof. Dr. Joy Archer, president) in co-operation with the Polish Society of Pathologists and the Polish Small Animal Veterinary Association. The congress will focus on macroscopic, microscopic, ultrastructural, molecular and experimental veterinary pathology as well as pathology of transgenic animals, toxicopathology, morphological sciences (animal histology and anatomy) and clinical pathology with the application of technology.

Manfred Reinacher, Józef Szarek

Meeting History of the European Society of Veterinary Pathology and the Predecessing Societies

No	Year	Location	Name of Society	President
1	1951	Hannover, Germany	Arbeitsgemeinschaft für Veterinärpathologen	Paul Cohrs
2	1952	Freiburg, Germany	- " -	Paul Cohrs
3	1953	Marburg, Germany	- " -	Paul Cohrs
4	1954	Hamburg, Germany	- " -	Albert Hjärre, Stockholm
5	1955	Zürich, Switzerland	- " -	Albert Hjärre, Stockholm
6	1956	Düsseldorf, Germany	- " -	Johannes Dobberstein, Berlin
7	1957	Giessen-Bad Nauheim, Germany	- " -	Jan Henrik ten Thije, Utrecht
8	1958	Wien, Austria	- " -	Georg Pallaske, Giessen
9	1960	München, Germany	- " -	Hans Sedlmeier, München
10	1961	Münster, Germany	- " -	Hugo Stünzi, Zürich
11	1962	Dortmund,	- " -	Walter Renk, Berlin

		Germany		
12	1963	Basel, Switzerland	- " -	Harro Köhler, Wien
13	1964	Salzburg, Austria	- " -	Paul Cohrs, Hannover
14	1965	Saarbrücken, Germany	- " -	Jan Henrik ten Thije, Utrecht
15	1966	Heidelberg, Germany	- " -	Kurt Potel, Leipzig
16	1967	Göttingen, Germany	- " -	Erwin Dahme, München
17	1968	Würzburg, Germany	- " -	Irmgard Gylstorff, München
18	1969	Mainz, Germany	Europäische Arbeitsgemeinschaft für Veterinärpathologen	Hans-Jörgen Hansen, Stockholm
19	1970	Berlin, Germany	- " -	Joseph Hoorens, Gent
1. Herbsttagung	1970	Bern, Switzerland	- " -	Leo-Clemens Schulz, Hannover
20	1971	Nürnberg, Germany	- " -	Leo-Clemens Schulz, Hannover
21	1972	Graz, Austria	- " -	Eugen Weiss, Giessen
22	1973	Karsruhe, Germany	- " -	Hansruedi Luginbühl, Bern
2. Herbsttagung	1973	St. Vincent, Italy	- " -	Gerhard Trautwein, Hannover
23	1974	Interlaken, Switzerland	- " -	Gerhard Trautwein, Hannover
24	1975	Kiel, Germany	Europäische Gesellschaft für Veterinärpathologie	Joachim von Sandersleben, München
25	1976	Freiburg, Germany	- " -	Paul Cohrs, Hannover
3. Herbsttagung	1976	Utrecht, The Netherlands	- " -	Franco Guarda, Turin
26	1977	Erlangen, Germany	- " -	Franco Guarda, Turin
27	1978	Wien, Germany	- " -	Johan Mouwen, Utrecht
4. Autumn Meeting	1978	Como, Italy	- " -	Rudolf Fankhauser, Bern
28	1979	Stuttgart, Germany	- " -	Rudolf Fankhauser, Bern
29	1980	Bremen, Germany	- " -	Horst Loppnow, Berlin
5. Autumn Meeting	1980	Gent, Belgium	- " -	Giancarlo Mandelli, Milano
30	1981	Innsbruck, Austria	- " -	Giancarlo Mandelli, Milano
31	1982	Göttingen, Germany	- " -	Samuel Lindt, Niederwangen/Bern
6. Autumn Meeting	1982	Alfort, France	- " -	Klaus Dämmrich, Berlin
32	1983	Luzern, Switzerland	- " -	Klaus Dämmrich, Berlin
33	1984	Berlin, Germany	- " -	Hans König, Bern

7. Autumn Meeting	1984	Utrecht, The Netherlands	- " -	Knut Frese, Giessen
34	1985	Köln, Germany	- " -	Knut Frese, Giessen
35	1986	Heidelberg, Germany	- " -	Roland Rudolph, Berlin
8. Autumn Meeting	1986	Cordoba, Spain	- " -	Wolfgang Drommer, Hannover
36	1987	Salzburg, Austria	- " -	Wolfgang Drommer, Hannover
37	1988	Hannover, Germany	- " -	Amador Jover, Cordoba
9. Autumn Meeting	1988	San Remo, Italy	- " -	André-Laurent Parodi, Alfort
38	1989	Koblenz, Germany	- " -	André-Laurent Parodi, Alfort
39	1990	Aachen, Germany	- " -	Joachim Pohlenz, Hannover
10. Autumn Meeting	1990	Zürich, Switzerland	- " -	Andreas Pospischil, Zürich
40	1991	Friedrichshafen, Germany	- " -	Andreas Pospischil, Zürich
41	1992	Graz, Austria	- " -	Hugo Burtscher, Wien
11. Autumn Meeting	1992	Zaragoza, Spain	- " -	Franz Hartig, Mannheim
42	1993	Würzburg, Germany	- " -	Franz Hartig, Mannheim
43	1994	Zürich, Switzerland	- " -	Franco Guarda, Turin
12	1994	Mondovi, Italy	European Society of Veterinary Pathology	Franco Guarda, Turin
13	1995	Edinburgh, UK	- " -	Franco Guarda, Turin
14	1996	Gent, Belgium	- " -	Franco Guarda, Turin
15	1997	Sassari-Alghero, Italy	- " -	Franco Guarda; Turin
16	1998	Lillehammer, Norway	- " -	Andreas Pospischil, Zürich
17	1999	Nantes, France	- " -	Andreas Pospischil, Zürich
18	2000	Amsterdam, The Netherlands	- " -	Andreas Pospischil, Zürich
19	2001	Thessaloniki, Greece	- " -	Richard Ducatelle, Gent
20	2002	Turin, Italy	- " -	Richard Ducatelle, Gent
21	2003	Dublin, Ireland	- " -	Richard Ducatelle, Gent
22	**2004**	**Olsztyn, Poland**	**- " -**	**Manfred Reinacher, Giessen**
23	2005	Naples, Italy		
24	2006	Edinburgh, UK		
25	2007	München,		

		Germany		

Manfred Reinacher

PLENARY LECTURES

LABORATORY AND PATHOLOGY INFORMATICS: MANAGING DATA FOR RESEARCH AND DECISION SUPPORT

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Laboratory and pathology informatics is a clinical and academic subspecialty of pathology that involves collecting, examining, reporting, organizing, storing, and retrieving large and complex sets of data from clinical and research laboratories. Data sets include laboratory test results, cytology and histopathology reports, image files, and experimental data, including genomic, proteomic and tissue array data. Clinical and anatomic pathologists must strive to maintain and improve data integrity, ensure data confidentiality and accountability, and facilitate the integration of laboratory, pathology, and clinical databases. Ultimately, the goals of pathology informatics are to improve clinical practice, enhance understanding of disease, and discover new therapeutic tools. Accomplishment of these goals requires: 1) the development and application of standards; 2) enhancing the value of data through the development of decision support tools; and 3) data mining to derive new information from existing data sources. Laboratory standards are critical to database quality and consistency and include controlled vocabularies and uniform terminology (i.e. SNOMED) for pathologic diagnoses. Standardized reporting guidelines for surgical pathology specimens have been recommended. The Logical Observation Identifier Names and Codes (LOINC), a Health Level 7 message standard is a free, public database that ensures unambiguous identification of a given test. Decision support tools include interpretive guidelines such as flags or coded comments, automatic notification of critical or threshold values, and cascade (reflex) testing. Expert and machine learning systems have been developed for decision support, including both Web and wireless applications, and may facilitate evidence-based medicine through linkage with journal databases. PDAs are an essential tool for rapid access to databases of test information and patient results. Data mining of medical and biological databases is a process that combines statistics, visualization, machine learning, and extraction techniques to gain insights into relationships and patterns hidden in data. The digitization and integration of genomic, tissue, and laboratory data are being used to facilitate drug discovery and toxicologic safety assessment. Challenges in pathology and laboratory informatics include scalability and financial constrains for veterinary laboratories, the integration of decentralized testing, and adequate training of clinical and anatomic pathologists in the principles and application of informatics (see Henricks et al., 2003, Arch Pathol Lab Med, 127: 1009-1018 for a comprehensive set of learning objectives). The Association for Pathology Informatics (API, www.pathologyinformatics.org), a division of the American Society for Investigative Pathology, is the main organization with a focus on pathology informatics. The API supports advances in the field through research, education, and scientific meetings, plays a leadership role in the promotion of data standards, and takes an active role in regulatory and governmental issues related to pathology informatics. The Pathology Informatics Committee of the American College of Veterinary Pathologists promotes similar goals in veterinary clinical and anatomic pathology.

KEY NOTE LECTURE: GENERATION AND PHENOTYPING OF GENETICALLY ENGINEERED ANIMALS

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Genetically engineered animals play an increasingly important role in biomedical research, such as, functional genomics, "gene farming", drug testing and animal models of human diseases.

Contemporary genetic engineering techniques include (1) overexpression of an artificial gene construct using DNA microinjection into the pronucleus or retroviral vectors and (2) targeted mutagenesis using homologous recombination of the construct with the target genomic sequence. The latter approach is used to generate the so-called knockout- or knockin- animals in which a specific gene is either inactivated or replaced by a different gene. The first approach (1) has been used in a large number of different species whereas the second approach (2) is usually restricted to the mouse. The two approaches are very different with regard to the definability of the obtained genetic manipulation, the required time and efforts and the interpretation of the phenotype. A recent and important extension of the knockout techniques is the use of conditional gene targeting using the Cre/loxP and Flp/FRT technologies. These systems are used to, for example, overcome the frequent problem of embryonal lethality by activating a knockout genotype only in a specific cell type or at a desired time point after birth.

"Phenotyping" describes the process of the analysis of changes and lesions that are induced by a specific genetic alteration. Such analyses may comprise biological data, behavioral phenotyping, gross and microscopical morphology, biochemistry and challenge experiments (infections, carcinogenicity studies, etc.). The pathology of genetically engineered animals usually plays the central role in the interpretation of a given phenotype.

Interpretation of lesions in a genetically engineered animal not only requires a profound understanding of the genetic manipulation but also critically depends on the awareness of factors that frequently modulate a given phenotype. Modulating factors include spontaneous disease unrelated to the manipulation (so-called background pathology), environmental factors, such as, microbial conditions, housing, nutrition and, particularly in mice, the genetic background (strain) of the examined animal. Importantly, the inclusion of appropriate control animals (usually wild type littermates) helps to differentiate specifically induced changes from background pathology. It is imperative that these factors be considered in the interpretation of changes observed in a genetically engineered animal.

CLINICAL AND DIAGNOSTIC APPROACHES TO RENAL DISEASES IN DOGS

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Kidney diseases are very common in the veterinary practice. Due to the role of kidney in homeostasis, excretion and haematopoiesis the clinical sings are variable and seldom misleading.
Like in other systemic diseases logical approach to the diagnosis is the principal key in successful solving the problem.
First step must be focused on signalement and history because some diseases are very closely breed and age related.
Lethargy, anorexia, vomiting, polyuria/polydipsia (pu/pd) and dysuria including haematuria and proteinuria are the most common signs of kidney diseases. The main diagnostic effort must be focused on establishing whether the clinical signs are associated with prerenal, renal or postrenal problem as well as whether they are primary or secondary disease.
The most common signs in chronic renal diseases include pu/pd, weight loss, anorexia, hypertension and sometimes vomiting. Each one may be seen in other than renal diseases; so additional tests must be performed.
Urinalysis, including quantitative protein content, protein/creatinin ration, and sediment examination must be a part of minimum database for any dog presenting with above-mentioned clinical signs. In selected cases, when infection of urinary tract is suspected, urine culture and sensitivity testing should be performed. For this examination urine must be collected by cytocentesis. This procedure is necessary to exclude pyelonephritis, potential cause of all clinical signs resembling other than infectious kidney diseases.
In advanced cases, with systemic sings, additional tests such as complete blood count and plasma biochemistry panel (BUN, creatinine, blood ammonia) must be performed.
Diagnostic radiography, ultrasound and computed tomography can also yield valuable information about urinary tract, especially kidney. These procedures may help to establish stage of kidney damage, developmental abnormalities or neoplastic diseases.
If potential cause of renal disease has not been identified after performing above diagnosticals a renal biopsy should be done.
All our efforts must be focused on determining the potential cause of disease or evaluating the stage of disease. This may help us to introduce specific treatment and to estimate prognosis as well as establish whether kidney disease is primary or secondary problem.
Renal amyloidosis and chronic glomerulonephritis are the frustrating idiopathic diseases in which careful clinical and laboratory examination must be performed. In the presented review the diagnostic steps in the diagnosis of these two diseases will be discussed.

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EXPLOITING CELL BIOLOGY TO ENAHNCE VACCINE EFFICACY

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Vaccination is without peer in control of infectious diseases of animals and humans. The development of attenuated live vaccines has led to remarkable control over viral infectious diseases during the past 50 years – an achievement often taken for granted. Extending this control to infectious diseases caused by microbial pathogens that cannot be attenuated and safely deployed as live vaccines remains a major scientific challenge. In contrast to live vaccines, in which in vivo replication leads to widespread uptake of microbial antigen by dendritic cells with subsequent activation and MHC-restricted antigen presentation, immunization with killed vaccines does not amplify antigen presentation and often fails to trigger priming of naïve lymphocytes. Recent advances in cell biology, including understanding of intracellular pathways and mechanisms of cell-cell spread, have provided new tools to develop non-living vaccines that mimic actual in vivo replication, activation, and amplification. Three specific developments key to this development are: 1) identification of cell-cell trafficking pathways; 2) identifying the role of toll-like receptors in antigen uptake and activation; and 3) dissecting the mechanisms of intracellular communication between MHC class I (for CD8+ cytotoxic T lymphocytes) and class II pathways (for CD4+ T lymphocytes and B lymphocytes). These advances, to be presented during the lecture, have allowed significant enhancement of both priming and expansion of antigen-specific immune responses in outbreed animals.

BONE MARROW CYTOLOGY

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Indications for bone marrow evaluation

The basic indication for performing a bone marrow evaluation is to answer questions that a routine hematology examination of a blood sample does not answer. One need not take the additional effort to take a bone marrow aspirate and biopsy if, for example, the blood already clearly indicated there was an immune mediated hemolytic anemia, typical inflammatory response or if even a leukemia with clearly diagnostic features in EDTA blood.

The most common indications for bone marrow analysis are a deficiency of cells from one, two or all 3 cell lines. Cytopenias suggest decreased bone marrow function so one should check for various bone marrow diseases. These would be called a non-regenerative anemia, thrombocytopenia and/or leukopenia. Additionally leukemia may be hidden in the marrow, in that blast cells may be numerous in the bone marrow but few or no blast cells are seen in the blood (aleukaemic leukaemia). Suggestions of leukaemia are dysplastic changes such as megaloblastic rubricytes, hypersegmentation or rare blast cells in the blood. Hypercalcemia is often caused by lymphosarcoma, which may be in the bone marrow. Plasma cell myeloma may be suggested by hyperproteinemia or lytic lesions in the spine.

Bone marrow profile

A profile of tests allows the most complete evaluation and best conclusions. The profile should include a complete blood count (CBC), bone marrow aspirate and bone marrow biopsy. The biopsy is often neglected but is especially important when evaluating cytopenia and when one expects low cellularity of the bone marrow. All too often one has a poorly cellular aspirate of bone marrow and is uncertain if the low cellularity of the sample reflects low cellularity of the bone marrow or simply a poor aspirate. A CBC gives excellent quantitative and morphologic information at the time of the bone marrow evaluation. An aspirate allows excellent morphologic evaluation of cells, differential count and myeloid:erythroid ratio (M:E). A histologic section of a biopsy sample gives best quantitative information on cellularity of the marrow, reveals myelofibrosis and architectural patterns. All three together usually provide the best answer possible. Neglecting one or two parts often leaves one with unanswered questions. Performing a test several days after the other may also leave some questions.

Answers from bone marrow evaluation

The usual answers one gets from bone marrow aspirates and biopsy is quantitative and morphologic information on the cell lines in the bone marrow. Typical answers include hypoplasia, hyperplasia or normal numbers of myeloid, erythroid, megakaryocytic and lymphoid cells. These changes are then interpreted with the cell numbers seen in blood.

Additional answers/conclusions/diagnoses are based on maturity and appearance of the cells examined. Increased immaturity usually indicates a hyperplastic/reactive change unless extreme. Presence of over 30% blast cells indicates an acute leukaemia. Hemosiderin amount helps diagnose iron deficiency anaemia (absent) or anaemia of inflammation (increased).
When interpreting a bone marrow report or reading a smear oneself, the first decision should be that the sample provided adequate cells of sufficient quality to indicate what the bone marrow looked like. Always realize that one is interpreting changes in a sample and one must first determine if the sample represents the bone marrow well. The next factor is predicting the cellularity of the bone marrow. Cellularity is best predicted by particles on an aspirate or by a histologic section. Cellularity of individual cells may be so dense as to also indicate normal to increased cellularity of the bone marrow. A differential count gives the percentage of various cell types which when compared to the estimate of total cellularity is used to predict hyperplasia or hypoplasia of a cell line. The M:E ratio is the percentage of myeloid cells divided by the percentage of erythroid cells. The M:E ratio is usually slightly over 1:1 in dogs and cats. Lymphocytes and plasma cells usually represent about 5% of the marrow cells.
Morphologic changes usually are increasing percentages of immature cells. The presence of atypical morphology indicates a dysplastic process. Dysplasia may be induced by drugs, infectious agents, toxins, nutritional problems or myelodysplastic or leukaemic diseases. Feline leukaemia virus causes a wide range of dysplastic changes in cell types or alteration in the number of various cells from nonregenerative anaemia to erythremic myelosis and neutropenia to leukaemia, from thrombocytopenia to megakaryocytic leukaemia. Thus FeLV and Feline immune deficiency virus (FIV) testing is indicated in any haematologic disorder of cats.

Specific bone marrow diseases
There are too many diseases to list all, but a few examples are as follows. Aplastic pancytopenia (aplastic anaemia, fatty marrow) is when a disease process has damaged haematopoietic cells so those cells are absent and increased fat remains in the marrow. Infectious causes include *Ehrlichia canis*, parvovirus, FeLV, FIV, septicemia and endotoxemia. Toxic causes include estrogen, phenylbutazone, meclofenamic acid, trimethoprinme-sulfadiazine, quinidine, griesofulvin, thiacetarsamide and the chemotherapeutic agents. Often the diagnosis is idiopathic. Myelofibrosis is somewhat similar in that haematopoietic cells have been damaged and fibrosis has replaced normal tissue.
Canine pure red cell aplasia can be immune mediated (primary) or secondary to parvovirus infection or vaccine in dogs or FeLV of FID in cats. The bone marrow has very few erythroid cells remaining while other cell lines are normal.
Ineffective haematopoiesis may be difficult to understand. The pattern seen is a deficiency of a cell line in blood (i.e. non-regenerative anaemia) exists yet normal to increased numbers of that cell line are found in the bone marrow (i.e. erythroid hyperplasia). Bone marrow cellularity and morphology in cytologic and histologic samples does not directly measure function. Various chemical, infectious, nutritional and other causes prevent even increased numbers of cells in the marrow from effectively producing cells that are released from the marrow. Ineffective erythropoiesis may be

caused by inflammatory diseases or iron deficiency. Ineffective neutropoiesis may be caused by drugs (i.e. phenobarbital) or viruses (i.e. FeLV).
Leukaemia is a neoplastic process of hematopoietic cells in the bone marrow. The neoplastic cells are often seen in the blood giving the name "white blood" one can appreciate by looking at buffy coats in microhematocrit tubes from leukaemic patients. But the blast cells may be trapped to a great extent in the bone marrow and one may be surprised to see a bone marrow filled with blast cells where the blood had so few blast cells that one could not rule out only the presence of reactive lymphoid cells. Leukaemia diagnosis is a broad topic but most commonly there is over 30% blast cells in the bone marrow to allow an easy basic diagnosis. Specific diagnosis of types of leukaemia is useful in countries where treatment of leukaemia in dogs and cats is well accepted.

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METHODS IN ANALYSIS OF APOPTOSIS BY FLOW AND LASER SCANNING CYTOMETRY

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Numerous methods have been developed to identify apoptotic cells and analyze morphological, biochemical, and molecular changes that occur during apoptosis. Reviewed will be methods applicable to flow cytometry and slide based laser scanning cytometry (LSC) aimed to identification and enumeration of apoptotic cells as well as assessment of selected mechanisms controlling cell death.

Apoptotic cells can be detected by: 1) changes in nuclear chromatin condensation after staining with DNA specific fluorochromes such as propidium iodide in the presence of RNase and evidenced by new and unique to LSC parameter called maximal pixel; 2) collapse of mitochondrial transmembrane potential – one of the early events of apoptosis that can be proved by several fluorochromes such as DiOC6, Rh123 and JC-1; 3) loss of asymmetry in the distribution of plasma membrane phospholipids namely exposure of phosphatidylserine on the outer leaflet of the plasma membrane that is probed by combination of annexin V-FITC and analysis of exclusion of the plasma membrane integrity probe – propidium iodide; 4) decreased cellular DNA content (sub-G_1 peak); 5) "*in situ*" labeling of DNA strand breaks; and 6) immunohistochemical staining of the product of poly-(ADP) ribose polymerase (PARP) cleavage (p89) that results from activation of caspases.

Unlike flow cytometry, LSC offers the opportunity to correlate the molecular and functional changes that occur during apoptosis with cell morphology that still remain the gold standard for identification of apoptotic cells, thus confirming the mode of cell death. In flow cytometry the detection of apoptotic cells relies on a single parameter reflecting a change in biochemical or molecular feature of the cell, assumed to represent apoptosis.

LSC allows also the measurement of translocation of the proteins involved in the regulation of apoptosis such as nuclear factor kappa B, p53 or Bax within the cell. Translocation of Bax from cytoplasm to mitochondria leads to accumulation of Bax between the mitochondrial membranes that appears to be the decisive event triggering the release of cytochrome C. In addition "file merge" function of the LSC software permits the correlation of changes that can be measured only in live cells with the changes that can be detected only after fixation.

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LECTURES

STEM CELLS AND THEIR ROLE IN REGENERATIVE PROCESSES OF THE LIVER

(presented during Pathology Symposium)

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For decades, the role of stem or progenitor cells in liver regeneration has been controversial. Observations of mitotic division by hepatocytes and cholangiocytes in human and other vertebrate livers confirmed that these cell compartments had regenerative potential. It was assumed by many, though not all, investigators that this regenerative capacity of mature cells was sufficient to explain the extensive regenerative capacity of this organ.

The current concept of liver regeneration can be modelled as at least a three-tier system of cell replacement. First, mature hepatocytes and cholangiocytes contribute to normal cell turn over and responses to certain types of injury and most mild injuries. Mitotic figures, nuclear vacuolization and hyperchromasia, cytoplasmic basophilia, etc., confirm their ability to repopulate in response to injury.

Second, there is an intra-organ stem cell compartment, located mostly, if not entirely, in the smallest, most proximal branches of the biliary tree, i.e. the canals of Hering and the intralobular bile ducts. Reconstructive studies of human livers in massive hepatic necrosis demonstrated that the proliferating cells of the ductular reaction represented complex arborizing networks of cells budding from the canals of Hering and differentiating into hepatocytes. The same is true in case of cirrhosis where activation of an intrabiliary stem cell compartment give rise to newly formed hepatocytes.

A third tier of liver cell replacement has recently been identified, consisting of cells entering from the circulation. This cell compartment, probably of bone marrow origin (though other contributing sources have not been ruled out), enters the liver in a seemingly random distribution, as isolated mature cells, or in a portal and periportal distribution when there is marked injury. In this latter mode, responding to severe injury, they enter first as an intermediate cell population (“oval cells” in rodents, “ductular reactions” in humans), which then mature into hepatocytes and cholangiocytes. The point of entry in this more robust response is probably through the canals of Hering and the smallest bile ducts.

A source of controversy surrounds the issue of whether plasticity events are in fact occasions of circulating cells fusing with end-organ cells, such as hepatocytes, leading to the appearance of plasticity where exists

The mechanisms of engraftment from the circulation remain uncertain. Work in other organs suggests that factors that can mobilize stem cells from the bone marrow, such as g-csf, may be important. The finding that canals of Hering and progenitor cells which can be isolated from human livers are c-kit positive, suggests that the c-kit/stem cell factor receptor-ligand system may also play a role. Other factors responsible for homing of circulating cells to the liver as well as for their coordinated integration and differentiation into functioning liver parenchyma remain uncertain. This area is one

where exciting developments can be expected to appear in the near future.

Other sources of hepatocytes

Other, non-physiologic sources of cells for therapeutic needs are not limited to those that participate in physiological repair processes. Alternates may include stem cells from other adult populations such as bone marrow stromal cells, from fetal liver tissue, or from *ex vivo* differentiation of embryonic stem cells. Isolated, cultured, and expanded *ex vivo*, may be able to produce greater numbers of cells for rapid therapeutic use. Of these populations, fetal hepatoblasts and fetal hepatocytes were the first to be studied. It was presumed that these cells were already "committed" to a hepatic lineage, bidirectional in the case of hepatoblasts (i.e. toward both cholangiocytes and hepatocytes) and hepatocyte-committed in the case of fetal hepatocytes. Differentiation of mouse embryonic stem (ES) cells into mature hepatocytes has now been readily demonstrated by a number of groups.

Cell based therapies

Whatever the source of these cells, how they can be manipulated for therapeutic interventions in a variety of diseases is of course of great interest. The most commonly touted practical implications of hepatic stem cell discoveries are that they might serve as a source of cells for cell transplantation, for the necessary biological components of artificial livers, and as targets for gene therapies.

The diseases which are potentially most likely to show real benefit from such a procedure include primary liver diseases or diseases where extra-hepatic manifestations arise from abnormal gene expression or defective protein production by the liver (Wilson's disease, α-1-antitrypsin deficiency, tyrosenemia type I, hyperlipidoses, and porphyria, metabolic deficiencies i.e. Crigler-Najjar syndrome, familial hypercholesterolemia and amyloidosis, oxalosis and coagulation defects like hemophilia A, Factor IX deficiency).

Acquired liver diseases, particularly acute failure secondary to toxic or viral injury, have been treated in limited clinical trials with fetal and adult hepatocytes. The efficacy of these treatments in helping patients to survive until a donor organ became available, with improvement of clinical measures such as hepatic encephalopathy and cerebral perfusion pressures, is promising, but not yet clear.

Bioartificial liver devices (BLDs) require an enormous number of cells for full metabolic support and functioning of patient's liver with acute fulminant failure to survive or transplant. A stable and expandable stem cell in culture may provide the means to creating such numbers of cells.

Intriguing possibilities even include seeding the artificial devices with stem cells directly and allow the serum of the patient, with all its circulating chemokines, cytokines, increased bile salts, etc, assist in the differentiation of the cells into functional hepatocytes phenotypes.

Needless to say, our understandings of liver biology and pathobiology are rapidly evolving. With these changes, new therapeutic modalities are being imagined and, in some cases, brought to fruition. Under the combined pressures of increasing incidence of liver disease (hepatitis C!) and recent, sudden leaps in understanding of how our bodies function and regenerate, we can expect these exciting changes to escalate.

DIAGNOSIS OF RED BLOOD CELL PARASITES

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RECENT ADVANCES IN THE PATHOLOGY AND PATHOGENESIS OF TUBERCULOSIS

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WRITING MICROSCOPIC DESCRIPTIONS IN CYTOPATHOLOGY

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RECENT PROGRESS IN THE USE OF ACUTE PHASE PROTEIN MEASUREMENT IN ANIMALS

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DISEASES OF LABORATORY ANIMALS; WHY CARE?

Feinstein R
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PATHOLOGY OF LAGOMORPHS

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WHITE CELL PARASITES OF DOGS AND CATS

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ANIMAL CLINICAL BIOCHEMISTRY: THEN, NOW AND IN THE NEW MILLENNIUM

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INTERNAL ORGAN CYTOLOGY: A PREBIOPSY DIAGNOSTIC TOOL

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USE OF FRACTIONAL EXCRETION TESTS

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ARCHITECTURAL PATTERNS IN CYTOLOGY: A CORRELATION WITH HISTOLOGY

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PLATELET ACTIVATION, NEW WAYS TO LOOK AT IT

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ORGAN SPECIFIC ACUTE PHASE PROTEINS IN ANIMALS

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NEW DEVELOPMENTS IN CARDIAC TROPONIN ASSAYS

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AUTOMATED PLATELET ANALYSIS

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CANINE MALIGNANT LYMPHOMAS: A MORPHOLOGICAL, IMMUNOLOGICAL AND EPIDEMIOLOGIC STUDY OF 608 NEW CASES – COMPARISON WITH HUMAN'S NON HODGKIN'S LYMPHOMAS

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APPLICATION OF CLINICAL PATHOLOGY IN DRUG RESEARCH AND DEVELOPMENT

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MYSTERY SKIDE SEMINAR ON REPRODUCTIVE TRACT PATHOLOGY

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PHENOTYPE-APPLICATIONS OF MULTI-ANALYTE PROFILING TECHNOLOGY

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OVERVIEW OF AVIAN PATHOLOGY

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PRION PATHOGENESIS: A JOURNEY FROM THE MUCOSA TO THE BRAIN IN NATURAL AND EXPERIMENTAL MODELS

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COMPARATIVE IMMUNOPATHOLOGY – ACTUAL ASPECTS

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HISTOPATHOLOGICAL PATTERN IN DERMATOPATHOLOGY: THEIR USE IN DETERMINING LESION PATHOGENESIS I

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PIG PATHOLOGY 1: RESPIRATORY TRAKT

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PIG PATHOLOGY 2: ALIMENTARY TRAKT – THE DECISION-MAKING TREE

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TOXICOLOGY STUDIES IN LABORATORY ANIMALS

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BODY CAVITY FLUIDS CYTOLOGY

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HISTOPATHOLOGICAL PATTERN IN DERMATOPATHOLOGY: THEIR USE IN DETERMINING LESION PATHOGENESIS II

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WORKSHOPS

MYSTERY SLIDE SEMINAR ON REPRODUCTIVE TRACT PATHOLOGY

(European Society of Veterinary Pathology)

Convenor: Maja Suter, European College of Veterinary Pathology
Chair: Donald H. Schlafer, College of Veterinary Medicine at Cornell University, USA

CASE REVIEWS

(European Society of Veterinary Clinical Pathology)

Convenor: Joy Archer, ESVCP
Chair: Ilse Schwendenwein, Austria
Zoe Palizopoulou, Greece
Kirsten Tolling, Sweden

ABSTRACTS

HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF SCRAPIE-AFFECTED SHEEP IN DIFFERENT STAGES OF THE DISEASE

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Introduction: Scrapie is a fatal, neurodegenerative disease that affects sheep and goats and belongs to the transmissible spongiform encephalopathies (TSEs) or prion diseases.Strain characterization of transmissible spongiform encephalopathies (TSEs) has been carried out by the transmission of isolates in several mouse lines. Recent investigations asses the vacuolar lesion profile, the glycoprofile and the phenotype of disease-specific PrP deposition in the brain and other tissues of affected sheep. On this sense, in this study it is described the histopathological and immunohistochemical profiles showed in 9 animals on different stages of the disease that had the same genotype and breed.

Materials and Methods: Animals: Nine sheep on three different stages of the disease (preclinical, clinical and terminal) were studied. Genotyping: All the animals of the study were genotyped by sequencing techniques. The whole coding region was amplified using the following primers: 20 fwd: 5' ATGGTGAAAAGCCACATAGGCAGT 3' (codons 1-8) and 767 rev: 5' CTATCCTACTATGAGAAAAATGAG 3' (250-stop codon). The same PCR primers were used for bi-directional sequencing and chromatograms were analysed. Histopathological and immunohistochemical profile: Histopathological analysis was performed as described by Ligios et al. (2002) and immunohistochemical one was based on González et al. (2002) using L42 and R145 antibodies.

Results and Discussion: The animals of the preclinical stage shown perivascular and perineuronal patterns. This could be due to the preliminary deposition of PrP^{sc} in the first stages of the disease, without invading the neuronal pericarion, and ascending through the astrocytic cells of the vessels. In the animals of the clinical stage, they shown the same pattern described by González et al. (2002) in the Welsh Mountain PrP^{VRQ}/PrP^{VRQ} breed. That could imply the presence of a specific strain in that focus.

The aim of this study was the development of histopathological and immunohistochemical profile in order to relate them with the scrapie strain. Nevertheless, as described by other authors, there were several features that could not be controlled, as the age, dose, route and age at infection. It is possible that these aspects could modify the characteristics of the lesional and immunohistochemical patterns.

ANGUILLA ANGUILLA L. OXIDATIVE STRESS BIOMARKERS RESPONSES TO COPPER EXPOSURE WITH OR WITHOUT β-NAPHTHOFLAVONE PRE-EXPOSURE

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Introduction: The assessment of the interactions between heavy metals and other classes of contaminants represent a major exposed area of research among various essential trace metals, copper (Cu) is abundantly present at low concentrations in the aquatic environment. Cu is a classic limiting factor for fish, as it is both essential and toxic. The redox cycling of Cu and the interactions between heavy metals and organic pollutants can greatly modify the toxic effects measured at different biological levels. Therefore, in order to evaluate Cu-induced oxidative stress after pre-exposure to β-naphthoflavone (BNF) research was carried out in European eel (*Anguilla anguilla* L).

Materials and Methods: Eels (length 25±3 cm, weight 30±5 g) were captured and acclimated for 7 days under standard conditions (APHA, 1998) in aquaria containing 80 liters fresh water, each. After acclimation, 7 groups (10 eels each) were exposed. Three groups were reserved for 24 h in clean water, and then exposed with two concentrations of Cu^{2+} as copper dichloride. Concurrently, a control group of eel was kept in clean water for a 48 h period with a 24 h water renovation (CW+CW). Another four groups were exposed to 2.7 µM BNF during 24 h. Following 24 h exposure, two groups were transferred to CW and BNF (BNF+BNF), and the remaining two groups were exposed during 24 h to Cu with their respective concentrations. *A. anguilla* were killed after 48 h exposure and fish tissues (gill and kidney) were collected for various biochemical analyses. Lipid peroxidation (LPO), glutathione peroxidase (GPX), catalase (CAT), glutathione S-transferase (GST), total reduced glutathione (GSH), total protein contents were measured.

Results: BNF+BNF exposures caused induction of CAT, GPX and GST in gill. The induction of these antioxidant enzymes in kidney was not very prominent. However, GSH showed a significant decrease in gill. BNF+BNF exposure also induced LPO in gill and kidney. Cu exposures (24 h) without BNF pre-exposure (24 h) reduced CAT and GPX activity (kidney), enhanced GST activity (gill and kidney), depleted GSH levels (gill and kidney) and no LPO change in gill and kidney was observed. However, its exposure to Cu with BNF pre-exposure showed no effect on gill and kidney CAT activity, decreased gill GPX activity, increased kidney GST activity, depleted kidney GSH contents and increased gill and kidney LPO.

Conclusion: BNF pre-exposure with subsequent Cu exposures modulate the fish LPO and induces an array of antioxidants that may be beneficial to fish in the case of oxidative stress resulting from copper contamination.

STUDY THE EFFECTS OF *HEMISCORPIOS LEPTURUS* SCORPION VENOM ON THE LEVEL OF SOME SERUM ENZYMES IN RATS

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Introduction: In Iran severe complications are known from the sting of *Hemiscorpius lepturus* in animals and man. Severe and fatal hemolysis, secondary renal failure, deep and necrotic ulcers, psychological problems and death are reported complications.
Materials and Methods: Experiment was carried out *in vivo* by injecting *H. lepturus* scorpion venom in male rats. The different sub lethal concentrations of venom (20, 40, 60, 80 and 100 μg/kg body weight) were injected subcutaneously in 5 groups of rats (each group containing 10 mature male rats). The control group was injected with normal saline. Blood samples were taken to measure the levels of blood serum enzymes include AST, ALT, ALP, CPK and LDH in different times after envenoming (0, 30 and 60 min., 3 and 12 h, 2, 3 and 7 days).
Results: Our results showed that there was a significant increase in the circulating levels of all serum enzymes following envenoming in comparison with control group ($p \leq 0.5$). There was a significant positive correlation between the concentration of the venom and the levels of all serum enzymes especially in AST, ALT and LDH in different groups of rats ($p \leq 0.5$).
Conclusion: This alteration showed that the serum enzymes are elevated because of the necrotic effects of *H. lepturus* scorpion venom. So the elevation of these serum enzymes showed that the liver and blood can be the most important target organ of this scorpion venom.

DIFFERENTIAL EXPRESSION OF MATRIX-METALLOPROTEINASES AND THEIR INHIBITORS IN ACUTE AND CHRONIC DEMYELINATING THEILER'S MURINE ENCEPHALOMYELITIS

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Introduction: Theiler's murine encephalomyelitis (TME) is caused by TME virus (TMEV), a cardiovirus of the *Picornaviridae* family. TME represents an important model for human demyelinating diseases like multiple sclerosis (MS) as following intracerebral infection of susceptible mice with certain strains (BeAn) a biphasic demyelinating encephalitis develops. Matrix-metalloproteinases (MMPs) are zinc dependent enzymes that are able to disintegrate myelin. Their activity is controlled by tissue inhibitors of matrix-metalloproteinases (TIMPs). Based on these facts, our interest was to create a molecular biological profile of MMP and TIMP expressions in different stages of TME with the future objective of a therapeutical modulation of the disease.
Materials and Methods: Five weeks old female SJL/JHanHsd mice were inoculated intracerebrally with the BeAn strain of TMEV and killed 0, 1, 4, 7, 14, 28, 56, 98, and 196 days post infection (dpi). Morphological analysis included routine histology and immunohistological/lectin histochemical evaluation of brain and inflammatory cells. For molecular studies RNA was isolated from frozen spinal cord specimen. Quantitative polymerase chain reaction (qPCR) was performed with the Brilliant® SYBR® Green QPCR Core Reagent Kit using a MX4000™ Multiplex Quantitative PCR System. Primer pairs for the detection of MMP- (MMP-2, -3, -7, -9, -10, -11, -12, -13, -14, -15, -24), TIMP- (TIMP-1, -2, -3, -4) and TMEV- specific RNAs were selected from the murine GenBank® reference sequences. A $\log_{10}$ dilution series of purified specific cDNA was used as external standard for an absolute quantification. Values were normalized employing 4 different housekeeping genes.
Results: Histologically, biphasic demyelinating encephalitis was characterized by demyelination peaks around 28 and 98 dpi that coincided with the peak influx of T and B cells starting at 7 dpi TMEV RNA persisted in the spinal cord throughout the observation period. qPCR revealed, that MMP-12 was the most prominently up-regulated enzyme starting at 28 dpi with highest copy numbers at 98 dpi MMP-2 was increased from days 28 until the end of the observation period. MMP-3 was up-regulated at all time points investigated. MMP-11 was slightly increased in the early phases of the disease until 56 dpi MMP-7, -9, -10, -13, 14, -15, and -24 as well as TIMP2-4 expressions were comparative to control levels. TIMP-1 was up-regulated from 1-196 dpi peaking at 1 dpi.
Conclusion: The present study resulted in the elaboration of a specific expression pattern of MMPs and TIMPs in various phases of TME. Accordingly, up-regulation of TIMPs is restricted to the first days after TMEV infection pointing to a possible impairment of MMP inhibition as the motor for demyelination progression. Cellular source of MMPs and TIMPs and enzyme activity will be object of future studies.

THE EFFECT OF *UNCARIA TOMENTOSA* WATER EXTRACT ON SOME PARAMETERS OF CELLULAR IMMUNE RESPONSE IN MICE

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Introduction: *Uncaria tomentosa* (Wild.) D.C. also known as „vilcacora", „una de gato" or „cat's claw", is a liana belonging to the family of *Rubiaceae*, growing in humid tropical forests of Middle and South America. It is one of the most popular Peruvian medicinal plants, and preparations of its bark, leaves or roots have been the basis of local natural medicine for ages. This plant displays a drivers range of bioactive secondary metabolites including tetracyclic and pentacyclic oxindole alkaloids, triterpenes, glycosides, flavonoids, catechins and procyanidins. Organic solvent extracts of *Uncaria* were shown to have cytostatic, contraceptive, anti-inflammatory, anti-mutagenic and anti-viral activities. The aim of the work was evaluation of the effect of water extract of *Uncaria tomentosa* (W1/04) on some parameters of cellular immunity in Balb/c mice.

Materials and Methods: Water extract: The preparation was obtained by extraction of bark *Uncaria tomentosa* with water (37°C, 24 h). Total content of alkaloids in extract was estimated on the level 0.43% dry weight. The dominant alkaloids were uncarine C and isomitraphylline. Mice: Inbred Balb/c mice 7-9 weeks old, approximately 20 g of body mass, females, were fed W1/04 for 7 days (10 mg/kg), or water (controls). On the 8th day mice were anaesthetised, bled from retro-orbital plexus and sacrificed with Morbital. Spleens were excised, spleen lymphocytes suspension prepared. The following tests were performed: evaluation of blood granulocytes chemiluminescent activity, evaluation of splenic lymphocytes chemokinesis in 24-hour cultures *in vitro*, evaluation of splenic lymphocytes graft-versus-host activity after grafting intradermally to F1 hybrids (LIA test).

Results: In all tests applied significant stimulation of blood and spleen leukocytes activity were observed in W1/04 fed mice in comparison to the controls.

Conclusion: Water extract of *Uncaria tomentosa* W1/04 possess immunostimulatory properties in mice.

COMPARATIVE STUDY OF MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL FINDINGS INDUCED BY DIFFERENT ENERGY SOURCES AND APPROACHES OF SURGICAL ATRIAL ABLATION IN SHEEP

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Introduction: Surgical atrial ablation is performed to produce transmural lesions for preventing the re-entry into circuits of atrial fibrillation, the most common cause of arrhythmia in humans. In this study the cardiac alterations induced by different energy sources and approaches of surgical ablation in an experimental sheep model were investigated.

Material and Methods: In 33 sheep circular lesions were created endo- or epicardially in the left atrium and the pulmonary veins by the use of cryoablation, microwave, laser, unipolar or bipolar radiofrequency. Post treatment electrophysiological examinations were performed to evaluate conduction block. The sheep were sacrificed two hours after ablation procedure, and the hearts were fixed in formalin, embedded in paraplast and stained with haematoxylin and eosin, Masson Goldner trichrome (MG), aqueous Congo red (CR), Picrosirius red (PSR) and Luxol Fast Blue (LFB). Additionally desmin and laminin were detected by immunohistochemistry.

Results: Endocardial ablation resulted predominantly in transmural lesions, which were confirmed by electrophysiological examinations. Epicardial ablation was mainly not successful, due to the contemporary blood flow, causing a heat sink phenomenon. Endocardial thrombi, transmural necroses, bleedings and inflammatory reactions were observed varying in degrees, depending on the ablation technique. Endocardial application of laser and microwave resulted in the most extended coagulation necroses and lots of endocardial thrombi. Radiofrequency and cryoablation induced more or less circumscribed coagulation necrosis surrounded by well defined borderlines of myofibrillar contraction band necroses. In general, coagulation necrosis was characterized by a failure of desmin detection. Cardiomyocytes were coloured pale greenish in MG, pale bluish in LFB and deeply red in CR staining. Necrotic collagen fibres showed a loss of birefringency in PSR stained slides. In contrast, cells with myofibrillar contraction band necrosis were intensively labelled by desmin and they stained red in MG, deeply blue in LFB but pale in CR stain. These staining patterns were related to the type of necrosis but not to the different energy sources.

Conclusion: Surgical ablation using different energy sources and approaches resulted in various electrophysiological effectivity and histomorphological lesions. Diverse (immuno)-histological methods were useful in detecting the different types of myocardial necroses but the causal energy sources could not be concluded from a certain staining pattern.

RE-ROUTING INTRACELLULAR TRAFFICKING OF PRION PROTEIN IN NEURONES: A NOVEL THERAPEUTIC APPROACH?

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Whilst direct interaction of various compounds with PrP^{sc} may lead to disruption of prions and loss of infectivity, other therapeutic approaches include inhibiting the mechanisms by which prions infect neurones and cause their degeneration. In the present studies, the squalene synthase inhibitor squalestatin was used to reduce the cholesterol content of three prion-infected cell lines and dispersed lipid rafts on their plasma membrane. This action prevented the accumulation of PrP^{sc} in all three cell lines. The effects of squalestatin were dose-dependent and evident at nanomolar concentrations. Squalestatin also prevented the killing of ScN2a cells by microglia and non-infected neurones treated with squalestatin became resistant to the otherwise toxic effect of prion preparations or PrP peptides. The protective effect of squalestatin coincided with a reduction in the activation of the phospholipase A2 pathway and the prostaglandin production that is associated with neuronal injury in prion disease. Furthermore, squalestatin treatment altered the intraneuronal trafficking of prion peptide away from its normal recycling pathway and into degradative lysosomes. The neuroprotective effects of squalestatin treatment were reversed by the addition of water-soluble cholesterol to neurones. These studies indicate a pivotal role for cholesterol sensitive lipid rafts in controlling PrP^{sc} formation, prion neurotoxicity and in the activation of pathways leading to neuronal death, and provide a further avenue for developing therapeutic strategies.

PATHOLOGICAL EVALUATION OF NATURAL CASES OF SHEEP SHOWING THE NERVOUS FORM OF MAEDI-VISNA INFECTION

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Introduction: The nervous form – or Visna- of Maedi-Visna infection has been considered as a rare manifestation of the disease. Besides the Icelandic epidemics, only sporadic cases have been recorded in different countries and not detailed pathological studies have been carried out. This form is characterized by a non-suppurative meningoencephalitis that classically has been seen as located in the subependymal areas of the brain. In our laboratory, several cases of Visna are diagnosed every year, and not all of them fit with the topographic distribution already described. In this work, we made a detailed study of the lesions encountered in confirmed natural cases of Visna.

Materials and Methods: A total of 71 sheep showing a non-suppurative meningoencephalitis in which Maedi-Visna virus was demonstrated by immunohistochemistry or PCR were examined. Histopathological evaluation was made at different levels of the Central Nervous System.

Results: In most of the animals, lesions appeared in more than one of the examined locations. The cerebellar peduncles, pons and medulla oblongata were the most common and severely affected sites, followed by the corpus callosum area. Whereas in the latter lesion had a periventricular pattern, in the cerebellar peduncles affected mostly the cerebellar white matter without involving significantly the parenchyma surrounding the ventricle. Lesional intensity varied from scattered foci of gliosis to diffuse areas of inflammation and demyelination. According to the histopathological findings, three different patterns were considered: vascular, with the predominant presence of perivascular cuffs; malacia, when this was change the most oustanding feature; inflammatory, characterized by a diffuse mononuclear infiltrate of the nervous parenchyma. Mixed forms were commonly seen in the same sample. The presence of perivascular cuffs was the most frequent feature, accompanied by a diffuse inflammatory infiltrate in the most cranial parts of the brain or malacia in the brain stem. Positive cells by immunohistochemistry were only seen related to lesions. They appeared associated with the inflammatory pattern whereas perivascular cuffs were mostly negative. Although different degrees of positivity were seen, they were not related to the intensity of the inflammatory lesion.

Conclusion: Natural cases of the nervous form of ovine Maedi-Visna infection present a variety of lesions according to their location, intensity and histological features. The most common sites are the cerebellar peduncles and medulla oblongata, where distribution of lesions does not follow the classical periventricular pattern of this disease. Presence of viral antigen in the cells is related to the histological type of lesions but not to their intensity.

A CASE OF *RHINOSPORIDIUM SEEBERI* IN AN IMPORTED HORSE IN THE UNITED KINGDOM

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Introduction: Fungal, bacterial infections, chronic irritants and neoplasias are potential causes of nasal polyps in animals and humans. *Rhinosporidium seeberi*, belonging to the class mesomycetozoa, is an organism involved in the infection of the nasal and ocular mucosae, frequently associated with the development of polypoid growths. The condition is rarely seen in the United Kingdom and has only been documented in imported horses.
Materials and Methods: A small polyp and detached fragments of tissue from the nasal mucosa of a Polo pony were submitted for routine histopathological examination. The pony had a history of bilateral epistaxis and apparent recovery. Progressively expansile masses were noted months later on the nasal mucosa at the external nares with bouts of epistaxis. The tissues were fixed in formalin and paraffin embedded. Sections were cut and stained with haematoxylin and eosin and examined under a light microscope.
Results: Examination revealed infiltration and disruption of the nasal mucosa with numerous round structures (sporangia) containing large numbers of small round endospores. The histological appearance of the sporangia is considered pathognomonic for *Rhinosporidium seeberi*.
Discussion: Rhinosporidium infections are very rarely seen in the United Kingdom. In other countries it is an important condition of predominantly humans and to a lesser extent horses, mules and cattle. Goats, dogs and waterfowl are also reported to have been affected. *R. seeberi* occurs endemically in India and Sri Lanka, but occurs in the southern United States, South America, South Africa, Japan and parts of Europe.

CHARACTERISATION AND DISCUSSION OF CYCLOPIA IN A KITTEN

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Introduction: Cyclopia as a subset of the spectrum of pathological changes associated with holoprosencephaly is seen in a variety of domestic animals and humans. In sheep and occasionally cattle ingestion of the alpine legume *Verratum californicum*, at a specific stage of gestation, can result in cyclopia of the foetus. Mutations in the sonic hedgehog signaling pathways have been documented in cases of holoprosencephaly in humans. Cyclopia is rare in cats.
Materials and Methods: A stillborn, full term kitten, with severe cranio-facial malformation was submitted for *post mortem* examination together with a normal stillborn littermate.
Results: The kitten was male, and had a single enlarged central eye, with deformities of the nasal cavities, maxilla and mandible. There was a single narrow optic foramen and nerve with a shallow orbit. The brain lacked normal division of the cerebral hemispheres, with dilation of the ventricles, and the pituitary gland was absent. There was no microscopic or historical evidence of any current viral or toxic insults to the queen.
Discussion: The sonic hedgehog gene is integral in the development and orientation of various organs, and has an important role in cranio-facial development and patterning. Abnormalities in, or mutations of this transduction signaling pathway can result in various grades of holoprosencephaly, of which cyclopia the most severe is. We are currently undertaking further studies to determine the nature of any genetic anomaly in this kitten.

MALIGNANT HISTIOCYTOSIS IN THREE CATS

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Introduction: Malignant histiocytosis (MH) or disseminated histiocytic sarcoma is a systemic neoplastic proliferation of macrophages that is well characterized in dogs, but rare in cats, as only eight cases have been published in the international literature. In this report the clinical course and diagnostic findings of three feline patients with MH are presented, along with differential diagnosis considerations and a comparison with other cases described in the literature.

Materials and Methods: Samples for cytology were taken by fine needle aspiration and stained with May Grünwald-Giemsa. Tissue for light microscopy was fixed in buffered formalin. Sections were stained with haematoxylin and eosin, PAS, Masson trichrome, toluidine blue, Perls for haemosiderin, methyl green-pyronin and Buffalo black for haemoglobin. Immunohistochemistry was performed using an avidin-biotin-peroxidase technique for vimentin, CD3, CD79α, cytokeratin AE1/AE3, MAC387, MHC-II, and lysozyme. Small pieces of tissue were also post-fixed in osmium tetroxide, epon embedded, sectioned and stained for transmission electron microscopy (TEM).

Results: Case 1 was a 13 years old european male neutered cat presented with a laryngeal mass and moderate lymphadenopathy; case 2 was a 10 years old European male neutered cat with marked jaundice and hepatomegaly; case 3 had lumbar lymphadenopathy and hepato-splenomegaly. The FIV-FeLV status was negative in case 1, FIV+ in case 2 and unknown in case 3. All patients were markedly anaemic and the disease rapidly progresses to death. The main post mortem findings common to all cases were marked hepatomegaly and splenomegaly and moderate lymphadenopathy. Histology and cytology showed the infiltrative proliferation of large mononuclear and multinucleate round-to-polygonal atypical discrete cells, with huge and pleomorphic nuclei and nucleoli. These cells had abundant vacuolated cytoplasm and occasionally displayed erythrophagocytosis. Immunohistochemistry (vimentin and lysozyme expression) and TEM supported the morphologic hypothesis of histiocytic malignancies, and MH diagnosis was then formulated.

Conclusion: Diagnostic alternatives in our cases included systemic histiocytosis, histiocytic (anaplastic large cell) lymphoma, anaplastic systemic plasmacytoma and visceral histiocytic mast cell tumour, but histological, histochemical and immunohistochemical figures ruled them out.

MH has been extensively described in dogs, and a genetic predilection has been recorded in Bernese mountain dogs, Rottweilers and Golden retrievers. Although no predisposing factors seem to be evident for feline MH, this disease should be considered in the differential diagnosis for cats with anaemia and hepato-splenomegaly.

IMMUNE RESPONSE IN PIGLETS EXPOSED TO AFLATOXINS: EFFECTS ON PLASMA ZINC AND THYMIC HORMONE (FTS)

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Introduction: It is well known that pigs are very susceptible to aflatoxins and that their biologically active compounds (B1, B2 and G1, G2) are presumed to negatively influence immune system function and consequently piglets welfare and growth performances (Mocheggiani et al. 1998, Cabassi et al. 2002). Previous results (Cabassi et al. 2002, Grugliasco 2002, Cabassi et al. 2004 – in press) about T lymphocytes subsets showed that 23 days aged piglets born of sows fed daily with a diet containing a pool of aflatoxins (B1, B2, G1, G2) during pregnancy, had a delay of CD4+ and CD8+ differentiation and probably this is induced by the presence of aflatoxin M1 in milk (Cabassi et al. 2002). In this work a further study on immune response efficiency was performed by means serum zinc and thymic hormone (FTS) determinations.

Materials and Methods: Six healthy 2-3 years old sows, Duroc x (LW x L), with previous normal pregnancy history, ecographically pregnant detected at 30 day (d), were fed daily (n=4) or not (n=2) with feed containing a pool of 300 ppb of aflatoxins (B1, B2, G1, G2) from d 40 of pregnancy up to d 23 of lactation. Zinc and thuymulin (FTS), active (AT) and total (TT), assay were performed as previously described (Mocheggiani et al. 1998) on plasma blood samples obtained from piglets (4/sow) collected by jugular vein puncture at 6 and 23 day after birth.

Results: Zinc and thymulin values were lower in all 6 days old piglets, but this effect appeared more severe in aflatoxins exposed piglets. Then, while in control animals serum zinc ion, AT and TT recovered, in piglets born from aflotoxins exposed sows remained significantly lower until the 23 day of age (*$p<0.01$).

Piglets	Plasma zinc ion (µg/dl)	Active thymulin (log_{-2})	Total thymulin (log_{-2})
Control	94±10.5	6±0.3	6.2±0.1
Aflatoxins exposed	63.33±8.2*	2±0.2*	4.2±0.2

Conclusions: The data confirmed our previous (Mocheggiani et al. 1998) results of toxic effects of aflatoxins in piglets, born from aflatoxins exposed sows, during pregnancy and weaning period. At birth the high levels of cortisol can negatively influence the zinc bioavailability and the active (AT) thymulin level. A direct effect of aflatoxins, not detoxified by P450 system, furtherly associated with a high level of cortisolaemia, characteristic in this phase of life, can reduce strongly intestinal zinc absorbtion and its bioavailability with negative effects on the efficiency of thymic endocrine activity. Further correlation between cortisol, zinc and thymic hormones and also growth hormones could better explained, not only the negative effects of aflatoxins on immune response, but also the potential influence on growth performances and so justifies possible zinc supplementation in the diet during weaning period.

BPV E5 ONCOPROTEIN BINDS TO AND ACTIVATES PLATELET-DERIVED GROWTH FACTOR RECEPTOR β IN NATURALLY OCCURRING BOVINE URINARY BLADDER TUMOURS

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Introduction: In cattle, tumours of the bladder are commonly encountered in animals suffering from chronic enzootic haematuria (CEH). Recently, *Bovine Papillomavirus* type-2 (BPV-2) DNA infection has been found in high percentage of naturally occurring urinary bladder tumours in cows. Moreover, E5 oncoprotein expression was also found suggesting a possible role in bovine urinary bladder carcinogenesis (Borzacchiello et al. 2003*). In vitro* molecular studies suggest that BPV E5 oncoprotein induces transformation by binding to and activating the platelet-derived growth factor (PDGF) β receptor (DiMaio et al. 2001). In an attempt to validate the role of the E5 oncoprotein in bovine urothelial carcinogenesis, we investigated the interaction E5 – PDGFR-β in BPV-2 positive naturally occurring bovine urinary bladder tumours.

Materials and Methods: Tumour samples were collected at slaughterhouses from cows suffering from CEH. Viral analysis to detect BPV-2 DNA was assesses by PCR as elsewhere described (Borzacchiello et al. 2003). For immunofluorescence and confocal microscopy as well as molecular analysis a rabbit antiserum anti-PDGFR-β (kindly provided by Dr. Daniel DiMaio, Yale University, USA) was used. For co-immunoprecipitation an antiserum anti-E5 (kindly provided by Dr. Richard Schlegel, Georgetown University, USA) was used and the samples were analyzed with an anti-PDGFR-β. Furthermore, the same samples were analyzed by Western blot with an anti-phosphotyrosine antibody.

Results: All the examined tumour samples gave a positive PCR result. A 307 bp DNA sequence encompassing the BPV E5 sequence was amplified. The tumour samples were tested by Western Blot for the reactivity to the anti-PDGFR-β antibody. This antibody recognized a band of the right molecular weight. The coexpression of E5 and PDGFR-β was also detected by confocal double color immunofluorescence. After co-immunoprecipitation with antiserum anti-E5 the PDGFR-β was recovered in the samples. Finally, western blot with anti-phosphotyrosine antibody showed that the PDGFR-β is tyrosine phosphorylated.

Conclusion: It has been shown that BPV E5 interacts with PDGFR-β inducing cell transformation *in vitro*. Our data demonstrate, for the first time, that E5 – PDGFR-β interaction occurs also in naturally occurring bovine urinary bladder tumours, thus supporting the idea that this molecular pathway could underline also E5 – mediated carcinogenesis. Finally, our study seems to validate the cow as a suitable naturally occurring animal model to verify the potential of E5 vaccination as antiviral vaccine.

POSSIBLE HEALTH EFFECTS OF DEPLETED URANIUM (DU): EXAMINATION OF PERIPHERAL BLOOD OF RUMINANTS IN EXPOSED AREAS

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Introduction: According to the official records, during the NATO attack in former Yugoslavia in 1999 more than 500 000 DU missiles were used, yielding a total activity of 18.3 x 10^{10} Bq. In South Serbia, eleven sites were hit by DU ammunition. The paper presents the preliminary results on the health status of ruminants (Ru) in the exposed region.

Materials and Methods: The samples of animals' blood, (20 cows and 20 sheep) soils and feed were collected in the region of Bujanovac, Serbia. Clinical examination of Ru was performed by standard procedures. Ceruloplasmin (Cp) and hemoglobin (Hb) concentrations were determined spectrophotometrically. Leukocytes (Le) and erythrocytes (Er) count and hematocrit values (Ht) were obtained by standard manual procedures. Leukocyte different functional activities were estimated by modified method described by Oez (1990) and Monboisse (1991). Cytogenetic analysis of blood Le was assessed according to the modified protocol by Evans and O'Riordan (1975). Activity of the radionuclides was determined by standard gamma spectrometry.

Results: Cp concentration in cows' plasma was significantly lower, while Cp level in sheep plasma was insignificantly decreased in comparison to the control group of animals from the non-exposed areas. All examined animals, especially sheep, had severe anemia. Number of Le was on the lower physiological value. No changes in spontaneous and PMA-stimulated NBT reduction in cow and sheep Le suggested that no Le oxidative stress mechanism was activated. Stimulated adhesiveness of sheep Le was significantly increased**,** while the same parameter in cows' Le was decreased vs control. Significantly increased number of chromosomal breaks and gaps detected in cows and sheep Le suggested presence of some genotoxic agents in the environment. The activity of $^{238}U/^{235}U$ in all investigated samples of soils and feed was below the minimal detectable concentrations MDC (10^{-3} Bq/kg).

Conclusions: Although clinical examination of local veterinarian revealed no apparent health problems in Ru, further investigation of hematological parameters demonstrated that animals had mild to severe anemia. Different responses in cow and sheep Le functional activity can imply different mechanism of activation. Low concentration of Cp could point out liver malfunction. Detected chromosomal aberrations indicate presence of some genotoxic agents in exposed areas. Therefore, the results indicate some changes in the health status of examined animals, but however, we cannot conclusively correlate them with the effects of DU that entered the environment. The study is in progress.

IMMUNOHISTOCHEMICAL INVESTIGATION OF AMYLOID β PROTEIN (Aβ) IN THE BRAIN OF AGED CATS

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Introduction: In human Alzheimer's disease (AD) amyloid deposition, neurofibrillary tangles (NFTs) and loss of neuronal connections are the most prominent histologic lesions in the brain. Senile plaques (SP), cerebral amyloid angiopathy (CAA) and neurofibrillary tangles (NFTs) are well known forms of cerebral amyloidosis of aged humans and old animals. The aim of this study was to examine the positive reacting deposits of Aβ in the brain of aged cats after staining with various anti-Aβ antisera, and to compare them with Aβ deposits found in the brain other species.
Materials and Methods: The brains of 14 cats range in age from 7.5 to 21 years old were examined. The cats were euthanised or died spontaneously suffering from a variety of disorders. The brains were routinely fixed by immersion in 4% buffered formaldehyde, then embedded in paraffin wax and cut into 5 μm sections. Paraffin sections were stained with haematoxylin and eosin (HE) and alkaline Congo red. Several immunohistochemical staining methods were performed in order to detect accumulations of Aβ amyloid in the neuropil and in the wall of the vessels using a panel of six antibodies (anti-Aβ 8-17, anti-Aβ 40, anti-Aβ 42, anti-Aβ 43, anti-APP, anti-Pan β-amyloid).
Results: Aβ immunostaining labeled 2 types of Aβ deposits in the neuropil representing senile plaques. The majority of the plaques represented Aβ positive antigenic material (first type) and the minority diffuse (preamyloid) plaques (second type). Amyloid deposition was also detected in the wall of the cerebro-meningeal vessels and cerebral arterioles and around the wall of cerebral capillaries. The plaques and the Aβ deposition in the wall of the vessels were intensively labeled by the antibody specific for the C-terminus of Aβ 42. By the comparison, the immunoreactivity of the Aβ (8-17) and Pan β-amyloid was weaker than with Aβ 42. No staining was detected by using the Aβ 43.
Conclusion: The current findings indicate that the feline appears to represent a useful spontaneous model for understanding the early changes either of normal aging or neurodegenerative diseases. Further research is needed to investigate the subsequent events in the development of this disorder.

HEPATIC IRON DEPOSITION IN DONKEYS AND HORSES

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Introduction: A study of was carried out as part of an investigation into aspects of hepatic iron deposition in donkeys and horses.

Materials and Methods: Serum and liver samples were obtained from 12 donkeys; comparisons were made of serum iron levels, liver iron and copper concentration, morphometric histochemistry and histopathology. Serum and histological samples were available from 11 horses.

Results: Liver iron and copper: In donkeys liver iron levels ranged from 524 to 5010 µg/g (mean 1723±SD 1258). Copper levels ranged from 2.7 to 4.8 µg/g (mean 0.353±SD 0.052). There was no correlation between iron and copper content. Serum iron: Donkey serum iron levels ranged from 0.22 to 46.7 µmol/l (mean 18.8±SD 14.7). Horse serum iron levels were generally similar (ranging from 8.8 to 42.1; mean 55.5±SD 61.8) with the exception of two horses with serum iron levels in excess of 100. Serum total iron binding capacity (TIBC): TIBC in the donkeys ranged from 40.9 to 78.9 µmol/l (mean 59.8±SD 13.5). Most horse TIBC levels were generally similar (ranging from 28.2 to 235.2; mean 84.6±SD 58.9); in the two animals with high total serum iron, TIBC levels were also very high (143.6 and 235.2). Percentage iron saturation: Percentage iron saturation in donkey sera ranged from 3% to 78.8 % (mean 35.4±SD 28.6). In horse sera levels were generally similar range (21.0% to 92.7%); highest % saturation values were obtained in the two horses with high total serum iron and TIBC but were not very different from other horses. Hepatic iron histochemistry: There was a wide range in the extent and intensity of iron staining in liver sections.

Conclusion: There was no correlation between measured parameters and age or sex of the animals. Accumulation of large amounts of hepatic iron may be a normal feature in donkeys and horses at various ages, rather than an ageing change.

INCREASED EXPRESSION OF NADPH-d/NITRIC OXIDE SYNTHASE AFTER FACIAL AXOTOMY; ULTRASTRUCTURAL AND LIGHT MICROSCOPIC ANALYSIS

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Introduction: Recent light microscopical examinations revealed an increased NOS expression in motor, sensory and neurosecretory neurons following traumatic injury. In our study we applied light and electron microscopy for a comparative assessment of the intensity of NADPH-d expression in control and axotomized rat facial motor neurons.
Materials and Methods: The experimental material consisted of 11 adult male Wistar rats (200-250 g) of our own breeding stock. Following facial axotomy or sham operation the rats were allowed to survive 28 days. Four of them were processed for light microscopy and the remaining three for electron microscopy. The light microscopy control group included two sham-operated animals sacrificed 28 days after operation and two intact rats. Animals were anesthetized with chloral hydrate (400 mg/kg *i.p.*) and subjected to unilateral transection of the left facial nerve. All the animals were perfusion fixed and processed for light and electron microscopic NADPH-d histochemistry. The total number of stained neurons as well as numbers of intensely, moderately and weakly stained cells were compared between the affected versus unaffected side. Statistical differences were analyzed using Student's t test and considered significant if $p<0.05$.
Results: Facial nuclei of all control, sham operated as well as experimental animals contained NADPH-d-positive motoneurons. Electron microscopic examination of subcellular distribution of the NADPH-d activity in intact controls, sham-operated as well as axotomized animals revealed apparent histochemical staining of the facial motoneurons. The fine BSPT formazan precipitate labeled numerous mitochondria, bands of endoplasmic reticulum, nuclear envelope and membranes of the Golgi apparatus as well. Following the nerve transection, there was a significant increase in number of intensely and moderately stained neurons on the affected side versus contralateral unaffected side. These changes were accompanied by a distinct, but not statistically significant, decrease in population of weakly stained neurons and a significant increase in the total number of stained motoneurons on the transected side comparing to the contralateral side.
Conclusion: It is likely that axotomy-induced NOS expression observed in the present study might play an important role in the recovery of synaptic function. Our electron microscopical findings fully confirm light microscopical observations on high NOS activity in facial motoneurons.

CHLAMYDIAE IN SWISS BREEDING SOWS WITH INCREASED RATES OF RETURN TO OESTRUS

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Introduction: Recent studies in Germany have shown a high prevalence of *Chlamydiaceae* in the genital tracts of breeding sows with reproductive disorders including sows with increased rates of return to oestrus. This study was designed to estimate the influence of chlamydial infections on sow fertility in Swiss sows.

Materials and Methods: Cervical swabs and sera were taken from sows from problem herds with increased rates of return to oestrus (group A, n=65) and from control herds without reproductive problems (group B, n=128). In addition, the genital tract of 21 slaughtered sows of group A was examined. Swabs and genital tracts were screened for *Chlamydiae* by a new 16S rRNA PCR and sera by ELISA for *Chlamydiaceae* LPS. The genital tracts of the slaughtered sows were also examined anatomicopathologically and bacteriologically.

Results: *Chlamydophila* (*Cp.*) *abortus* was found by PCR in 7 (10.8%) swabs from 65 sows from 24 farms of group A and in none from 128 swabs from 14 farms taken from group B (0%) (p=0.0007). *Chlamydia suis* was present equally in swabs from both groups A (1.5%, 1 of 65) and B (2.3%, 3 of 128). *Cp. abortus* was detected in 33.3% (7 of 21) of the genital tracts. Of the 193 sera tested, 61.7% were positive with no significant difference between group A (52.3%, 34 of 65) and B (66.4%, 85 of 128). Chlamydia-like organisms were detected in 28.2% of the swabs from group A and in 22.0% from group B. Twenty of the twenty one uteri revealed no histopathological changes, one showed a pyogranulomatous endometritis due to a *Staphylococcus aureus* infection.

Conclusion: These results imply that Swiss pig herds are regularly challenged by *Chlamydiaceae*. The presence of *Cp. abortus* could account for some of the reproductive failures in problem herds, albeit at a substantially lower prevalence than in German pig farms. The role of the heterogeneous Chlamydia-like organisms requires further investigations.

CUTANEOUS LESIONS ASSOCIATED WITH VIRAL INDUCED VASCULITIS IN A CAT WITH FELINE INFECTIOUS PERITONITIS

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Introduction: One of the hallmarks of feline infectious peritonitis (FIP) is a monocyte-mediated, granulomatous to necrotising phlebitis and periphlebitis. It is mainly observed in leptomeninges, renal cortices and eyes, but has also been described in other organs, such as lungs and liver. Cutaneous lesions have not previously been reported.
This report describes a case of FIP with multisystemic involvement, including multiple nodular cutaneous lesions due to a granulomatous-necrotising dermal phlebitis and periphlebitis. Immunohistology confirmed the diagnosis by demonstrating coronavirus antigen in macrophages within these lesions.
Materials and Methods: Skin and renal biopsies were taken from a cat with suspected dry FIP and multifocal nodular skin lesions. Histological examination and imunohistology for feline coronavirus (FCoV) antigen, using a mouse monoclonal anitbody (FCV3-70, Custom Monoclonals International, West Sacramento, USA), were performed on renal and skin biopsies.
Results: Grossly, the skin lesions presented as multiple, well circumscribed, elevated, up to 2 mm in diameter, red nodules with partial alopecia that were non-painful and non-pruritic. Histologically, multifocal pyogranulomatous perivascular infiltration and phlebitis was seen in mid and deep dermis. In the kidney, severe extensive pyogranulomatous infiltration was seen. Both in skin and renal lesions, FCoV antigen-positive macrophages were demonstrated.
Conclusion: This report describes the first case of FIP with phlebitis and periphlebitis in the dermis, clinically presenting as nodular erythematous skin lesions.
FIP is a systemic, FCoV-induced disease. One of its morphological hallmarks is a granulomatous to necrotising phlebitis and periphlebitis which was recently shown to be triggered by activated monocytes which attach to venous endothelial cells, migrate out of the veins, thereby destroying the basal lamina, and then accumulate perivascularly. The process appears to be cytokine-mediated. Animals with FIP exhibit generalized activation of venous and, to a lesser extent, arterial endothelial cells, and likely mediating selective adhesiveness of monocytes. Selective endothelial cell reactivity to systemic cytokines could explain why FIP-associated phlebitis is not seen in all organs, but predominantly occurs in leptomeninges, renal cortex and eyes. However, phlebitis in i.e. lungs and liver has been shown and the present report demonstrates that even dermal veins can be affected.

FELINE NEUROPATHOLOGY: A RETROSPECTIVE STUDY ON NEUROLOGICAL DISORDERS 1995-2004

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Introduction: The identification in Europe of cats with Feline Spongiform Encephalopathy (FSE) stimulated a retrospective investigation on feline neurological disorders in the Department of Animal Pathology of Torino. The aim of this study is to evaluate the most frequent nervous pathologies in cats with progressive neurological diseases observed between 1995 and 2004.
Materials and Methods: A total of 192 cases were identified in the archive. Of these 190 derived from animals submitted by clinicians of this Department or by private practitioners and 2 were surgical removals. The animals aged between 1 month and 20 years, were mainly European and Persian breeds. Histology, histochemistry, immunohistochemistry and electron microscopy techniques permitted to classify the lesions.
Results: Diagnoses were divided in 6 groups: malformative (3%) – hydrocephalus, syringo-hydromyelia, inflammatory (20%), traumatic/degenerative (16.6%), neoplastic (9.9%) and not classified or minor lesions of uncertain significance (13%). Moreover, 37.5% of cats didn't reveal any histological nervous lesions. The most important group is represented by cats with inflammatory lesions, including Feline Infectious Peritonitis (30.76% of flogosis), non suppurative encephalitis suggestive of viral infections (43.6% of flogosis), suppurative leptomeningoencephalitis and granulomatous encephalitis (by *Toxoplasma gondii* and *Cryptococcus neoformans*). Metabolic and traumatic diseases were the most frequent degenerative pathologies. Meningiomas and lymphomas were the most important neoplasia followed by metastatic tumours, a papillary ependimoma and a malignant peripheral nerve sheath tumour (MPNST). No cases of FSE were identified.
Conclusion: This presentation summarizes the data obtained during 10 years of activity on feline neuropathology surveillance. Inflammatory processes result the most frequent pathologies involving the nervous tissue in cats. This result suggests the importance of a constant surveillance especially on viral infections of this species.
Acknowledgements: This work has been performed in collaboration with Italian Association of Feline Pathology (AIVPAFE).

ENCEPHALITOZOON CUNICULI INFECTION IN RABBITS: PATHOLOGICAL AND SEROLOGICAL INVESTIGATIONS

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Introduction: *Encephalitozoon cuniculi* is a protozoan that affects a wide range of laboratory and domestic animals causing granulomatous lesions in many organs, but especially in the brain and kidneys. Aim of this research is to evaluate the prevalence of this parasitosis in rabbits regularly slaughtered in Piemonte region with serological and histological investigations.

Materials and Methods: The investigations were carried out on 320 rabbits, mixed breeds of both sexes selected from different areas of Piemonte. The animals tested ranged from 90 days to 2.5 years of age (226 breeders, 50 and 44 respectively of 90 and 120 days). Sera were collected during slaughter and kept at -20°C. Serological studies were performed using carbonimmunoassay test from Medicago Laboratories of Uppsala (Sweden). Tissue samples from kidney, brain and eyes were 10% neutral buffered formalin fixed and processed to obtain histological sections stained with haematoxylin and eosin. Specific staining methods (Giemsa and Good-Pasture) were also performed on selected sections.

Results: Serological studies reveal a seropositivity of 73.75% (80.08% on breeders and 30% on 90 days aged animals). Only 7% of seronegative animals showed slight inflammatory lesions both in the brain and in the kidneys. In seropositive rabbits moderate to severe microscopical lesions were present in 81.41% of animals. Chronic non suppurative interstitial nephritis, focal or multifocal granulomatous or non suppurative encephalitis were the most important observed lesions. No significant lesions were detected in the eyes. Brain granulomas, located especially in the hippocampus and in the cerebral cortex showed frequently a necrotic centre surrounded by macrophages, lymphocytes and glial cells. Reactive cells were scattered in the peripheral neuroparenchima, too. Numerous parasitic forms and rare pseudocysts were observed in the brain granulomas using specific stains.

Conclusion: This investigation indicates that *E. cuniculi* infection is diffused in rabbits of Piemonte causing frequently moderate to severe systemic lesions. However clinical signs were not reported by veterinary clinicians. Serological data merit some considerations. In fact the percentage of seropositivity is low (30%) in 90 days aged rabbits related to seropositivity of other categories. Further investigations are necessary for a better understanding of this result.

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PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS): ULTRASTRUCTURAL STUDY OF LUNG DURING AN EXPERIMENTAL INFECTION WITH PRRS VIRUS

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Introduction: The aim of our study was to study the possible implications of pulmonary macrophages (pulmonary intravascular macrophages and alveolar macrophages) in the pathogenesis of PRRS.

Materials and Methods: Four groups of three 3 weeks old piglets were employed. One group was used as control and slaughtered at the beginning of the experiment, the other three groups being inoculated with a strain of PRRS isolated in Chile and killed at 7, 14 and 21 days post inoculation. Lung samples from all the animals were fixed 10% formalin and glutaraldehyde, being routinely processed for light microscopy, immunohistochemistry and ultrastructural studies. The immunohistochemical study was performed employing the avidin-biotin-peroxidase complex (ABC) method with the monoclonal antibody against PRRS SDOW-17.

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

Conclusion: The increase and activation of pulmonary intravascular macrophages are responsible of the interstitial pneumonia characteristic of PRRS.

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DETECTION OF A NOVEL PAPILLOMAVIRUS IN GENITAL PAPILLOMATOUS LESIONS OF BOTTLENOSE DOLPHINS

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Introduction: Genital papillomavirus infections have been observed in cetaceans, and are in general self-limiting and regress spontaneously. However, chronic slow progressing condyloma-like lesions were observed in 4 dolphins with no or minimal related clinical signs. The purpose of this study was to describe these chronic lesions and to identify the etiological agent.
Materials and Methods: Two male and two female bottlenose dolphins (*Tursiops truncatus*) with an age between 8 and 36 years presented with condyloma-like lesions on the penis and in the vagina. The lesions were slow developing and present since several years. Biopsies were taken under voluntary behaviour in 3 animals. In 1 animal a sample was obtained at necropsy. Samples were taken for microscopical examination and for DNA sequencing. Immunohistochemistry (IHC) was performed using a polyclonal rabbit anti-bovine papillomavirus 1 (BPV1) antibody, which reacted with genus-specific structural papillomavirus antigens. "*In situ*" hybridization was performed on IHC positive cells using a labelled, bovine papillomavirus probe under stringent conditions.
Results: The lesions were single, soft, whitish, exophytic, plaque to cauliflower-like, non-ulcerative, non-painful and ranging from 0.5 cm up to 4 cm. Microscopically, a severe diffuse epithelial hyperplasia with marked rete ridge formation and intracellular oedema was found. Occasionally, large amorphous eosinophylic intranuclear inclusions were present. In all samples, several nuclei stained positive in immunohistochemistry using the anti-BPV1 antibody. After in situ hybridisation with a BPV-1 DNA probe, no reaction was observed. Using degenerate primers, partial sequences of the L1 capsid gene of a novel papillomavirus were determined from the penile papillomatous lesion. Comparison with known papillomavirus sequences in GenBank showed the highest degree of similarity with the corresponding region of the *Phocoena spinipinnis* papillomavirus type 1 (PsPV1), isolated from a Burmeister's porpoise.
Conclusions: This is the first time in which papillomavirus infections were detected in biopsies of chronic condyloma-like lesions in dolphins. The genital location suggests that the disease is venereally transmitted. Since animals were chronically infected and viral replication was present, they may constitute a risk to other animals. Therefore, an accurate diagnosis is indispensable. Since some condylomas in other animal species and humans can contribute to reproduction disorders or can develop into malignancies, regular follow-up is recommended in order to evaluate the progression of the lesions.

MOLECULAR BASIS OF ANGIOGENESIS – IMPLICATIONS TO NOVEL THERAPEUTIC METHODS DEVELOPMENT

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Angiogenesis, the process of new blood vessels formation, is a crucial mechanism required for a number of physiological and pathological events. It normally occurs during embryonic development, wound healing and the menstruation cycle. On the other hand, unregulated, pathologic angiogenesis is seen in pathological conditions, such as psoriasis, diabetic retinopathy, chronic inflammation and particularly cancer. Neovascularization is required for continuous tumour growth, its local invasion and metastasis formation. New blood vessels formation is regulated via multiple factors, mostly cytokines, secreted by both endothelial cells, tumour cells and surrounding stroma, but also through the proteolytic system activity. Since angiogenesis is the result of an intricate balance between pro- and anti-angiogenic factors, it was recognized as a powerful control point in tumor development. The number of anti-angiogenic approaches has been developed, many of them based on the inhibition of the pro-angiogenic factors functional activity.

NATURAL SCRAPIE PHENOTYPE IN ITALIAN OUTBREAKS: A RETROSPECTIVE STUDY

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Scrapie in sheep and goats is the longest known Transmissible Spongiform Encephalopathy (TSE) and to date it is considered non pathogenic for humans at least under natural conditions. However, under experimental conditions, sheep are easily orally infected by the agent of Bovine Spongiform Encephalopathy (BSE) and, in contrast to cattle, carry abundant amounts of infectivity throughout most body tissues even early after infection. The attention towards scrapie and its discrimination from BSE has increased because a natural transmission of BSE to sheep can't be excluded, with a high risk of exposure for humans. Furthermore, the biological basis of etiology, in TSE, remains controversial and at the moment the classification of the PrP^{sc} phenotype plays a major role. A classification of such phenotypes, in natural conditions, relies on genomic differences and clinico-pathological features: clinical signs, neuropathological changes, detection of diseases related PrP (PrP^{sc}) by both immunohistochemistry (IHC) and Western blot (WB). The aim of this study is to provide a phenotype definition of natural scrapie in Italy. Therefore a retrospective study on 6 natural scrapie outbreaks in Italy was carried out. Clinical signs were assessed according to a pre-established schedule. Neurohistological changes of scrapie affected sheep were semi-quantitatively assessed using a 5 (0-4) score. The PrP^{sc} phenotype was studied by immunohistochemical distribution in 15 SNC areas and interpretation of the molecular weight using a highly sensitive Western blot using sodium phosphotungstic acid (NaPTA) in the same areas. PrP-gene genotype was determined at codons 136, 154, 171. IHC and WB allowed us to identify in all cases a single phenotype of PrP^{sc}, no relationship was established between clinical signs and severity of neuropathological changes. All sheep, but one (ARQ/AHQ) were of the same genotype (ARQ/ARQ).

HELICOBACTER FELIS BUT NOT *HELICOBACTER BIZZOZERONII* INDUCES SEVERE PARIETAL CELL LOSS IN MONGOLIAN GERBILS

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Introduction: Non-pylori Helicobacters are found in approximately 0.3% of human gastric biopsies. These infections are associated with gastritis, gastric ulcers and MALT lymphomas. Using PCR analyses we showed that approximately 50% of these are Helicobacters commonly found in dogs and cats, including *Helicobacter (H.) felis*, *H. bizzozeronii* and *H. salomonis*. In contrast to *H. pylori*, the virulence mechanisms of these species are largely unknown. It was the purpose of the present study to find differences in virulence between *H. felis* and *H. bizzozeronii* using an *in vivo* experimental model in Mongolian gerbils.
Materials and Methods: Fifteen 6 weeks old female SPF gerbils were inoculated intragastrically three times with 48 h interval with *H. felis* ATCC 49179 (n=10) or *H. bizzozeronii* CCUG35545 (n=5). Three gerbils were inoculated with sterile culture medium. All animals were sacrificed three weeks later. A longitudinal strip covering all regions of the stomach was taken for histology. Samples from fundus and antrum were taken for PCR. Gastric colonization by the bacteria was assessed using Giemsa staining, immunohistochemistry (anti-*H. pylori* antibody) and a multiplex PCR. The histologic samples were evaluated for inflammation on haematoxylin and eosin stained sections. Apoptosis was visualized using immunohistochemical staining for caspase-3. Parietal cells were identified using immunohistochemistry for hydrogen potassium ATP-ase.
Results: All gastric samples from infected gerbils were positive for the bacteria by PCR. Giemsa staining and immunohistochemistry revealed mainly colonization of the gastric antrum. The control animals were negative by all three techniques. Inflammation consisted mainly of lymphocytic and neutrophilic infiltrates in the propria mucosae of the antrum, and to a much lesser extent of the fundus. All animals infected with *H. felis* showed a complete loss of parietal cells extending from the limiting ridge into the fundus. In this region the mucosa was not reduced in thickness. In these same animals a dense band of apoptotic cells and large numbers of *Helicobacter* bacteria were seen at the transition between the zone of complete parietal cell loss and the zone with normal parietal cells. The bacteria were seen in close contact with the parietal cells. In the *H. bizzozeronii* infected gerbils, there was only mild loss of parietal cells.
Conclusion: At 3 weeks post infection, focal apoptotic loss of parietal cells was spatially associated with the presence of bacteria in *H. felis* infected gerbils but not in *H. bizzozeronii* infected gerbils. This strongly suggests a difference in virulence between the two non-pylori *Helicobacter* (species) strains in the gerbil model. This loss of parietal cells may lead to intestinal metaplasia, as already reported for *H. pylori* infection in the gerbil model.

SCRAPIE CASE SIMILAR TO NOR98 DIAGNOSED IN BELGIUM VIA ACTIVE SURVEILLANCE

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Introduction: Scrapie is a fatal transmissible spongiform encephalopathy caused by prions, similar to Bovine Spongiform Encephalopathy (BSE) in cattle. Typical features of these diseases are a long incubation period and the gradual vacuolation of brain neurons and neuropil. The pathogenesis of the diseases is believed to due to the conversion of the normal protease-sensitive prion protein, PrP^{sc}, into a partly protease-resistant isoform, PrP^{sc}, which accumulates progressively in the central nervous system of the affected animals. Several (classical) scrapie strains have been described based on lesion profiling.
Materials and Methods: In Belgium, since April 2002, all sheep older than 18 months are tested with a rapid test (Bio-Rad) through the active TSE surveillance program (EC regulation 1999/2001).
Results: In 2002, five of the 6 outbreaks showed a classical scrapie lesion profile, but one showed special features. The ewe was apparently healthy and presented for slaughter. According to the active epidemio-surveillance protocol only part of the medulla oblongata around the region of the obex is taken out. The sample was repeatedly tested positive with the rapid test. The histopathological investigation was negative. No detectable PrP^{sc} were revealed by immunohistochemistry. The detection of scrapie associated fibrils (SAFs) was also negative. Ovine western blot (WB) analysis was positive showing a PrP^{sc} glycoprofile with a strongly marked 4^{th} lower band at ~ 12 kDa, compared to a classical scrapie glycoprofile. The whole flock was culled, but no other animal of the flock tested positive.
Discussion: The unusual characteristics of the present case are: 1) only one animal of the flock (n=55) was affected; 2) no lesions were present in the brainstem (obex) as compared to classical scrapie cases; 3) the absence of PrP^{sc} immunolabelling in the area of the obex; 4) the PrP^{sc} glycoprofile of the present case differed clearly from the glycoprofiles found in isolates of classical scrapie strains and the BSE strain, and is indistinguishable from the Nor98 glycoprofile. Nor98 cases are characterized by ataxia, the onset of disease at older age, the presence of neuropil vacuolization and PrP^{sc} deposits mainly localized in the cerebellar and cerebral cortices respectively on histopathology and immunohistochemistry.
Conclusion: In the present case, histopathology, immunohistochemistry and SAF were all negative. Only the WB confirmed the scrapie positivity of the present case and the diagnosis of scrapie could therefore have been overlooked. Nor98 cases may question the significance and efficiency of the scrapie active epidemio-surveillance protocol, because from a diagnostic point of view, the positive results obtained with a rapid test require to be confirmed by standard methods like the histopathological examination and the immunohistochemical detection of PrP^{sc} at the level of the obex.

"STAGGERING DISEASE" IN A CAT: FIRST CASE OF BORNA DISEASE VIRUS INFECTION IN A BELGIAN CAT

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Introduction: Borna disease virus (BDV), is named after a city in Germany where severe outbreaks in horses with progressive neurological symptoms occurred. However many animal species, including humans, are susceptible for the disease with clinical or subclinical infections. It has generally been accepted that BDV causes a persistent infection of the central nervous system (CNS), which might express in behavioural changes up to severe neurological disease. In cats, a spontaneous non-suppurative meningo-encephalomyelitis, characterized by ataxia and behavioral changes may be etiopathogenically linked to BDV. This is the first BDV infection in a cat in Belgium.
Materials and Methods: Performed tests were histopathology; immunohistochemistry (FSE) and ELISA (BDV antigen). Other tests on infectious agents were: rabies, feline infectious peritonitis (FIP) virus, feline leukemia virus (FeLV), feline herpes virus (FHV) and feline immunodeficiency virus (FIV).
Results: A 1 year old female domestic cat showed progressive ataxia, hypermetria, hyperaesthesia, pruritus and "madness". The cat was euthanized. Brain histopathology revealed a moderate to severe perivascular cuffing with lymphocytes, plasma cells and some macrophages of the meningeal blood vessels of the cerebellum and cerebrum. A widespread lymphoplasmacytic cuffing, multifocal astrocytosis, gliosis and neuronophagia were present in the cerebrum and brainstem. FSE could be excluded histologically and immunohistochemically. Rabies, FIP, FeLV, FHV and FIV examinations were all negative. BDV-specific protein antigens using a highly specific antigen ELISA tested positive.
Discussion: The described symptoms and lesions correspond very well with those reported in literature. The mechanism of BDV induced brain pathology may be the following: astrocytes play a major role in maintaining the appropriate microenvironment in the CNS required for normal neuronal activity. Astrocytes regulate the level of extracellular glutamate. Excessive levels of extracellular glutamate often result in neuron toxicity and death. Astrocytes are one of the target cells during BDV infection in the CNS and show severe and specific impairment in the ability of glutamate uptake.
Conclusion: BDV infection of cats should carefully be followed, as cats are one of human's closest companions and a zoonotic spreading of BDV is presently not known.

QUANTITATIVE HER2/NEU EXPRESSION IN FELINE MAMMARY TUMOURS

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Introduction: HER2/neu gene is a member of the Erbb-like oncogene family. It is commonly overexpressed in 20-30% of breast carcinomas and this has been shown to correlate with poor prognosis. We have previously shown that feline HER2 is expressed in 37% of feline mammary carcinomas (FMC) and that has the same molecular weight of the human HER2. We have also partially determined the nucleotide sequence corresponding to kinase domain of receptor from exon 16 to 19. Aim of this study is to complete the sequence of the kinase domain of feline HER2 receptor and to evaluate by real time PCR its expression in normal, benign and malignant feline mammary tumours.

Materials and Methods: To determine the sequence from exons 16 to 23 of feline HER2 we designed primers according to homology of canine and human HER2 sequence. Specific amplified fragments were than directly sequenced. To determine HER2 gene expression in feline mammary tumours we extracted total RNA by phenol-chloroform method from 8 feline mammary carcinomas, 2 fibroadenomas, 1 hiperplasia and from 3 primary feline mammary carcinoma cells lines. Total RNA was than subjected to cDNA synthesis by reverse trascriptase. To evaluate HER2 expression by real time PCR we designed a TaqMan probe specific for the kinase domain of feline HER2 sequence and we used as house-keeping the feline β-glucuronidase gene.

Results: We determined the complete sequence of feline HER2 from exon 16 to exon 23 and we found a different expression level of HER2 in feline mammary samples. In particular we showed that feline mammary carcinoma cells line and feline mammary carcinomas express more HER2 gene copy numbers than benign tumours and normal mammary glands.

Conclusions: These data show that, like in humans, HER2 is important in the tumour progression of feline mammary tumours and that this tumours is a suitable model in comparative oncology to test innovative approaches for the therapy of aggressive human breast cancer.

PATHOMORPHOLOGIC AND EXPRESSION PATTERN ANALYSIS OF HUMAN CD59 – TRANSGENIC PIGS DESIGNED FOR XENOTRANSPLANTATION

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Introduction: Progress in the field of organ transplantation has led to a shortage of human donor organs. Pigs are considered as appropriate organ donors for xenotransplantation. However, hyperacute rejection mediated by the complement cascade represents the main problem in xenotransplantation. To overcome the hyperacute rejection, pigs were produced that are transgenic for the human complement regulator CD59 which inhibits the formation of the membrane attack complex. Here, the transgenic pigs were morphologically examined to find out whether a specific phenotype is linked with the CD59 transgene. Furthermore, the expression patterns of human CD59 mRNA and human CD59 protein were systematically analyzed in a variety of organs and tissues.

Materials and Methods: Human CD59 transgenic pigs were produced by microinjection of a CMV-promotor driven human CD59 construct into the pronuclei of porcine zygotes. Sixteen CD59 transgenic pigs (aged 4-32 months) were necropsied and a complete pathomorphological evaluation was conducted. Formalin-fixed, paraffin-embedded samples from organs and tissues were haematoxylin and eosin stained and histologically examined. RNA-extraction, cDNA-synthesis and qualitative polymerase chain reaction of formalin-fixed, paraffin-embedded samples were performed to analyze the expression of CD59 mRNA. To evaluate the expression of the human CD59 protein, an immunohistochemical analysis with a monoclonal mouse-anti-human-CD59 antibody was performed.

Results: No specific pathomorphologic phenotype was detected in any of the examined transgenic pigs. Reverse transcriptase – polymerase chain reaction identified expression of CD59 mRNA in all organs in 3 pigs and 8 pigs had expression only in a subset of the examined organs. Immunohistochemistry revealed expression of the human CD59 protein in cardiomyocytes, skeletal muscle cells and endothelial cells of the transgenic pigs. In each animal, only a subset of organs expressed the human CD59 protein and these organs varied from pig to pig. In the heart and in skeletal muscle, a patchy expression pattern was observed, i.e., only part of the cells showed a positive staining.

Conclusions: Human CD59 transgenic pigs had no specific pathomorphologic phenotype. Expression of human CD59 mRNA was demonstrated in a variety of organs and tissues of the transgenic animals, suggesting a successful integration of the human CD59 construct. Surprisingly, the transgene was expressed with great variations between different organs as well as between different animals. The possible reasons for the inconsistent expression patterns give rise to speculations on the mechanisms responsible, including chimerism or tissue specificity of the CMV-promotor.

NEUROPATHOLOGICAL FINDINGS IN TRANSGENIC MICE EXPRESSING A BOVINE PRION PROTEIN WITH FOUR EXTRA OCTAPEPTIDE REPEATS

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Introduction: Prion diseases are neurodegenerative disorders of infectious, spontaneous or inherited origin. Some inherited prion diseases in humans have been associated with an increased number of octapeptide repeats within the PrP open reading frame (ORF). In animals, it has been shown that the region comprising the octarepeats (OR) is not essential for mediating scrapie pathogenesis and prion replication but certain role in modulating the extent of incubation times has been suggested.

Materials and Methods: To gain more insight in the role of the octapeptide repeat region in pathogenesis of bovine spongiform encephalopathy (BSE), transgenic (Tg) mice expressing PrP containing four extra octapeptide (bo10ORTg) repeats were developed and inoculated with BSE brain homogenates. We show results of neuropathology, after performing histopathological and immunohistochemical (IHC) studies in paraffin embedded tissues along with Western blot analyses.

Results: In these mice, the course of infection progressed faster than boTg mice expressing six-octapeptide protein (bo6ORTg mice). Moreover, bo10ORTg showed spontaneous neuropathological alterations. Typical CNS spongiform degeneration was detected by histopathological analysis in all mice tested although only inoculated animals showed the presence of bovine PrP^{res} by immunohistochemistry. The PrP^{res} distribution pattern was indistinguishable from that of bo6ORTg mice, consisting of fine granular and punctuate neuropil labelling, and stellate labelling foci, which seemed to be associated with glial cells, but we also observed granular staining in the neuronal bodies and perineuronal staining, and occasionally as a plaque-like deposits. 10ORBo-PrP non-inoculated-Tg mice showed early neurological dysfunction which increased at later times. The onset of signs could be correlated with the expression levels of the transgene. On the other hand, when these animals were inoculated they developed a fast disease with deposition of PrP^{res} after less time post-inoculation bo6ORTg mice.

Conclusion: These results revealed that the number of octapeptide repeats in the PrP gene might change the development of prion diseases but not the pattern of PrP^{res} deposition.

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A GENOME-WIDE ANOIKIS SUPPRESSION SCREEN IDENTIFIES A POTENT INDUCER OF METASTASIS

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Introduction: Metastasis commonly underlies the malignancy of cancers, representing the principal cause of cancer treatment failure. Distant site colonization by tumours, in particular those of epithelial origin, is prevented by several physiologic barriers, including anoikis; i.e. apoptosis resulting from lack of adhesion. Hence, anoikis is considered to act as a biological hurdle, preventing survival or primary tumour cells upon dissemination and entry into blood and lymph criculation. Indeed, acquiring resistance to anoikis has been proposed to represent a general prerequisite for survival of metastases during circulation.
Materials and Methods: Given this critical role of anoikis in cancer, we asked whether anoikis suppression could serve as the basis for an unbiased, functional genome-wide screen to identify genes that induce metastasis. For this purpose, we have designed an *in vitro*, epithelial anoikis system that allows the rapid screening of cDNA expression libraries.
Results: This approach has led to the isolation of an anoikis-resistant cell clone. The molecular and pathological characterization of this clone will be presented. Consistent with our objective, both intravenous and subcutaneous administration of this cell clone to mice caused highly invasive metastases with a short latency.
Conclusion: With an anoikis suppression screens genes and pathways can be detected and identified that contribute to the metastatic phenotype of tumour cells.

PATHOLOGICAL STUDY OF GOUT IN LAYERS FLOCKS

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Introduction: Kidneys are one of the important organs that excrete the excess uric acid. Renal dysfunctions cause the high mortalities follow gout syndrome. Gout includes visceral and synovial forms. The visceral form was discovered 30 years ago. The other name of the gout is acute toxic nephritis, renal gout, renal uroliths, and etc. This disease causes atrophy in one or both of the kidneys, dilated pelvis that mixed with uroliths and different degree of visceral gout. The mortality can exceed 3% in several months that 50% of it is due to uroliths. The other aspects of this syndrome are decreasing reproductive performance in layer flocks and weight loss in broiler flocks. The most causative factor in recurrence of gout syndrome is: 1) vitamin A deficiency; 2) IBDV and IB; 3) nephritis due to sulfonamide overdose; 4) food mycotoxins; etc.

Materials and Methods: We decide to evaluate effects of gout in layer flock in Tabriz city. The suspected cases choose and after taking photos we put the affected organ in 10% formalin. Macroscopic and microscopic cut were prepared with 5 to 6 thickness. The silver, haematoxylin and eosin staining is used.

Results: Macroscopic findings: renal swelling and atrophy, marbleizing appearance, gray to whitish color, dilated pelvis that occur concurrence pasty white substance, chalky urate depositions in cardiac, liver, splenic, mesenteral serosis, synovial fluid specially hock joint. Microscopic findings: located urate depositions (Fenal urate tophus) with inflammatory cell infiltration (lymphocytes, heterophiles, giant cells and macrophages) that follows inflammatory and fibrotic reactions. Degenerations and necrotic lesions in renal tubules and interstitial nephritis were seen.

Conclusion: In this syndrome because of destructions in renal parenchyma, dysfunction of uric acid excretion causes urate deposition in visceral and non visceral organs. We suggest with control of hydrations situation, viral disease (IBDV, IB) and increasing of diet from viewpoint of vitamin A can reduce prevalence of this syndrome.

PREVALENCE OF CANINE PROSTATIC LESIONS IN STRAY DOGS OF TABRIZ (IRAN)

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Introduction: Prevalence of canine prostatic disease is reported to be 2.5%. Prevalence of all types of prostatic diseases increase with age, with a mean reported age of 8.9 years for onset of clinical signs. Common canine prostatic disorders include benign prostatic hypertrophy (BPH), prostatitis, prostatic cysts and prostatic adenocarcinoma. Canine BPH is a benign alteration of prostate gland characterized by diffuse hypertrophy and hyperplasia of the prostatic glandular epithelium and mild stromal proliferation. Bacterial infection of the prostate gland can be subdivided into acute and chronic bacterial prostitis (ABP) and (CBP).

Materials and Methods: Fifty adult male stray dogs were selected for necropsy examination and determining the all types of prostatic disease. After remaining of prostatic samples, we fixed them in 10% formalin for 24 h, and then haematoxylin and eosin was used for staining of tissue sections.

Results: We recognized that almost all of the necropsy dogs had prostatic lesions. In our study, the prevalence of CBP was 37.5% and in the case of BPH 36%, prostatic cysts 11%, squamous metaplasia 7%, prostatic papillary adenoma 6.4%, prostatic calculi 2.1%.

Conclusion: In our study, we found that the prevalence of chronic infectious disease was high and attention to infectious disease at high ages must be more indicated. Finally, using of appropriate antibiotics is recommended.

EXPERIMENTAL STUDY OF APOPTOSIS INDUCED BY INFECTIOUS BURSAL DISEASE VIRUS, USING TUNEL ASSY, ELECTRON MICROSCOPY TECHNIQUES AND RT-PCR

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Introduction: Infectious bursal disease virus is an important immunosuppressive virus of chickens. The virus is ubiquitous and, under natural conditions, chickens aquire infectious by the oral route. IgM cells sever as targets for the virus. The most extensive virus replication takes place in the bursa Fabrici (BF). 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-VP5. The large segment A encodes VP2-VP5, the smaller segment B encodes VP1 which has polymerase and capping enzyme actvities. Lymphoid cells in the bursa Fabrici and the target cells of IBDV. Infectious results in lymphoid deplation and the final destruction of the BF as the predominant feature of the pathogenesis of infectious bursal disease. Besides necrosis, marked atrophy of infected BF without sever inflammatory response was also reported. This suggests the involvement of apoptotic processes in the pathogenesis of the disease.
Materials and Methods: We decided to evaluate the apoptotic effects of IBDV on bursa Fabrici. We organized three groups, of SPF chicken with 21 days old, naming TEST-IBDV, TEST-VAC, CONT-SERUM. Group TEST IBDV recevied VVIBDV (IR499), group TEST-VAC recevied D78 intermediate vaccine and group CONT-SERUM recevied saline normal by oral and nasal route. After 3 days that we had clinical signe and mortality, the bursa Fabrici of all treatment and control group SPF chickens we ectomized and were provided for preparing light microscope (LM) and electronic microscope section, tunel detection technique and RT-PCR techniques.
Results: LM and tunel studies result in apoptotic bodies appear as round or oval cytoplasmic mass with or without contained basophilic nuclear material and well defined cresent clamps of chromatin in lymphocyte of bursa Fabrici and electron microscope (EM) studies resulted the peripheral nuclear chromatin forms aggregates of osmophilic granules (x 6200) which from the fibrilar core and errigular cell gut line nuclear fragments and whiriling endoplasmic reticulum (x 8200) also RT-PCR technique confirm of IBDV in bursa Fabrici.
Conclusion: In this study we showed that may be IBDV induce of apoptosis in lymphocyte of bursa Fabrici. But in order to determine the significance of apoptosis in the pathogenesis is of IBD, simillar investigations must be performed on the target cells in the BF of infected chickens.

CHANGES IN THE DENSITY OF SYMPATHETIC NERVE TERMINALS AND STEROIDOGENIC ACTIVITY OF PORCINE OVARIES AFTER DEXAMETHASONE-INDUCED POLYCYSTIC OVARIAN SYNDROME

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Introduction: The role of the nervous system in the control of normal ovarian secretory activity and follicular development has already been documented in part. We studied the possibility whether cysts formation may be associated with derangement of the sympathetic control of the ovary in gilts.
Materials and Methods: Ovarian cysts in the sexually matured gilts (gr. I, n=6) were induced by *i.m*. injections of dexamethasone (DXM; Dexasone®, 3.3 μg/kg b.m., every 12 h), on days 7-21 of the first estrous cycles. Using the same paradigm, the gilts of gr. II (n=6) received saline. Ovaries were dissected out on day 11 of the next cycle and their macroscopic evaluation was performed. Cryostat ovarian sections were stained by means of double-immunofluorescence to investigate the number and the distribution pattern of tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DβH) and/or neuropeptide Y-immunoreactive (NPY-IR) nerve fibers. The contents of progesterone (P4), androstendione (A4), and estradiol-17β (E2) in follicular and cystic fluid and wall as well as in corpora lutea (CL) were estimated by RIA. Expression of cytochromes P450scc and P450arom in ovarian structures was determined by Western blot. Immunoblots were quantitated by scanning on Kodak 1D Image Analysis Software (USA).
Results: The ovaries in the group II were heavier ($p<0.05$) than after DXM injections. In both groups, the number of follicles (F) with diameter 1-3 mm and CL were not different. In the ovaries of the I group, F measuring 3-6 mm in diameter were not found, while 2.2±0.4 of follicular cysts, 1-3 cm in diameter, was encountered per ovary. An increase in the number of TH/DβH- and/or NPY-IR nerves in the vicinity of F, CL, cysts and blood vessels was observed in the I group, when compared with the II group. DXM injections lead to an increase ($p<0.05$) in the content of P4 and a decrease of A4 and E2 ($p<0.05$) in the cystic fluid, compared to 3-6 mm F in the II group. The concentrations of P4, A4 and E2 in the cystic wall were higher ($p<0.05$-0.001) than those found in these F of control gilts. In CL of the gilts receiving DXM, an increase ($p<0.001$) in the content of P4 and A4 was observed, when compared to that detected in the II group. Expression of cytochromes P450scc and P450arom in CL of DXM-treated animals was higher (130% and 104.7% of control values, respectively). In the cystic wall, the staining intensity of the P450scc band was lowered (by 8.2%), whereas intensity of cytochrome P450arom staining was enhanced by 7.9%, as compared to the 3-6 mm follicles of control gilts.
Conclusion: The present study revealed that in the polycystic ovaries in gilts an increase in the number of the sympathetic nerve terminals was associated with changes of the steroidogenic activity, what suggests the important role of the sympathetic neurons in the etiology and/or progression of ovarian cysts.

HEREDITARY HYDROCEPHALUS INTERNA IN LABORATORY REARED SYRIAN HAMSTERS

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Introduction: In a study of effects of housing changes on the behavior of hamsters, three animals died acutely without signs. The animals had hydrocephalus. In the colony that provided animals, a problem of hydrocephalus had been documented. In an attempt to clean the colony of the problem, animals for the ongoing experiment were offspring of new breeders from another supplier.

Materials and Methods: At the termination of the behavioral study, all brains were collected and examined macroscopically and by routine light microscopy of paraffin embedded tissues. Based on the data from the brain examinations, three trial matings from breeders expected to produce hydrocephalic offspring and one mating of breeders expected to produce normal offspring were made, and the offspring were examined. Feed and liver samples from animals were tested for teratogens, and sera from breeders of hydrocephalic offspring were tested for antibodies to viruses known to cause hydrocephalus in hamsters.

Results: Although no animals showed obvious behavioral changes, many males and females in control and manipulated groups had hydrocephalus interna. Of 35 hamsters, 26% had severe, 29% had moderate, and 29% had mild hydrocephalus. Only 6 (16%) animals had normal brains. Lesions were not noted in other organs. In 3 controlled breedings for hydrocephalus, 100%, 100% and 75% of offspring had hydrocephalus while in a mating for normal animals, 100% of offspring were normal. One animal in a litter bred for hydrocephalus where all 5 had hydrocephalus had a domed skull and was runted. No metals, or mycotoxins were detected in tissues or food, and sera were tested negative for Sendai virus, reovirus 3, and LCM virus. Retrospectively, hydrocephalus was confirmed in breeders.

Conclusion: Hereditary hydrocephalus appears to be widespread in hamster stocks supplied by reputable suppliers in Europe. Affected animals are asymptomatic and usually die without obvious premonitory signs. Despite severe hydrocephalus, the animals can breed, and perform well in tests of balance and behavior. This entity is unlike the previously described, hamster, hereditary hydrocephalus which is phenotypically identifiable and usually is lethal before attaining breeding age. In experiments using hamsters where brain lesions are part of the measured outcome, a colony should be certified free of hydrocephalus. This form of hereditary hydrocephalus provides a model to study the pathogenesis of hydrocephalus.

ATYPICAL SCRAPIE IN SWITZERLAND: CASE REPORT WITH DIAGNOSTIC AND EPIDEMIOLOGICAL RELEVANCE

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Introduction: Scrapie incidence appears to be very low in sheep and goats in Switzerland. Seven single cases have been diagnosed since 1981, five in sheep and two in goats. In recent years, surveillance programs were started in order to substantiate the low prevalence of PrP^{sc}-positive animals in the Swiss sheep and goat population.

Materials and Methods: An adult sheep (breed: Spiegelschaf, Mirror Sheep) was presented with a history of slowly progressive ataxia, stumbling and hard of hearing. The animal was euthanized due to bad bodily condition and prognosis and submitted for necropsy. At post mortem, the animal was emaciated and had pointed teeth. Histological findings were confined to the CNS. The presence and the distribution of PrP^{sc} were investigated by immunohistochemistry (IHC) using 2 mouse monoclonal andibodies (mAbs: 34C9 and 6H4) and a polyclonal antibody (pAb: C15S). In addition, brain samples from medulla oblongata, cerebellum and cerebrum were investigated by a Rapid Western blot technique (Prionics® test).

Results: Histopathological changes consisted of an extensive diffuse vacuolation of the grey matter neuropil, mainly in the cerebellar molecular layer, less severe in the cerebral cortex, basal ganglia and in thalamic and hyothalamic areas, but only minimal in the medulla oblongata. Gliosis was moderate and diffuse. A strong PrP^{sc} immunostaining was detected in brain (grey matter neuropil of cerebellum), and retina (inner and outer plexiform layer), but not in lymphnodes, spleen or other lymphatic tissues including nictating membrane follicles.

Western blot analysis correlated well with the immunhistochemical PrP^{sc} distribution, in particular they were interpreted negative in samples from the caudal brain stem.

Conclusion: Histopathological findings and PrP^{sc} distribution within the brain is similar to the so called Nor98 cases reported from Norway. The atypical lesion and PrP profile as well as the restriction of PrP to brain and eyes differs clearly from classical scrapie. In conclusion, the selective sampling of medulla oblongata (obex region) for surveillance studies as well as biopsies of nictating membranes could miss atypical scrapie cases as the one reported here.

TOXICITY FOLLOWING DELIVERY OF NAKED PLASMID DNA AND CATIONIC LIPOSOME-DNA COMPLEXES TO THE OVINE LUNG

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Introduction: Cystic Fibrosis is a human genetic disease; its basis is a deficiency of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The sheep is used as an animal model in the development of a gene therapy delivery system. As part of the study to assess the efficacy of liposome mediated (pDNA:GL67) and naked DNA gene transfer, toxicity was assessed through histopathological examination of lung tissue.
Materials and Methods: Fibre-optic endoscopy was advanced through the endotracheal tube of an anaesthetised sheep into defined lung segments. Various transgene doses of naked pDNA, pDNA:GL67 and water (control) were instilled into different specific lung segments. At post-mortem, each lung segment was identified, removed and transversely sectioned; samples were taken for assay and histopathological examination. Toxicity was assessed histopathologically by using a semi-quantitative scale, evaluating the inflammatory response. The grades were given from – to ++++; the pathological changes identified were mild, moderate to severe levels of suppurative, airway centred inflammation, extending to architectural destruction and oedema in the most severe cases.
Results: Histopathological changes were characterised by a neutrophils predominant, acute inflammatory response. The severity of changes for naked pDNA and pDNA:GL67 correlate with dosage; the response to pDNA:GL67 was generally most severe. The response was predominantly bronchiolocentric in origin, extending into the interstitium in the most severely affected areas. Lesions often appeared as discrete, well demarcated foci adjacent to areas of normal lung. Controls showed no/incidental changes.
Conclusion: Delivery of gene therapy agents by direct instillation into lung segments is associated with significant toxicity. The agents were instilled into large airways, but an inflammatory response in seen further distally, suggesting a pooling of material. A similar effect has been seen in mouse models. It is important to note that segments within the same lung cannot be considered entirely biologically separate, although different responses are seen to according to the dose administered. It is important to assess the toxicity of the gene therapy agents as their use may be limited if they cause significant levels of inflammation, particularly as repeated administration may be necessary for the therapy's efficacy.

RUMINAL ENZYME ACTIVITIES OF SHEEP IN INDUSTRIALLY EXPOSED AREA

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Introduction: Rumen microbes can synthesize enough amino acids and peptides from the inorganic nitrogen in ammonia or other nitrogen source and carbon skeletons and sulphur precursors. Ammonia assimilation by rumen microbes depends on rumen pH, rumen ammonia concentration and ruminal ammonia-assimilating enzyme activity. In the recent past, factories producing copper and mercury have altered the agricultural environment. It has been reported that soil and plant biomass sample analyses from localities situated maximally 10 km from the copper and formerly mercury producing factories showed siginficant soil and biomass contamination by mercury, lead, cadmium, copper, and zinc ions. The objective of the present study was to determine whether various ions causing air, soil and biomass contamination in industrially exposed area would affect some enzyme activities of the rumen fluid.

Materials and Methods: The experiments were carried out on 8 ewes from the industrially exposed area (E) and 8 ewes from control area (C). The ewes weighted between 30 to 35 kg. Rumen fluid was collected in the morning 2 h after feeding. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT) and glutamate dehydrogenase (GDH) enzyme activities of rumen fluid were determined by spectrophotometric kits. Ruminal copper, zinc, cadmium and lead concentrations were determined by atomic absorption spectrophotometry.

Results: Ruminal enzyme activities in sheep is shown in the table:

	ALT (μkat/L)	AST (μkat/L)	GGT (μkat/L)	GDH (μkat/L)
Experimental	0.998±0.383	2.549±0.069	3.250±0.527	1.216±0.118
Control	0.585±0.049	1.562±0.0499	1.365±0.307	0.299±0.027

The concentrations of copper, zinc and cadmium were significantly higher in rumen fluid in sheep from industrially exposed area in comparison with sheep from control area with no significant differences for lead.

Conclusion: The results of this experiment indicate that the ions copper, zinc and cadmium affect the activity of several rumen enzymes, playing important roles in metabolism of nitrogenous compounds. They can further alter nitrogen metabolism in the rumen.

STUDY OF HISTOPATHOLOGIC CHANGES ON RESPIRATORY TRACTS OF CHICKENS, AFTER VACCINATION AGAINST INFECTIOUS BRONCHITIS (H_{120})

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Introduction: The histopathological changes in the respiratory tracts of chickens were evaluated after vaccination against infectious bronchitis virus with live attenuated vaccine (Massachusetts Serotype).
Materials and Methods: One day old chickens were housed in two isolation units, in controlled environmental conditions, with *ad libitum* access to food and water. Each isolation group composed a group:
1 – control group (C-group),
2 – test group (vaccinated birds).
All birds of both groups were free from *Mycoplasma gallisepticum* and *Mycoplasma synovia*. After necropsy on the 5th, 10th and 21st day of life, samples from lung and upper portion of trachea were prepared for light microscopy tissue response was monitored by microscopic examination of trachea and lungs.
Results: In 5th day samples, there was a mild deciliation in trachea, and a moderate edema and congestion, in addition to infiltration of mononuclear cells in lungs. In 10th day trachea was deciliated and mucous secretions was increased, and edema and proliferation of goblet cells and increased activity of these cells were observed. In 21st day of trachea persisted and mucous secretions were present, too.
In lungs changes included deciliattion of bronchiols, mucosal hyperplasia and sever infiltration of mononuclear cells, in addition to proliferation of goblet cells; increased inflammatory exudation, bleeding and bronchitis. Also hyaline cartilage nodules and their ossification were observed. In control group significant and meaningful changes was not observed. Tracheal injuries due to vaccination were mild to moderate, but in lungs these injuries were more sever and longer.
Discussion: In this study after vaccination with H_{120} strain there was a mild deciliation in trachea and a moderate edema and congestion and these lesions continued until 21st day. Stressor factors, i.e. furmaline with high concentrations in hatcheries caused more reactions in respiratory tract after vaccination with H_{120}, thus in this situation we commend that vaccination used in another way for example water drinking.

A COMPARATIVE STUDY OF THE SERUM PROFILE OF CYTOKINES IN THE PATHOGENESIS OF EXPERIMENTAL ASF AND CSF

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Introduction: Classical Swine Fever (CSF) and African Swine Fever (ASF) are two viral haemorrhagic diseases of swine caused respectively by an enveloped RNA virus belonging to the genus *Pestivirus* (*Flavivirodae*) and by a large icosahedral DNA virus member of the new family *Asfarviridae*. These diseases are both characterised by haemorrhages and lymphopaenia.
Materials and Methods: In this study, we inoculated 4 pigs with the virulent strain Alfort 187 of the CSF virus and 4 pigs with the highly virulent isolate E-70 to study the serum profile of cytokines during the diseases. Animals were painlessly slaughtered, at 23 days post inoculation (dpi) in the case of CSF, and at 6-7 dpi the animals with ASF or culled before because of ethical reasons. Blood samples were taken daily and the level of IL-1β, IL-2, IL-4, IL-8, IL-10, TNF-α and IFN-α was measured using ELISA commercial kits. Samples for histopathology and immunohistochemical detection of viral antigens were fixed in 3.5% formalin and embedded in paraffin wax.
Results: Animals developed clinical signs characteristics of these diseases. At necropsy, we observed the typical lesions of acute ASF and CSF such as haemorrhagic lymphadenitis (particularly evident in gastro-hepatic and renal lymph nodes in ASF) and kidney (a pale kidney with petechiae, "turkey-egg kidney" in CSF), splenic infarcts in CSF and haemorrhagic splenomegaly in ASF. Viral antigen were widely distributed using monoclonal antibodies against E2 (CSF) and vp73 (ASF) by immunohistochemistry. Proinflammatory cytokines were highly expressed in sera of infected animals with CSF and ASF from the beginning of the experiment. Cytokines expressed mainly by lymphocytes increased at the beginning or latter stages of the diseases.
Discussion: Proinflammatory cytokines secreted by macrophages play an important role in the lymphocyte apoptosis and haemorrhages observed in these diseases, showing a decrease in latter stages of CSF. The latter increases of IFN-γ and IL-4 in CSF and IL-10, IL-4 in ASF might be related to B and T cell differentiation and the inhibition of the hyperproduction of proinflammatory cytokines (from 6-8 dpi in CSF) and suggest the role for T lymphocytes in the immune response against CSFV. The expression of chemokines such as IL-8 could induce leukocyte attraction, phenomena observed in different tissues such as lung in acute ASF and CSF. Cytokines produced mainly by Th1 lymphocytes showed an increase in the first stages of the CSF as well as in ASFV infection. Some cytokines related to Th2 lymphocytes showed an increase in the latter stages of the ASF and middle phases of CSF.

EFFECT OF INSULIN-LIKE GROWTH FACTOR (IGF) BINDING PROTEIN (IGFBP)-2 ON RENAL GROWTH PROCESSES: FINDINGS IN TRANSGENIC MOUSE MODELS

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Introduction: The growth hormone (GH)/insulin-like growth factor (IGF)-system plays a crucial role in renal growth and function and is intimately involved in the pathogenesis of various kidney diseases. GH transgenic (G) mice exhibit kidney and glomerular hypertrophy and regularly develop chronic renal failure (CRF) due to progressive glomerulosclerosis. IGF binding proteins (IGFBPs) are capable of modulating IGF effects, however, their functions are largely unclear. IGFBP-2 acts as a growth inhibitor *in vivo* in mice and is elevated in the serum of CRF patients. The present study investigates the effects of IGFBP-2 overexpression on pathological growth processes of the kidney.
Materials and Methods: Heterozygous G mice were crossed with heterozygous IGFBP-2 transgenic (B) mice generating four groups of offspring: nontransgenic controls (C), G and B mice, and double transgenic (GB) mice. Perfusion fixed kidneys from 38 and 75 days old male mice of the four genetic groups were analyzed. Using quantitative-stereo-logical methods, the volumes of renal zones and of nephron segments as well as the mean volume and number of proximal tubule epithelium (PTE) cells were determined.
Results: Renal enlargement was greatly reduced in GB vs G mice, whereas kidney volume did not differ between B and C mice. In G mice, stimulated cortical growth contributed mostly to renal enlargement, whereas growth of medullary zones was only slightly increased. Cortex and outer stripe of medulla of G mice exhibited an increased total volume of the PTE (V(PTE)). Coexpression of IGFBP-2 in 38 days old GB mice completely abolished the GH/IGF-I-induced increase in V(PTE) to control levels. As shown in 75 days old G mice, the increase in V(PTE) was exclusively due to PTE cell hyperplasia (90% increase in cell number), whereas mean PTE cell volume was unaffected. IGFBP-2 coexpression in GB vs G mice reduced V(PTE) by significantly inhibiting GH/IGF-I-induced hyperplasia of PTE cells. Other tubular segments were not influenced by IGFBP-2 coexpression in GB vs G mice. Glomerular enlargement was only slightly reduced (~ 10%) by IGFBP-2 coexpression in GB vs G mice. Glomerular damage (glomerulosclerosis index) did not differ between G and GB mice. No significant effects of IGFBP-2 overexpression on the investigated parameters could be found in B vs C mice.
Conclusion: Several studies indicate inhibitory effects of IGFBP-2 on IGF-I actions. The presented findings thereby suggest a functional dissociation of GH and IGF-I effects in the kidney *in vivo*, with glomerular hypertrophy and sclerosis being predominantly mediated by GH. On the other hand, the stimulated growth of the PTE in high GH/IGF-I conditions is suggested to be primarily mediated by IGF-I and is exclusively attributed to PTE cell hyperplasia.

A MYXOID EPITHELIOID MESOTHELIOMA OF THE *TUNICA VAGINALIS TESTIS* IN A DOG

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Introduction: Mesotheliomas are tumours arising from the mesothelial cells lining coelomic cavities, including the *tunica vaginalis testis*. The majority of mesotheliomas in animals and humans arise from the pleural mesothelium. In animals, three main histological types are described: epithelioid, sarcomatoid/fibroblastic and biphasic. In humans similar histological types are described. However, in human patients, mucin producing epithelial mesotheliomas has been reported and they often represent a diagnostic challenge when distinguishing them from pulmonary and other adenocarcinomas. Here we report a 10 years old, entire, male, Miniature schnauzer, which presented with a sudden onset, clinically palpable, but indistinct scrotal mass. Initial light microscopy revealed an anaplastic tumour of uncertain origin. The report describes the unusual histological features of this tumour, which was finally diagnosed as a myxoid epithelioid mesothelioma after immunohistological and electron microscopic examination.
Materials and Methods: Surgically excised scrotal and testicular tissue was fixed in 10% buffered formalin, routinely embedded in paraffin wax and prepared for light microscopic examination (haematoxylin and eosin stain, Alcian blue-periodic acid Schiff (Ab-PAS) stain). Further sections were stained immunohistologically for cytokeratin and vimentin expression. Following post fixation in 2.5% glutaraldehyde, the tissue was routinely embedded in epoxy resin; ultra-thin sections were obtained and examined with a transmission electron microscope.
Results: Light microscopic examination of the scrotal mass revealed an epithelioid neoplasm growing invasively into the deep and mid dermis. Neoplastic cells formed loosely packed islands, separated by myxoid stroma, and rare papilliform structures. Ab-PAS staining revealed that well differentiated neoplastic cells were occasionally aligned upon basement membranes and separated by plentiful stromal acid mucin. Immunohistology showed that tumour cells co-expressed cytokeratin and vimentin. Ultrastructurally, pleomorphic tumour cells with primarily perinuclear cytoplasmic tonofilaments and numerous well developed desmosomes were identified. Cells displayed long thin microvilli along the majority of their plasma membranes, which formed numerous interdigitations, and they formed intercellular microcavities lined by microvilli.
Conclusion: Histological, immunohistological and electron microscopic examination identified an infiltrative myxoid epithelioid mesothelioma of the *tunica vaginalis testis*. Myxomatous/myxoid epithelioid mesotheliomas have been described in the pleura of various domesticated species, including the dog, but to the authors' knowledge this is the first report of this variant of mesothelioma in the *tunica vaginalis testis* in a dog. Similar epithelioid mesotheliomas of the pleural and peritoneal mesothelium have been reported in humans, where they are regarded as a diagnostic challenge due to the fact that they.

THE STUDY ON THE NADPHd-POSITIVE INNERVATION OF THE PORCINE MAMMARY GLAND

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Introduction: Accumulating evidence suggests that NO may participate in the regulation of morphological and functional features of the mammary gland. Although, application of the NADPHd histochemistry revealed NOS-positive, non-neuronal structures in the mammary gland of human, goat, cow and rat there is complete lack of information on the NO-ergic innervation of the gland. Therefore, while applying fast blue (FB) retrograde tracing method and NADPHd histochemistry, the origin and distribution of NOS nerve supply in the porcine mammary gland were the objective of this study.
Materials and Methods: Three sexually immature female pigs (3 months, 50-55 kg body weight) of the Large White Polish breed were anaesthetized with sodium pentobarbital (Vetbutal, Biovet, Poland; 20 mg/kg, *i.v.*). The retrograde tracer FB was injected into the nipple (10 µl) and parenchyma (20 µl) of the right last abdominal mamma. After three weeks, the animals were reanaesthetized and transcardially perfused with 4% buffered paraformaldehyde solution (pH 7.4). Subsequently, the right and left last abdominal mamma, and DRG including thoracic, lumbar and sacral left and right ganglia were dissected out. The samples were cut into 10 µm thick cryostat sections and processed for NADPHd histochemical staining procedure.
Results: Retrograde tracing studies have revealed that NADPHd-positive innervation supplying the porcine last abdominal mamma originates from L1-L4 DRG. The NADPHd-positive nerves were found in the dermis of the nipple with single nerve terminals penetrating into the epidermis. Smooth muscle cells (SMC) located in the nipple were supplied with few NADPHd-positive fibres. Small arteries of this region received abundant NADPHd innervation, while large arteries, veins and lactiferous ducts (LD) possessed relatively weaker supply. Similarly, the parenchyma was provided with moderate number of NADPHd-positive nerve fibres which surrounded SMC, blood vessels and LD.
Conclusion: The acquired data concerning the origin of the NADPHd innervation of the gland indicate that it may be sensory in nature, however, distribution of the fibres may suggest its role in motor function and control of the local blood flow in the mammary gland.

IMMUNOHISTOCHEMICAL DETECTION OF PrP^{res} IN THE INTESTINE OF CATTLE FROM BOVINE SPONGIFORM ENCEPHALOPATHY POSITIVE HERDS IN SPAIN

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Introduction: The first case of Bovine Spongiform Encephalopathy (BSE) in Spain was diagnosed in November 2000. In this period, after the detection of a BSE outbreak, different control measures have been adopted. Several authors have pointed out the importance of the intestinal lymphoid tissue in the uptake, persistence and propagation of prion protein towards the central nervous system. In this work we evaluate the role of the intestine (gut associated lymphoid tissue and enteric nervous system) in the persistence of PrP^{res} in healthy cows from the same farms where a clinical case of BSE was detected.

Materials and Methods: A total of 142 bovines from 6 different herds in Castilla and León (Spain) have been studied. Three categories of cattle were analysed: the age cohort of the clinical case, familiar cohort and randomly selected animals. Samples were taken from the small intestine (distal ileum and ileocaecal valve) and medulla oblongata. Furthermore, samples from jejunal Peyer's patches were collected in 13 animals. The same samples from 18 cows aged 12 mounts (born in 2001) fed in a controlled natural system were also studied. Conventional histologic and immunohistochemical staining, using monoclonal antibodies against PrP^{res} P4 and L42 (R-Biopharm, Germany) were performed.

Results: No immunopositivity was observed in the central nervous system. Positive immunostaining was detected in 69 animals (48.59%): in the domes (M cells, dendritic and macrophage-like cells) of gut associated lymphoid tissue (46 cows, 66.67% of the positive); in the enteric nervous system (9 animals, 13.04%); and in both locations (14 cows, 20.29%). L42 was used in 37 P4 positive and 4 P4 negative animals. All L42 positive cattle were also P4 positive. Positive cases were more frequent in 4-7 years old animals (21 immunopositive cows), although 9 cows younger than 1 year old were detected.

Conclusions: This work demonstrates the presence of PrP^{res} in intestine of subclinical cases of BSE by immunohistochemical methods. Positivity observed in young animals (less than 1 year old), some of them born after mammalian meat and bone feed ban, indicates the lack of compliance to the feed ban or the existence of an alternative route of infectivity. P4 Mab showed a better sensitivity than L42 Mab, since the former detected positivity in epithelial cells of the dome, that were negative using L42.

ASSOCIATION OF CT-GUIDED AND TISSUE CORE BIOPSY IN THE DIAGNOSIS OF LUNG NEOPLASIA IN DOG

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Introduction: Diagnostic imaging is very important in the diagnosis of pulmonary lesions. Radiology has traditionally been considered the elective diagnostic procedure but it is often not possible to discriminate inflammatory lesion from neoplastic disease. A histopathological diagnosis is needed for the prognosis and for the therapeutic plan. In human computed tomography CT-guided biopsy is indicated to discriminate lung lesions not adequately imaged with other diagnostic procedure. Pneumothorax is a risk when computed tomography CT-guided biopsy is performed.

Aim of the present study is to asses the accuracy of percutaneous CT-guided biopsy in the diagnosis of lung neoplasia in dog.

Materials and Methods: Thirty two dogs with suspect of lung tumour, of different sex, breed and size, underwent CT and in 4 animals in which CT was not conclusive also CT-tissue core biopsy (CTB) was carried out. CTB was performed with a 16 G spring loaded needles for tissue-core biopsy, calibrated in a 14 G guide to get into the lesion allowing then the biopsy with an automatic needles. Biopsies were immediately fixed in a formalin buffered solution.

Formalin fixed paraffin embedded sections were stained for histological routine methods and for immunohistochemistry AE1 and AE3 (1:50, Dako) using Dako LSAB2 HRP kit – DAB (Dako) system.

Results: After biopsy the dogs were kept under clinical and radiological controls and in 3 cases a moderate to severe pneumothorax was recorded. At histological examination 3 biopsies have shown proliferating tubular structures covered by multilayer of atypical epithelial cells immunopositive for AE1 and AE3 diagnosed as adenocarcinoma. In one case the histological and immunohistochemical investigations were inconclusive for an adequate diagnosis. The specimen was characterized by the presence of lymphomononucleate aggregate in absence of any structure of lung parenchyma.

Conclusions: The relative procedural consequence of pneumothorax when CTB is carried out has limited the CTB approach only in animal with a risk history for lung neoplasia with inconclusive CT diagnosis. Histopathology and immunohistochemistry have shown to be very useful support in inconclusive diagnostic imaging case of lung neoplasia.

DUPLICITAS AEQUALES COMPLETA OF DOMESTIC BOVINE – ANALYSIS OF CERVICAL AND THORACIC PART OF SKELETON

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Introduction: In present days increasing influence of environment contamination on farm-animals manifests among other things in increasing number of congenital defects in fetuses. In Poland loses caused by this problem are reckoned about 2-3% total number of dead new-born animals. A try of analysis and finding of common aspects of such cases may be crucial in preventing such situations and decreasing economic loses.
Material and Methods: Caracas of domestic bovine, red race, female, weight ~ 40 kg. Anatomical dissection followed by preparation. Histopathological researches of chosen tissues were made.
Documentation:
1. photographical specification,
2. RTG examination with Prestige VH General Electrics,
3. CT examination with CT/e General Electrics.

Caracas was macerated. The analysis of changes in anatomical structure of cervical and thorax segments of spine together with thorax skeleton was the aim of the study.
Results: Doubled segments of cervical and thorax spine were observed. In both cervical segments number of vertebras was correct (7). Minor structure disorder and asymmetry were observed in respective parts of vertebras. Vertebras C6 and C7 were knitted together.
During analysis of thorax skeleton numerous structure anomalies were observed. Both calves had correct number of thorax vertebras. Vertebras showed numerous morphological disorders. External costae growing from each spine were in correct number and did not show any changes. Distal parts of those costae were connected with sternum. Sternum was built of two separate structures. Particular parts of sternum shown serious disorders. Anomalies are illustrated with respective photographs.

DIROFILARIA REPENS INFECTION IN THREE DOGS IN CROATIA

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Introduction: Dirofilariosis due to *Dirofilaria repens* as a helmintic zoonosis represents underestimated problem in veterinary medicine and public health in Croatia. Although the human case in Croatia is already noted, this work describes the first case of this disease in dogs in Croatia.

Materials and Methods: During the March 2004, a military dog (Rottweiler, 9 years old) was sectioned at our Department, and histopathological (haematoxylin and eosin, and DIFF Quick stain) examination of subcutaneous tissue, brain, pancreas, kidney, lymph nodes, liver, stomach, lung, myocardium and small intestine was performed. Also, blood and tissue smears (liver, lymph node and subcutaneous tissue) was stained with DIFF Quick and examined. Two other dogs which lived in cohabitation with the first one (both sarplaninac, 11 and 9 years old) was examined and treated at the Clinic of Internal Diseases. For the parasitological examination, a 3-5 ml of whole blood was drawn from the cephalic vein of each dog. Modified Knott test was performed within 3 h of the venepunction. All microfilariae in every sample were counted. In this case study, *s.c.* therapy with ivermectin (Iverktin® 1%, VETERINA d.o.o.) 300 μg/kg b.w. twice (q 7 days) was introduced.

Results: Necropsy revealed severe cachexia with the phlegmonous subcutaneous inflammation in the cubital, coxal and carpal regions. The cause of dead was peritonitis caused with perforation of the intestinal diverticulus. Liver cirrhosis with icterus was also seen. Histopathological examination showed numerous microfilariae in all examined organs predominantly intravasculary located but in the phlegmone, liver and lungs they were located in the tissue. Parasitological examination showed severe microfilariosis (12000 and 3700 microfilaria per ml of blood). The length and width of all microfilariae in each sample were measured under magnification x 400 using an ocular micrometer. The average width of microfilariae was 7.45 μm, and average length was 3.95 μm. On the basis of the observed characteristics (length, width and general morfology) the microfilariae were interpreted as species *Dirofilaria repens*. Haemathological and biochemical examination showed leucocytosis, and increased serum AST and CPK concentrations in the more severe infected dog. Applied therapy managed to clear the *D. repens* infection during 48 h of the first aplication. Both dogs remained negative (Knott test) 35 days after the second aplication. Also, haemathological and biochemical parameters were normal in the same period after the therapy.

Conclusion: This findings have shown that severe dirofilariosis due to *Dirofilaria repens* infection could cause very significant pathological lesions as well as clinical signs of the disease.

DICENTRARCHUS LABRAX BIOTRANSFORMATION AND GENOTOXIC RESPONSES AFTER EXPOSURE TO A SECONDARY-TREATED INDUSTRIAL/URBAN EFFLUENT

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Introduction: The present research work was designed to study sea bass biotransformation and genotoxicity responses to the soluble fraction of a secondary treated industrial/urban effluent (SF-STIUE) discharged through a submarine pipe outlet into the Aveiro coastal area. This secondary treated industrial/urban effluent (STIUE) is a complex mixture of pulp and paper mill, industrial and urban effluents.
Materials and Methods: Sea bass was exposed during 4, 8, 16, 24, 48 and 96 hours to 0, 0.1 and 1% SF-STIUE and the following biological responses were measured: 1) liver cytochrome P450 (P450) content and ethoxyresorufin-O-deethylase (EROD) activity, as phase I biotransformation parameters; 2) liver gluthathione-S-tansferase (GST) activity as phase II conjugation enzyme; 3) biliary and liver cytosol naphthalene- (Naph) and benzo(a)pyrene- (BaP) type metabolites, by fixed wavelength fluorescence detection (FF); 4) liver DNA strand-breaks, erythrocytic micronuclei (EMN) and erythrocytic nuclear abnormalities (ENA) as genotoxicity parameters.
Results: Both SF-STIUE dilutions (0.1 and 1%) failed to significantly increase liver EROD activity, despite liver P450 significant increase at 16 and 48 hours exposure to 0.1%. Liver GST activity increased significantly at 4 hours sea bass exposure to 1% SF-STIUE, being inhibited at 96 hours exposure to this SF-STIUE dilution. Naph- and BaP-type metabolites content were not significantly increased in bile. However, Naph-type metabolites content increased significantly in liver cytosol at 4 hours exposure to 1% SF-STIUE, and at 24 hours exposure to 0.1 and 1% SF-STIUE. Furthermore, BaP-type metabolites increased significantly in liver cytosol at 4 hours exposure to 1% SF-STIUE, and 16 hours exposure to 0.1 and 1% SF-STIUE. EMN and ENA frequencies increased significantly at 4, 8, 16, 24, 48 and 96 hours exposure to 0.1 and 1% SF-STIUE. Liver DNA integrity decreased significantly at 96 hours sea bass exposure to 1% SF-STIUE.
Conclusions: STIUE presents a high genotoxic potential measured as liver DNA integrity decrease as well as EMN and ENA frequencies increase in juvenile sea bass. The STIUE seems to contain phase I and phase II biotransformation inhibitors, which impair detoxification and biliary excretion. Therefore, the presence of genotoxic compounds in the STIUE discharged into the sea is of great concern to the Aveiro coastal area. This study shows that sea bass EMN, ENA and liver DNA strand-breaks frequencies are sensitive and suitable responses to assess the genotoxic potential of pollutant mixtures.
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GIANT CELL DERMATOSIS IN A CAT – DETECTION OF FELV BY IMMUNOHISTOCHEMISTRY AND POLYMERASE CHAIN REACTION

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Introduction: A 3.5 years old male castrated cat with a 3 month history of nailbed inflammation was presented due to acutely arising ulcerative lesions at the head, limbs and in the interdigital area. The cat was febrile and anaemic.
Materials, Methods and Results: Bacteriological and cytological examinations revealed staphylococcal infection. An ELISA for FeLV antigen was positive. Histological examination of skin biopsies revealed a severe, subacute, ulcerative dermatitis with purulent folliculitis. A moderate number of syncytial cells was found in epidermis, hair follicles, and sebaceous glands. Immunohistochemical staining with monoclonal antibodies against the envelope protein gp70 and the group specific protein p27 of FeLV (Custom Monoclonals International, West Sacramento, USA) revealed positive epithelial cells in epidermis, hair follicles and sebaceous glands. In addition, PCR analysis was performed on lesional skin. DNA was extracted from deparaffinized tissues and a 131 bp long proviral DNA fragment was amplified by real-time PCR. A diagnosis of FeLV-associated giant cell dermatosis with secondary bacterial folliculitis was made. Despite treatment with cefalexin and temporary improvement the cat deteriorated and was euthanized. Necropsy was not permitted.
Conclusion: Syncytial cell dermatitis is a rare presentation of FeLV infection in cats. Six cases have so far been described (Gross et al. 1993), with a pruritic crusting dermatitis, often involving the head, as the main feature. Different conditions have been associated with syncytial cell formation in man and animals, namely retroviral infection and herpesvirus infections. The exact mechanism of FeLV induced syncytial cell formation is currently unknown.
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THREE IMPORTANT CAUSES OF HORSE DEATH IN PROCESS OF SNAKE ANTIVENOM PRODUCTION

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Introduction: Snake venom is a complex mixture of proteins with enzymatic and/or toxic effects; non-protein substances like as metallic ions, lipids, carbohydrates and salts. These substances, especially proteins, encounter the horses, in process of snake antivenom production, to both local and/or systemic toxicity and amyloidosis.
Materials and Methods: A retrospective study was performed on 50 necropsy reports and histopathologic examinations of horses used for production of snake antivenom production during a period of 5 years, from 1998 until 2003.
Results: In histopathological examination of horses, had died in early periods of snake antivenom production, there were foci of hemorrhage and myocardial cell necrosis in myocardium of some horses. Thrombosis in small arteries inside the myocardium was also observed. In areas, where, necrosis had progressed the lesions consisted of persistent interstitial fibroblasts, collagen and capillaries. In the kidneys congestion, hemorrhage and diffuse tubular necrosis were the prominent features. In most of livers, there were congestion of sinusoids, hemosiderosis, necrosis of hepatocytes. In these cases, frequently, portal sclerosis in some parts, which formed bridging fibrosis, was evident. In the lungs severe congestion and hemorrhage were common and destruction of alveolar walls caused marked emphysema. However, death due to amyloidosis and its consequences; deposition of amyloid materials, commonly, in the livers and with lesser degree in spleens, impaired liver function and its rupture were the most prominent consequences of longer duration of using the horses for production of snake antivenom.
Conclusion: Early death of some horses during the snake antivenom production is commonly as the results of, cardiovascular insufficiency and renal failure, whereas, amyloidosis and its consequences; impaired liver function and rupture, are the most important causes of later death.

SEVERE NEPHRITIS AND PANCREATITIS IN CHICKEN FOLLOWING INTRAVENOUS INOCULATION WITH AVIAN INFLUENZA VIRUS A/CHICKEN/IRAN/259/1998(H9N2)

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Introduction: In late 1997 and early 1998 avian influenza (AI) outbreak was reported at layer farm in Tehran province, Iran, with 10% mortality and severe drop of egg production. The viruses isolated from three farms identified as non-highly pathogenic (n-HPAI) H9N2 AI virus and confirmed by CVL (Central Veterinary Laboratory, Weybridg, Surrey, UK). The purpose of the present study was to determine the type, severity and frequency of gross, histopathologic changes and tissue tropism in some of chicken's tissues following intravenous (*i.v.*) inoculation of Iranian AIV isolate.
Materials and Methods: Forty 5 weeks old chickens hatched from SPF eggs were randomly divided into two equal groups (treated and control groups). The treated group was subsequently inoculated intravenously with type A AIV [A/Chicken/Iran/259/(H9N2)] in chorioallantoic fluid (CAF). The control group received sterile CAF on the same manner. Five chickens from each group were randomly sampled on day 1, 3, 6 and 10 post inoculation (pi). They were humanely slaughtered and necropsy was performed, subsequently, gross lesions were recorded and samples of different organs were collected for virus isolation, histopathology and immunohistochemistry studies.
Results: Data related to infected chicken were summarized in the table.

Tissue	Frequency of lesions (%)	Predominant lesions type	Category	Nucleo-protein detection
Kidney	75	necrosis, nephritis	specific	+
Pancreas	53.3	necrosis, pancreatitis	specific	+
Spleen	46.7	lymphoid and RE hyperplasia	non-specific	-
Liver	38.8	lymphocyte infiltration	non-specific	-
Heart	31.5	lymphocyte infiltration	non-specific	-
Cloacal bursa	20	lymphoid atrophy	non-specific	-
Lung	16.7	lymphocyte infiltration	non-specific	-

Tubulointerstitial nephritis and pancreatitis were the most frequent specific histopathologic changes. Influenza nucleoprotein was demonstrated in renal tubule epithelium and in acini of pancreas/foci of necrosis in both organs. Common nonspecific histopathologic changes were lymphoid and reticuloendothelial cell hyperplasia in spleen, leukocyte cell infiltration in myocardium and lymphocyte infiltration in liver.
Conclusion: This study showed that A/Chicken/Iran/259/1998(H9N2), an Iranian AIV isolated, is a non-highly pathogenic virus, which in IV inoculation has tissue tropism to kidney and pancreas of chickens, and could produce severe damage in these organs.

INCIDENCE OF SALMONELLA IN BROILERS AND COMPARISON BETWEEN PCR AND BACTERIOLOGICAL METHOD FOR DIAGNOSIS

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Introduction: Studies were conducted to estimate the prevalence of different Salmonella among 150 broiler flocks. Seven Salmonella isolates were identified: *S. typhimurium*, *S. enteritidis* (SE), *S. montevideo* with percent of 28.57% for each and 14.28% for *S. kentucky*. Also, experimental infection to study the pathogenicity of these Salmonella isolates was carried out. All isolates showed highest mortalities during 1st week (13.54%) followed by 2nd week (5.8%). Colonization of Salmonella in liver was 96.6% and intestine 66.6%. The second experiment was carried out to compare between PCR and conventional method in detection and identification of *S. enteritidis*. PCR used primers from genomic sequences amplified a 312 bp fragment specific for SE within sef A gene and samples were collected from cloacal swab and internal organs (liver, cecum).

Materials and Methods: Cloacal swabs were enriched in selenite F broth for 8, 16 and 24 h and then subcultured on selective media and simultaneously examined using PCR method.

Results: PCR had a better sensitivity (100%) for detection of SE from cloacal swabs after 8 h of incubation, but bacteriological method failed to detect SE at the same time. While PCR could detect SE from liver, intestine (60%) after 6 h of incubation, bacteriological method could detect only (20%) from liver and from (40%) of intestine at the same time of incubation.

EFFECTS OF FABA BEAN (*VICIA FABA* L.) DIETS ON CHICKENS IN ORGANIC FARMING – A MORPHOLOGICAL AND MORPHOMETRICAL APPROACH

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Introduction: Several studies identified high quantities of faba beans (*Vicia faba* L.) in diets of chicken as a cause of reduced food consumption and reduced performance, probably due to physiological and morphological changes in the intestinal tract. Antinutritive substances, i.e. tannins, saponins and the two pyrimidinglycosides vicine and convicine, are suggested to play an important role in inducing these effects.
Materials and Methods: In different experiments the influence of diets containing different faba bean varieties with variable contents of antinutritive substances on small intestine, liver and pancreas of chicken was evaluated. The animals were raised under experimental conditions regarding the methods of organic farming, which among others means a prolonged duration of growing in comparison to conventional farming. The first experiment was performed to examine the effects of diets containing conventional faba beans on different broiler genotypes in comparison to congeneric control groups fed with faba beans free diets. In second experiment, effects of increasing quantities of two conventional faba bean variety with reduced pyrimidinglycoside content, a conventional faba bean variety and a faba bean free diet. In this experiment not only the influence of the broiler's diets was estimated, but also the effects of the parent's diets on the parameters examined in the broilers and in the parents themselves. Morphometry was performed measuring the layers of the wall of the small intestines using computer-aided microscopy. Specimens of liver, pancreas and small intestine were evaluated semiquantitatively for group differences.
Results: We identified the postmortal contraction of the intestinal muscle layers to be a disturbance variable, affecting the morphometric parameters. The following significant but slight effects were found: one genotype had a higher intestinal epithelium and a higher grade of periportal infiltration, conventional faba beans caused a decrease of fat storage in the liver, higher tannin contents produced a lower number of intraepithelial intestinal lymphocytes in one group and a decrease of periportal infiltration of the liver in another group, feeding vicin/convicin reduced diets results in higher intestinal villi and lower intestinal epithelium. Control diet fed animals showed a significant higher fat storage and periportal infiltration in the liver. Performance was affected only marginally.
Conclusion: Although growing period was prolonged, the feeding of faba beans only produced marginal, though significant effects. These effects were not only negative (i.e. tannins classified as antinutritive reduced periportal infiltration). Regarding the pyrimidinglycosides reduced bean variety the higher villi and the lower epithelium of the intestine may facilitate absorption.

TRANSGENIC MICE EXPRESSING A DOMINANT NEGATIVE GLUCOSE-DEPENDENT INSULINOTROPIC POLYPEPTIDE RECEPTOR ($GIPR^{dn}$): A NOVEL ANIMAL MODEL FOR STUDYING DIABETES MELLITUS

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Introduction: The postprandial secretion of insulin is regulated by hormonal factors released from the gut in response to nutrient ingestion, the incretin hormones. Two major incretin hormones have been characterised: glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide (GLP)-1. GIP is released from intestinal K cells and binds to the specific GIP receptor on pancreatic β cells, leading to the stimulation of insulin secretion.

Materials and Methods: In order to study the contribution of the GIP/GIPR axis to maintaining glucose homeostasis, transgenic mice were generated, expressing a dominant negative GIP receptor ($GIPR^{dn}$) under the control of the rat insulin gene promoter. Non-transgenic littermates served as controls. Several diabetes-relevant parameters were examined, including urine glucose, blood glucose, serum insulin and glucagon levels. Blood glucose curves and insulin secretion in response to parenteral glucose load with and without the concomitant application of GIP and glucagon-like peptide (GLP)-1 were examined. The endocrine pancreas was examined using quantitative-stereological methods.

Results: As shown by urine glucose excretion, $GIPR^{dn}$ transgenic mice became overtly diabetic between 14 and 21 days of age. Blood glucose and serum glucagon levels were significantly increased, whereas serum insulin levels were significantly decreased in transgenic animals, as compared to controls. The blood glucose curves during glucose tolerance testing were severely increased in transgenic animals, accompanied by the lack of glucose and incretin hormone induced insulin secretion. The distribution of endocrine cells within pancreatic islets was severely disturbed in $GIPR^{dn}$ transgenic mice. The total volumes of islets, β cells in the islets and isolated β cells in the exocrine pancreas were significantly reduced, as compared to controls.

Conclusion: We conclude that $GIPR^{dn}$ transgenic mice are a valuable model for studying the relevance of incretin hormones in pancreas development and glucose homeostasis, as well as for studying diabetes-associated organ lesions.

THE USE OF IN-SITU HYBRIDIZATION (ISH) FOR DETECTION OF *BRACHYSPIRA* IN PORCINE INTESTINES

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Introduction: Bacteria of the genus *Brachyspira* (*Br.*) are important pathogens in pigs. *Br. hyodysentriae* is the agent of swine dysentery, *Br. pilosicoli* causes intestinal spirochetosis and also *Br. intermedia* probably causes mild colitis. Two other species (*Br. innocens* and *Br. murdochii)* are described as non pathogenic. It is very important to differentiate between the pathogenic and non pathogenic species. Cultivation of *Brachyspira* from faeces is difficult and time-consuming. We investigated whether ISH is a useful method for detecting *Brachyspira* directly in formalin fixed and paraffin embedded tissue sections.

Materials and Methods: We investigated sections of the large intestine from 78 pigs with colitis. Sections from all samples were stained with haematoxylin and eosin (HE) and Warthin Starry (WSt.). For "*in situ*" hybridization (ISH) we developed a digoxigenin-labeled RNA-probe with 334 bp length, specific for all members of the genus *Brachyspira*. We also used a control probe, complementary to our RNA-probe. The sections were deparaffinized, denatured with 0.2 M HCl and treated with proteinase K. The hybridization with 3 µl probe/ml mixture, according to an RNA protocol was carried out at 50°C in a humid chamber overnight. The sections were treated with an anti-digoxigenin, alkaline phosphatase-conjugated antibody and were stained with X-phosphate-nitroblue tetrazolium chloride. From each sample two consecutive sections were subjected to ISH; one was treated with the RNA-probe and the second one with the control probe.

Results: Sixty four samples (82%) showed a positive ISH result: in these sections bacteria with the morphology of *Brachyspira* were stained within the crypts of the large intestine. In the parallel sections treated with the control probe the bacteria remained unstained. Five samples were negative: 3 of them showed no staining at all and in 2 samples there was also staining in the sections treated with the control-probe, indicative of an unspecific reaction. Nine samples showed a doubtful result and in 2 samples coiled bacteria in the crypts were clearly stained but seemed much shorter than in the other samples. Remarkably, ISH-positive crypts were generally few and disseminated. In contrast, the WSt. stained sections showed bacteria in the majority of crypts.

Conclusions: The results of this study show that ISH is a suitable method for detecting *Brachyspira* directly in the crypts of the large intestine of pigs. However, the quantity of bacteria identified by ISH is considerably lower than by WSt. Whether this reflects the reduced sensitivity of the ISH technique, or the fact that bacteria stained by WSt. represent no members of the genus *Brachyspira* is unknown as yet. Future directions of this study are generating specific probes to distinguish between pathogenic and non pathogenic *Brachyspira* species and probes also detecting bacteria which morphologically resemble *Brachyspira*.

CHRONIC *MYCOBACTERIUM TUBERCULOSIS* INFECTION IN AN ASIATIC ELEPHANT (*ELEPHAS MAXIMUS*)

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Introduction: In the past decade a renaissance in tuberculosis epidemiology has been witnessed, both in immunosuppressed and immunocompetent individuals. In order to show the possible zoonotic risks for susceptible species, the case of an Asiatic Elephant, participating in the shows of a travelling circus, with severe chronic tuberculosis is presented.

Materials and Methods: Full necropsy was performed on a 39 years old, female Asiatic elephant, 24 h after death. Organ material was routinely preserved for paraffin histology as well as for microbiology. From different organs isolated mycobacteria were cultivated and further characterized by biochemical and molecular techniques.

Results: About 70% of the lung parenchyma of the emaciated elephant showed a severe suppurative, partly abscessing to granulomatous bronchopneumonia with a massive interstitial fibrosis, including the pulmonary lymph nodes and extending into the adjacing thoracal vertebral column. Additionally there was a moderate to massive suppurative to necrotising, partly pyogranulomatous inflammation of mucosal tissues of the frontal sinuses, nasal cavity, larynx and trachea and regional lymph nodes. In macrophages in these mucosal tissues small amounts of acid fast bacilli were detectable. In addition to these findings, a suppurative to necrotising, partly pyogranulomatous chronic arthritis and osteomyelitis occurred in the cervical and thoracal vertebral column. The central nervous system showed multifocal moderate chronic pyogranulomatous meningitis and chorioiditis and numerous miliary perivascular granulomas.

Microbiology revealed a variety of facultative pathogenic to apathogenic bacteria in the severely altered lungs. Acid fast bacilli, further characterised as *Mycobacterium tuberculosis* were isolated in higher amounts from mucosal tissues of the upper respiratory tract (i.e. frontal sinuses, nasal cavity, larynx, trachea), and after several attempts in small amounts from the pulmonary parenchyma.

Discussion: The distribution and type of alterations show the case of a chronically tuberculoid elephant. Inflammatory processes ranged between an active subacute, pyogranulomatous to necrotising type of inflammation of the upper respiratory tract to a chronic, proliferative pneumonia extending into the thoracal vertebral column. The zoo-antroponotic, respectively anthropo-zoonotic potential of this tuberculoid circus elephant is discussed.

MORPHOLOGY AND DISTRIBUTION OF INCLUSION BODIES IN NEONATAL, JUVENILE AND ADULT *BOA CONSTRICTOR* WITH INCLUSION BODY DISEASE (IBD)

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Introduction: Inclusion body disease (IBD) is an infectious disease occurring worldwide mainly in boid snakes, from the genera Boa, Epicrates, Python and Morelia. A similar disease is reported from viperids and colubrids. The definitive etiopathogenesis of the disease is uncertain; a viral infection with an IBDV called retrovirus has been suggested. In order to give an overview over the symptoms, the intensity, the distribution pattern and the morphology of the characteristic, disease naming eosinophilic intracytoplasmic inclusions, IBD diseased *Boa constrictor* of different ages and of different clinical status were investigated.
Materials and Methods: Ten adult, five juvenile (3-4 months old, from one clutch) and two neonates (3 days old, from another clutch) *B. constrictor* of both sexes were euthanized due to an IBD-infection. Five juvenile to subadult non-infected *B. constrictor* (4-12 months old, from a third clutch) served as control. A variety of organ tissues, including the central nervous system, tissues from respiratory, gastro-intestinal and uro-genital tract, cardio-vascular system, endocrine organs, bone marrow and vertebral column was taken for both, routine paraffin histology and transmission electron microscopy. Additionally, bacteriology and parasitology were performed.
Results: Characteristic, variably sized inclusions were found in varying numbers in a variety of organs: nervous system (brain, spinal cord, retina, organ of Jacobson, peripheral nerves), respiratory tract (nasal, tracheal, bronchial and pulmonary epithelia), gastro-intestinal tract (epithelia of oral cavity, oesophagus, gut, small and large intestine, hepatocytes and exocrine pancreas), uro-genital tract (proximal and distal tubulus as well as ureter epithelium, spermatogonia, epithelia of sperm- and oviduct), endocrine organs (hypophysis, adrenal gland), lymphatic tissue (spleen, thymus, MALT, bone marrow), cardio-vascular system (endothelial cells), blood (erythrocytes, lymphocytes, plasma cells and heterophils). In contrast to the chronically infected juveniles and adults, inclusion bodies in the neonates occurred predominantly in the central nervous system. In most cases, microbiologically *Salmonella spp*. was isolated from a variety of organs, predominantly from the intestine. Parasitologically, a minimal to moderate infection with amoebae and nematodes was found.
Discussion: IBD infection affects a variety of neuroectodermal, endodermal and mesodermal cells in various organs. According to the findings in the neonates, a primary tropism for neuroectodermal tissues, i.e. brain and spinal cord, seems to be possible.

IMMUNOHISTOCHEMICAL CHARACTERISATION OF THE DISTRIBUTION OF *MYCOPLASMA BOVIS* MEMBRANE SURFACE PROTEINS AND OF THE LOCAL IMMUNE RESPONSE IN LUNGS OF EXPERIMENTALLY INFECTED CALVES

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Introduction: In calves and young cattle with pneumonia following infections with *Mycoplasma bovis*, despite of the presence of antibodies in the serum and proliferative responses of the bronchus-associated lymphoid tissue (BALT) of the lung, *M. bovis* can persist in the respiratory tract of infected animals for at least several weeks. The mechanisms by which *M. bovis* survives within the host are incompletely understood, but variable membrane proteins (Vsps, variable surface lipoproteins; pMB67, membrane surface protein unrelated to Vsps) are possible candidates being potentially involved in immune evasion of the host. Following natural or experimental respiratory infections of calves with *M. bovis* a local immune response with formation of lymphoid and plasma cell accumulations develop. The aim of this immunohistochemical study was to examine lung tissue samples from experimentally infected calves for the presence of variable antigens and to phenotype lymphocytes and plasma cells in the BALT of these animals.

Materials and Methods: Eight 3-5 weeks old calves were inoculated intratracheally with 10^8-10^{10} colony forming units of *M. bovis* strain 1067. Three weeks post infection all calves were euthanized and necropsied. Four non-inoculated calves served as controls. At necropsy, lung tissue samples were fixed in formalin or snap frozen. Paraffin sections were stained with haematoxylin and eosin for histopathology. Paraffin or frozen sections were stained immunohistochemically by using the avidin-biotin-peroxidase complex (ABC) method and different monoclonal antibodies to *M. bovis* Vsps and pMB67 antigens and antibodies to lymphocytes (CD3, CD79a, CD4, CD8) and immunoglobulins (IgG1, IgG2, IgA, IgM).

Results: Immunohistochemical staining revealed the presence of *M. bovis* variable surface protein antigens in lung tissue sections of all calves examined. The presence of variable antigens was associated with marked accumulations of CD3-positive lymphocytes in the BALT with CD4-positive T cells outnumbering CD8-positive T cells. Furthermore, an increase of immunoglobulin-containing plasma cells was noted in lung sections from infected calves being dominated by IgG1 containing cells.

Conclusion: The results suggest that expression of variable surface antigens of *M. bovis* occurs *in vivo* in the lungs of infected calves. The findings further indicate that variable antigens are inducing and continuously stimulating the proliferation of BALT in the respiratory tract of the host.

BORDER DISEASE IN A SHEEP WITH MUCOSAL DISEASE-LIKE LESIONS

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Introduction: Border disease is a pestivirus infection found in sheep and goats. This congenital disease was first reported from the border region of England and Wales in 1959 (Nettleton et al. 1998). Clinical signs in sheep include abortions, stillbirths and birth of weak lambs with hairy fleece (hairy shaker). In cattle, pestiviruses causes the illness bovine virus diarrhea with different clinical manifestations such as pneumonia, diarrhea or abortions. If an animal is persistently infected with a non-cytopathic biotype and subsequently superinfected with a cytopathic biotype, Mucosal Disease with erosions and ulcerations in different hollow organs develop. In sheep, a similar syndrome resembling Mucosal Disease in cattle has been published in 1997. In Switzerland this syndrome has not yet been described.

Materials, Methods and Results: A 28 weeks old sheep of a triplet birth was presented in the clinic with chronic emaciation. Due to bad general condition, the animal was euthanized and submitted for post mortem investigation. Macroscopically, erosions and ulcers of different size and shape were found in the dorsal aspect of the tongue, the hard palate, in the oesophagus and the rumen pillars.

Immunohistochemically pestivirus antigen was found in cryostat and paraffin sections of different organs such as tongue and thyroidea, using the EnVision-method (DAKO). On the cryostat sections, the following monoclonal antibodies were used: C16 and Ca3/34, but only C16 showed positive results. On paraffin embedded sections, C42 and 15c5 were applied, of which only 15c5 was positive. Sequencing revealed that the virus isolate resembles another ovine pestivirus strain previously isolated in Switzerland and belongs to the Border Disease-3 cluster (Becher et al. 2003).

The other animals of this flock consisted of the mother and the two other siblings. Immunohistochemically and virologically these three animals were negative for Border Disease virus.

Discussion: Based on this observation, we hypothesize that the infection of our sheep has most likely taken place postnatally, through contact with other sheep. It should be noted that, despite the similarity of the lesions observed in this sheep with Mucosal Disease in cattle, cytopathic pestivirus was not isolated. Hence, the pathogenesis of the condition observed in this animal may differ from that of Mucosal Disease, and may be explained by the virulence of this particular virus strain.

EVALUATION OF INTESTINAL PATHOLOGY IN *TRICURIS SUIS* INFECTED PIGS FED TWO DIFFERENT DIETS

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Introduction: *Trichuris suis* (*T. suis*) is an intestinal parasite in pigs and infections can cause important clinical symptoms and pathology. Previous studies have shown that fermentable carbohydrates, like inulin, in the diet have a negative influence on the establishment, persistence and fecundity of *Oesophagostomum dentatum* in pigs. The aim of the current study was to evaluate the intestinal pathology in *T. suis* infected pigs fed diets with either fermentable or non-fermentable carbohydrates. The emphasis of the evaluation is placed on the number, localisation, and type of inflammatory cells.
Materials and Methods: Macroscopical, histopathological and immunohistochemical quantitative and semi-quantitative evaluations were made of colon samples from 36 pigs along with parasitological examinations. Of these pigs, 29 pigs were allocated into 4 groups and infected with 2000 *T. suis* eggs. Groups A and C received a control diet supplemented of carbohydrate resistant to fermentation (oat husk flour) and were euthanized 7 and 9 weeks post infection (pi), respectively. Group B received a diet supplemented of fermentable carbohydrate (inulin) and was euthanised 7 weeks pi, while group D received control diet until week 7 pi and then changed to the inulin diet until euthanasia 9 weeks pi. The remaining 7 pigs were uninfected age controls fed control diet.
Results: All infected pigs developed a subclinical infection. Infected pigs had a higher degree of infiltration with mast cells, eosinophils and neutrophils than uninfected pigs 7 and 9 weeks pi. Also the number of IgG+ and IgA+ cells was higher in infected pigs than uninfected pigs at 7 weeks pi. Infected pigs fed the inulin diet had a lower number of *T. suis* than infected pigs fed the control diet. The infected pigs on inulin diet showed increased macroscopic mucosal hypertrophy but a decreased number of inflammatory cells in the *lamina propia* and *tela submucosa* compared to infected pigs on the control diet.
Conclusion: Infection with a low dose of *T. suis* results in a subclinical infection with diffuse infiltration of inflammatory cells in the colon. Diets with fermentable carbohydrate (inulin) have a negative influence on the establishment and survival of *T. suis.* An increased macroscopic mucosal hypertrophy along with a decreased infiltration of inflammatory cells in response to infection was seen in pigs fed fermentable carbohydrate. The mechanisms behind these effects can be discussed and are probably multifactorial.

T CELL LYMPHOMA OF GASTROINTESTINAL TRACT IN 10 DOGS

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Introduction: Among the gastrointestinal tumours of hematopoietic cell origin, malignant lymphoma is reported to be most common in the dogs. The origin of primary gastrointestinal lymphoma of dog was thought to be B cell, but immunophenotype of lymphoma has rarely been examined. The objective of this study was to characterize the clinical and pathological features of 10 dogs with T cell lymphoma of gastrointestinal tract.
Results: The average age of affected dogs was 9.5±2.1 years. No sex predominance was apparent. All tumours were located in small intestine. The prognosis was very poor. Grossly, the gastrointestinal wall was thickened prominently with marked narrowing of the lumen. Microscopically, there was transmural diffuse invasion of round to pleomorphic tumour cells. Tumour cells had small to abundant cytoplasm, round to ovoid nuclei with sparse or dense chromatin, resembling to those of mast cell tumour in gastrointestinal tract. In 7 cases out of 10, tumour cells consisted mainly of small-sized cell and infiltrated frequently into the mucosal epithelium. There was slight to heavy infiltration of eosinophils among the tumour cells in all cases. All 10 cases expressed a T cell phenotype (CD3+, CD79-), but c-kit and mast cell tryptase as the markers for mast cell, were negative in 90% and 100% of cases, respectively.
Conclusion: The morphological feature of T cell lymphoma of gastrointestinal origin was resembled to mast cell tumour of gastrointestinal origin. Therefore, immunostaining for mast cell and lymphocyte makers including mast cell tryptase, c-kit, CD3 and CD79 is thought to be essential for the differential diagnosis of gastrointestinal mast cell tumour and T cell lymphoma.

SHEEP PRION PROTEIN (PRP) GENE POLYMORPHISM IN POLISH SHEEP

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Scrapie is a fatal neurodegenerative disease of small ruminants belonging to the group of Transmissible Spongiform Encephalopathies (TSEs).
The TSEs are characterized by the accumulation of an abnormal isoform (PrP^{sc}) of the host-encoded cellular prion protein (PrP^{c}), in the central nervous system and in lymphoid tissues of affected individuals. Early epidemiological observations of sheep scrapie indicated that sensitivity or resistance to scrapie is associated with variability at codons 136, 154 and 171 of prion protein gene. Among five, most important for scrapie alleles: ARR, AHQ, ARH, ARQ and VRQ, ARR is linked to resistance and VRQ is linked to susceptibility to this disease. Susceptibility to scrapie is mainly associated with presence of valine (V) at codon 136 and glutamine (Q) at codon 171. But presence of alanine (A) at codon 136 and arginine (R) at codon 171 is associated with high resistance to scrapie. This study presents preliminary data on the polymorphism of prion gene in selected breeds of sheep occurring in Poland.
Polymorphisms at codons 136, 154, and 171 were identified by temperature gradient gel electrophoresis (TGGE) on Biometra apparatus (Goettingen, Germany). As a control samples from sheep with known genotype were used. Among examined ewes, nine had variation at codon 136 and only two had variation at codon 154. Significant variation was identified at codon 171 with 44% of the ewes being RR_{171}, 52% RQ_{171}, and 4% QQ_{171}.Analysis of sheep revealed also presence of VRQ allele which is linked to high susceptibility to scrapie. This allele is very rare or not present in some sheep breeds like Berichone du Cher, Massess or Suffolk. VRQ allele in high proportion was found in Icelandic sheep (about 80%) and in Norwegian Rygja breed of sheep. Study revealed very high proportion of ARR allele, which is linked to high resistance to scrapie. Given the fact that the most dominant allele in examined sheep was ARR the breeding strategy to increase genetic resistance to scrapie seems straightforward.

THE LEVEL OF SELECTED ANTIOXIDATIVE INDICES AND TESTOSTERONE IN BLOOD OF RATS INJECTED WITH VITAMIN E AND SELENIUM UNDER AN INCREASED OZONE CONCENTRATION IN THE AIR

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Introduction: Ozone, being a component of photochemical smog, induces oxidative stress in living organisms. Protective agents capable of quenching free-radical reactions include vitamin E and glutathione peroxidase (GPx) with selenium as a key-component. The aim of the research was to determine whether, and to what extent, an increased supply of selenium and vitamin E prevents the negative effects of oxidative stress in male rats.

Materials and Methods: The investigation was performed on 30 adult male rats randomly allocated to 3 groups: I K – control animals; II OEv – ozone-exposed animals additionally intramuscularly injected with vitamin E (22.5 mg/rat) and selenium (0.33 mg/rat) preparation Evetsel, for 25 days in 5 day intervals; and III O – ozonated rats without vitamin protection. The rats from groups II and III were exposed to 0.5±0.2 ppm of ozone 5 h a day for the period of 25 days. After termination of the experiment, blood samples of rats were determined for GPx activity and concentrations of testosterone (T), malondialdehyde (MDA) and glucose.

Results: Rats exposed to ozone (III O) were characterised by a decreased testosterone concentration, increased levels of MDA and glucose and elevated activity of GPx in blood (table). In the animals exposed to ozone and injected with vitamin E and selenium (II OEv), the level of MDA was also higher, however, it was apparently lower compared to rats exposed to ozone only. In this group, a statistically insignificant increasing tendency was observed for glucose level and GPx activity with reference to the control group (I K).

Groups/indices	I K (n=10)	II OEv (n=10)	III O (n=10)
T [ng/ml]	1.01±0.09	0.90±0.31	0.73**±0.09
Glucose [mg/dl]	77.02±3.22	82.4±5.09	92.42*±6.47
MDA [μm/l]	9.18±0.38	10.64**±0.24	12.26**±0.22
GPx [U/l]	2073±165	2274±87	2708*±168

**p≤0.01 – significance of differences compared to control group (I K)
* p≤0.05 – significance of differences compared to control group (I K)

Conclusions: In the rats injected with vitamin E and selenium, the changes referring to ozone-exposure were apparently less intensive, except for a high level of malondialdehyde. The results obtained suggest that Evetsel reduces, at least to some extent, the results of the detrimental activity of oxidative stress in male rats.

HORMONAL RECEPTOR IMMUNOHISTOCHEMISTRY OF CANINE INFLAMMATORY MAMMARY CARCINOMA

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Introduction: Inflammatory mammary cancer is the most aggressive spontaneous type of mammary cancer both in women and dogs (inflammatory mammary carcinoma, IMC). The clinical presentation is sudden and resembles an inflammatory process (dermatitis or mastitis) with or without the existence of mammary nodule. The only distinctive feature for the histological diagnosis is the observation of the massive invasion of dermal lymphatics by neoplastic cells. Hormonal receptor expression of ERα and PR has been studied in canine mammary tumours and IMC. However the role of ERβ, AR, IGF-1R and androgens in normal and neoplastic mammary growth is less known. Also the association among the hormonal receptors in the same neoplasm has not been reported. Special endocrine mechanisms have been suggested to participate in the development of canine IMC. The objective of this study was to investigate the immunophenotype of some hormonal receptors in canine IMC and to compare it with the rest of canine mammary tumours.

Materials and Methods: Mammary samples of 5 canine normal mammary glands, 15 dysplasias, 26 benign mammary tumours, 32 malignant non-inflammatory mammary tumours and 26 IMC have been included. Immunohistochemistry of estrogen receptor α (ERα), estrogen receptor β (ERβ), progesterone receptor (PR), androgen receptor (AR) and insulin-like growth factor 1 receptor (IGF-1R) was performed by streptavidin-biotin-peroxidase method. Tumours were considered positive when more than 10% of nuclei were stained. Intensity of the immunostaining was also evaluated by two observers.

Results: Both ER, PR and AR were decreased in non-IMC malignant mammary tumours respect the benign counterparts. All IMC tumours were ERα negative, and most were ERβ, PR, AR positive. IGF-1R was also more expressed in IMC tumours respect to benign and malignant non-IMC tumours. Normal and dysplastic mammary glands were AR-positive with low-moderate intensity. Benign mammary tumours had also a low-moderate AR-expression but few cases were AR-negative. Most of the malignant tumours were AR-positive. A high level of immunostaining (+++) was found significantly in IMC cases, where only 5.0% were AR-negative. Normal mammary glands had very low levels of IGF-1R immunostaining and they were considered negative. Dysplastic mammary glands and benign tumours were also mostly negative, although some cases showed IGF-1R expression of low and moderate levels. None of the malignant tumours non-IMC studied were negative and most of the cases had a low or moderate level of IGF-1R immunoexpression. Half of the IMC tumours had a high level of IGF-1R immunolabelling. Considering all the samples studied the expression of IGF-1R and AR was inversely correlated ($p < 0.05$).

Conclusion: Our results indicate a distinctive and decisive hormonal role in IMC.

IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY OF MEMBRANOUS GLOMERULONEPHRITIS IN IBERIAN LYNX (*LYNX PARDINUS*)

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Introduction: The Iberian lynx (*Lynx pardinus*) is the most endangered cat species in the world. There are less than 200 animals left in Spain and Portugal. There are very few published studies about Iberian lynx pathology. The aim of this work was to study the renal alterations found in these animals by immunohistochemistry and electron microscopy.
Materials and Methods: Between 1998 and 2003, necropsy samples of Iberian lynxes, found dead or that died in captivity, were submitted fixed in 10% buffered formalin from Doñana National Park to the Veterinary Pathology Service of the Veterinary Faculty of Madrid (Spain). In this study, renal samples of 16 Iberian lynxes were embedded in paraffin, sectioned at 4 μm and stained with haematoxylin and eosin, PAS, Masson trichromic and silver stainings. IgM, IgG, laminin, collagen IV and fibronectin immunohistochemistry was performed using the streptavidin-biotin-peroxidase complex method. Electron microscopy technical procedures were carried out from formalin fixed renal specimens.
Results: Focal to multifocal membranous glomerulonephritis was seen in all the animals studied. Focal glomerulorесclerosis was also seen in some cases. Glomerular membranous deposits expressed laminin, type IV collagen and fibronectin varying depending on the severity of the lesions, generally being more intense in chronic phases. IgM and IgG immunoreaction was present in membranous glomerulonephritis and absent in glomerulosclerosis. In spite of the technical limitations of the ultrastructural study, some electro-dense deposits, compatible with immune-complexes, were seen in the thickened glomerular basement membranes.
Conclusion: Our results reveal a high prevalence of immune-complex glomerulonephritis apparently not related to viral infections since the different tests used for feline immunosuppressor viruses (FIV, FeLV, FIP virus and Panleucopenia virus) were negative in most cases. In our series of cases, the glomerular lesions could be due to immune system alterations described in this species and attributed to consanguinity.
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STRUCTURAL AND BEHAVIORAL CHARACTERISATION OF MUSCLE SATELLITE CELLS SELECTED BY ELUTRIATION

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Introduction: Muscle satellite cells are small undifferentiated mononucleated cells, implicated in postnatal growth and muscle regeneration. Located between the sarcolemma and the basal lamina of the muscle fiber, they are mitotically quiescent in normal muscles. However, after activation, they proliferate, differentiate and fuse with differentiated muscle fibers or together to form nascent myofibers. Several studies suggested that satellite cells are highly heterogeneous in nature, with different sub-populations displaying distinct biological and biochemical properties. Here, we examined the characteristics of satellite cells extracted from avian skeletal muscles, in terms of cell size and ultrastructure, to define if the structural criterion could or not be associated with their *in vitro* behavior and so, could be used to separate early the satellite cells sub-populations displaying various properties. We tested Counterflow Centrifugal Elutriation (CCE) as a selection method.

Materials and Methods: Muscle satellite cells were extracted from the *pectoralis superficialis* muscle of 8 days old turkeys, by an enzymatic method. Using CCE, satellite cells were fractionated in size every micrometer, from 5 to 10 µm. The proliferation rate as the fusion ability (myotube number and shape, myosin heavy chain isoforms expression) and the Nicotanamide Adenine Dinucleotide-Tetrazolium Reductase (NADH-TR) activity of the satellite cells from each fraction were assessed *in vitro*, by using primary and clonal culture. Also, transmission electron microscopy was used to investigate the ultrastructure of the different cells from the elutriated fractions.

Results: Examination of the *in vitro* proliferation and fusion properties of the different elutriated satellite cells allowed us to define 3 groups of cells: cells smaller than 6.5 µm, cells with size comprised between 6.5 and 7.5 µm and cells bigger than 7.5 µm. The smallest ones exhibited a delayed and poor proliferative activity whereas the biggest ones are highly proliferative and fusing cells. The cells of the first group, that can't grow in clonal culture, display 4 times less NADH-TR activity compared to other cells. Ultrastructural analysis shows that small cells are characterised by a poorly-developped cytoplasm, containing swollen mitochondrias, and no visible cristae. The biggest cells (more than 10 µm) are characterised by an abundant and very dense cytoplasm, and an irregular nucleus. Cytoplasmic organelle density gradually increased with the cell size.

Conclusions: Our data demonstrate for the first time, that satellite cells with different proliferation and fusion abilities also differ in their size and ultrastructure, showing the presence in adult skeletal muscles of different types of satellite cells. CCE appears as a good way to select sub-populations of satellite cells. Further characterisation of the sub-population of small cells could show an interest in cell therapy.

IS THE FELINE CHOLANGIOHEPATITIS COMPLEX GENETICALLY RELATED

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Introduction: Feline inflammatory liver diseases according of histopathological feature has been reported as lymphocytic portal hepatitis, cholangiohepatitis, progressive lymphocytic cholangitis/cholangiohepatitis and etc. Etiopathogenesis of this feline cholangiohepatitis complex disease is poorly understood. Genetical predisposition is considered and our finding, in some aspects, support that hypothesis.
Material and Methods: Our material consist of 12 necropsied cats which have shown inflammatory lesion in liver. Ten animals were the Persian breed. Age of animals ranged between 1 and 12 years, but vast majority were over 4 years. Out of 12 animals 7 were female and 5 were male. Beside of the lesion in the liver 9 animals had polycystic kidney disease (PKD) changes. Formalin fixed and paraffin wax embedded tissues were stained with haematoxyline and eosin and Van Gieson methods.
Results: Gross pathologic findings included generalized icterus, dark brown discoloration of the liver, accentuation of the hepatic lobular pattern and thickening of the walls of the hepatic biliary ducts. Histopathological appearance is characterized by lymphocytes and plasma cells infiltration in portal region, bile duct proliferation, periportal fibrosis and intrahepatic cholestasis. Bridging between portal areas and central veins and pseudolobule formation were seldom seen.
Discussion: Feline cholangiohepatitis complex has a poorly defined etiopathogenesis. The nature of inflammatory infiltrate suggesting immune-mediated damage but nature of initiating agent remains obscure. The disease has been compared with primary biliary cirrhosis of human beings. Over-representation of Persian cats in our study (10 of the 12 animals) suggests a genetic predisposition. Simultaneous finding of the polycystic kidney disease (9 of the 12 animals), the lesion which is inherited as an autosomal dominant trait in families of Persian cats, support this hypothesis.

EXPRESSION OF BIOLOGICALLY ACTIVE SUBSTANCES BY INTRAMURAL NEURONS SUPPLYING THE STOMACH IN PIGS UNDERGOING DYSENTERY

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Introduction: It has been found that neurons can change their chemical phenotype under pathological conditions. Among peripheral autonomic neurons, enteric neurons are thought to be particularly highly plastic in their response to inflammation. However, no information is available on potential adaptive changes of gastric intramural neurons in large breeding animals in the course of infectious diseases affecting this organ. Therefore, the present study was designed to investigate the expression of biologically active substances by intramural neurons supplying the stomach in pigs undergoing dysentery.

Materials and Methods: Six juvenile female pigs were used. Three animals served as controls. The three remaining pigs were artificially infected (*p.o.*) with *Brachyspira hyodysenteriae* to evoke dysentery. Several days later, classical symptoms of the disease were observed in these animals. A few days (5-7) after the first symptoms were found, at the peak of their intensification, both infected and control pigs were deeply anaesthetized, transcardially perfused with 4% buffered paraformalehyde, and tissue samples comprising all layers of the wall of the ventricular fundus, revealing the clearly visible, characteristic pathological changes, were collected. The cryostat sections were processed for double-labelling immunofluorescence to study the distribution of the intramural nerve structures (visualized with antibodies against protein gene-product 9.5) and their chemical coding using antibodies against vesicular acetylcholine transporter (VAChT; marker of cholinergic nerve structures), nitric oxide synthase (NOS), galanin (GAL), vasoactive intestinal-polypeptide (VIP), somatostatin (SOM) and substance P (SP).

Results: In both inner and outer submucous plexuses of the control pigs, the majority of neurons were SP- (approx. 60%) or VAChT- (56%) positive. Some neurons stained also for GAL (15-18%) or SOM (solitary perikarya). No VIP- or NOS-immunoreactive neurons in the submucous plexuses were found. The myenteric plexus neurons stained for NOS (20%), VAChT (15%), GAL (9%), VIP (8%), or SP (8%) but they were SOM-negative. The most remarkable differences in the chemical coding of the enteric neurons between the control and dysenteric pigs included a very increased number of GAL- and VAChT-positive nerve cells (up to 63% and 86%, respectively) in submucous plexuses of the sick animals. The percentages of the neurons stained for the remaining substances in the submucous plexuses as well as the chemical coding of the nerve cells in the myenteric plexus were similar in the control and dysenteric pigs.

Conclusions: The present results suggest that acetylcholine and especially GAL have a specific role in the function of the inflamed porcine stomach during dysentery.

SPONTANEOUS EPITHELIAL PLAQUES IN THE UTERUS OF A NON-PREGNANT CYNOMOLGUS MONKEY (*MACACA FASCUCULARIS*)

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Introduction: Uterine epithelial plaques are a common but not ubiquitous endometrial response of primates in the early stage of pregnancy. They have not been observed in human beings. Epithelial plaques are transient structures during pregnancy and have been described in rhesus monkeys, baboons, marmosets, green monkeys, cynomolgus monkeys and dusky leaf monkeys. They occur as early as on day one of gestation adjacent to the primary implantation side. Cells of the epithelial plaques are derived from uterine luminal epithelium and the neck cells of uterine glands. Plaque cells start to degenerate at about day 16 of pregnancy and by day 30 only few intact plaque cells are still present. Epithelial plaques have been also induced in primates by various methods. Only a single spontaneous case has been observed in a rhesus monkey. In the present report histomorphological and immunohistochemical features of two spontaneous epithelial plaques occurring in the uterus of a cynomolgus monkey are described.
Materials and Methods: The 4 years and 8 months old female was used as a control animal in a toxicity study. A complete necropsy was performed on the animal at terminal kill. All organs were fixed in 10% neutrale buffered formalin. Samples of tissue were embedded in paraffin wax, sectioned at a nominal thickness of 5 µm and stained with haematoxylin and eosin. The uterus was additionally stained with the PAS reaction and Goldner stain. Immunohistochemically, the uterus was stained for pancytokeratin, ck7, ck8, ck18, ck19 and vimentin.
Results: Two epithelial plaques were observed microscopically in the uterus of a non-pregnant cynomolgus monkey. Plaques were located opposite to each other and extended from the lumen deep into the functional layer of this organ. Both plaques consisted of clusters and nests of epithelial cells that were separated by a scanty fibrous stroma. The plaque forming cells had large vesicular nuclei and abundant faintly basophilic cytoplasm. Frequently cells revealed giant nuclei and occasionally binucleated cells were found. Generally, marked cellular pleomorphism was one of the most prominent features of the lesion. Some cells had vacuoles. PAS reaction revealed PAS-positive granules (probably representing glycogen) in several cells. Mitotic figures were frequent and the plaque peripheries were slightly infiltrated by polymorphonuclear granulocytes. Plaque cells stained positively for pancytokeratin, ck7, ck8, ck18 and ck19 while they only had a weak unspecific reaction for vimentin (in contrast to strongly stained uterine stromal cells).
Conclusions: Uterine epithelial plaques can occur spontaneously in non-pregnant cynomolgus monkey and should not be misinterpreted as carcinoma "*in situ*" or early endometrial carcinoma.

LAFORA'S BODIES IN BORZOI DOG – CASE REPORT

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Introduction: The disease syndrome manifested by the presence of Lafora's bodies is extremely rare and the dog. The syndrome has the inherited genetic background, due to Lafora gene mutations and is manifested by extralysosomal storage of polyglucosan substances. The stored material is present in the brain, liver, striated skeletal muscles and myocardium, the skin tubular epithelium of apocrine sweat glands.
Materials and Methods: Female dog, Borzoi breed (Russian chart), 14 years old was necropsied. The histopathological routine examination of the following organs was performed: brain, heart, liver, lung, spleen kidneys, skeletal muscles, thyroid, parathyroid and adrenal glands. The tissue sections were stained haematoxylin and eosin. Brain section was additionally stained with PAS reaction.
Results: Histopathological changes were focused on the brain, liver and striated muscles, in which Lafora's bodies were found. The stored material was seen in Purkinye cells, cerebellum neuropil, and degenerated/necrotic hepatic cells areas. There were not well visible Lafora's bodies in the striated muscles fibres, however the degenerative changes were observed. In the kidney were interstitial and membranous-proliferative inflammation, with tubular epithelium necrosis and focal calcium deposits. It was accompanied by parathyroid glandular hyperplasia. In the adrenal gland there was adenoma located in zone fasciculate. The circulatory blood disturbances were manifested by the lung oedema and hyperaemia.
Conclusion: The Lafora's bodies were found in the cerebellum and the liver of the Borzoi dog. This inherited metabolic failure was responsible for the ailments and post-mortem diagnosed in the internal organs histopathological changes. The liver or skin biopsy procedure is proposed in the diagnosis of this disease syndrome in living animals.

THE EFFECT OF DIPEPTIDE ALANYL-GLUTAMINE ON RENAL TISSUES PROTECTION AFTER INTESTINAL ISCHEMIA-REPERFUSION

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Intoduction: Multiple organ failure (MOF) is a major cause of death in the surgical intensive care units. The study of the pathophysiology of this syndrome has shown that intestine is the most responsible organ for its cause and intestinal ischemia-reperfusion (IIR) activates a variety of inflammatory mediators and releases proinflammatory mediators which induce a generalized microvascular injury. Glutamin is an essential substance in the synthesis of glutathione, a ROI (reactive oxygen indermediates) scavenger during reperfusion. In this experiment, we studied the effect of dipeptide alanyl-glutamine on renal tissues protection after intestinal ischemia-reperfusion.

Materials and Methods: Thirty male Whistar rats, aged 3 months, weighing 250 g, were used for all experiments and animals were divided into three groups (10 animals/group). In group I (control group), animals underwent occlusion of anterior mesenteric artery for 30 minutes and then 60 minutes of reperfusion. In group II (glutamine group), animals received dipeptide of alanyl-glutamine, with intraperitoneal infusion, 24 and 48 hours before intestinal ischemia-reperfusion. In group III (sham group), animals were subjected to a sham operation of 90 minutes (anesthesia, laparotomy, dissection of anterior mesenteric artery). For the histological examination, from the anesthized animals tissue specimens from the left kidney about 1 mm thick were fixed with 10% paraformaldeyde, embedded in EPON 812, and ultrathin sections were cut for the observation by transmission electron microscopy. The rest of the left kidney was fixed with 10% neutral formalin and tissue sections 4 μm were stained for the observation by light microscopy.

Results: The main histological changes in group I were: capillary endothelial swelling, loosening of the intracellular attachments, and luminal reduction or obliteration of microthromboses. Glomerular capillary basal lamina thickening, podocytic foot processes fusion and pericapillary interstitial oedema were also prominent. Tubular endothelial cell swelling was mild. In group II the lesions were mild but some capillary microthromboses were shown. No abnormality in kidney fine structure of sham animals was observed.

Conclusion: Capillary microthromboses and interstitial oedema were more prominent in group I and basal lamina thickening was observed only in group I. These data support the aspect that in this model of IIR glutamine administration has beneficial effect on renal tissue.

A CASE REPORT OF FIBROMA IN VAGINA IN COW IN IRAN

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Introduction: Fibroma is one of the benign tumours that arise of mesenchymal cell (of the skin) and tumour of connective tissue cells. This is a well-circumscribed tumour of mature, richly collagenus fibrous connective tissue. It occasionally occurs in the oropharynx of all species except the pig it is affecting all domestic animals, but most commonly, horse and rarely occurs in cat. Affected animals are mostly adults or aged. There is no sex predisposition for fibroma. This tumour has been found in all areas of the connective tissue and subcutaneous of domestic animals. The skin of the face and legs, is particularly involved in the horse (Moulton 1978). Fibroma is encapsulated and circumscribed tumour and attachment to affective region epidermis and movement along a curve over its under tissue (Moulton 1978).
Materials and Methods: In January 2004 a 3 years old Holstein-Friesian cow was presented to veterinary clinic of Tabriz Islamic Azad University. Its weight was 300 kg. Temperature, heart rate and respiratory rate were normal. It had four time normal pregnancies in life cycle, but new clinical document showed us inability in next pregnancy. Clinical examination of reproductive system findings, were showed us; vaginitis and cervicitis and in the palpation the firm solitary mass in wall of vagina was determined. The surgery method for biopsy of this mass was in standing position. We used 0.3 mg/kg (*i.m.*) Rampon for sedative and 6cc Lidocaine (0.02 solution) in *hiatus sacrococcigial* (S5-Cy1). A piece of sample of mass referred to pathology laboratory. The coloure of mass was pink white. We used haematoxylin and eosin (HE) and Van Gieson staining.
Results: A histopathological finding in light microscopy includes fibrocyte and fibroblast. The cells had no mitotic figures, and polymorphic figures were very few cells had no haphazardly figure.
Conclusion: In this study we used two different staining (HE and Van Gieson) for compartments between fibroma and leiomyoma. As our sections that staining with Van Gieson had dark blue nuclei, and cytoplasm and fibers had red colure and this was synonyms haematoxylin and eosin staining results, this finding was confirmed the mass was not leiomyoma. In other hand cells have not more polymorphic figures we were understood this mass one of the benign mesenchymal tumour and fibroma has been diagnosed.

THE FIRST REPORT OF PREVALENCE OF BLADDER CALCULI IN THE IRANIAN CATTLE

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Introduction: The aim of the study was to examine the prevalence of bladder calculi in the cattle and recognize the composition of calculi.
Materials and Methods: In this survey, 250 bladders from slaughtered cattle were investigated at Tehran abattoir. All cattle had to pass an ante-mortem vet examination during which their age and sex were evaluated by the veterinary surgeon. The bladder of each cattle, immediately after slaughter was labelled and opened with a scalpel. Calculi in the bladder were collected and the chemical analysis of bladder stones was carried out by commercial kit. Also, the composition of calculi was compared for sex by t-test.
Results: Bladder calculi were found in the bladder of 39 cows (15.6%). There were calculi in one out of 24 calves and 38 out of 226 adult cattle, with higher frequency in the male sex in both groups. Analysis of the calculi showed that main composition of bladder calculi in the Iranian cattle is calcium and ammonium carbonate. In addition small amounts of calcium phosphate and trace amounts of calcium oxalate, uric acid and magnesium being detect by analysis. There was no statistically significant difference between the chemical compositions of the bladder calculi for sex.

AMYLOIDOSIS – AN EMERGING DISEASE IN HUNTING FALCONS IN THE MIDDLE EAST

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Introduction: Amyloidosis is an increasing problem not only in human beings, but also in hunting falcons in the UAE. Since this disease is new in falcons, a retrospective study was performed on 623 falcon necropsies, conducted at CVRL over the last 10 years. A drastic change in falcon species used for falconry in the Middle East was observed. More and more captive bred Gyr falcons (*Falco rusticolus*) and Gyr-hybrids are used instead of wild caught Peregrine falcons (*Falco peregrinus*) and Saker falcons (*Falco cherrug*), a development, which has a positive impact on the population of wild falcons. However, Gyr falcon and their hybrids appear more susceptible to amyloidosis then other falcon species.

Materials and Methods: A total of 623 falcon carcasses were submitted for necropsy to CVRL between August 1994 and April 2004. Organ samples from all cases were taken for histopathological and microbiological investigations, using routine methods. Amyloid suspicious organs were stained with haematoxylin and eosin as well as with Congo red. Two hundred and thirty liver biopsies, collected from sick falcons since 2002, were also included in the study. Statistical analyses were performed using a Chi-squared-test.

Results: Of the total number of falcons included in the study, 21.2% were Gyr falcons, 24.1% were Gyr-hybrids, 22.7% were Peregrines, 12.8% were Sakers and 19.1% were of unknown species. One hundred (16.1%) out of the 623 necropsied falcons were positive for amyloidosis. Forty percent of theses cases were pure Gyr falcons, 25% Gyr hybrids, 18% Peregrine falcons, 7% Saker falcons and 10% of unknown species. Statistical analyses demonstrated that Gyr falcons and Gyr-hybrids were significantly over-represented compared with other species in relation to the occurrence of amyloidosis. Three quarter of all amyloid cases were observed in the last 5 years (76 out of 100 cases). In this time period amyloidosis was diagnosed in 38.5% of the pure Gyr falcons (32 out of 83), in 21% of the Gyr hybrids (21 out of 100), in 17.2% of the Peregrine falcons (13 out of 62), and only in 10% of the Saker falcons (3 out of 30). The liver was the most targeted organ with amyloid deposits in 85 out of the 100 amyloid cases. Amyloid was found also in kidney (47%), spleen (31%), pancreas (7%) and adrenal glands (7%). Renal medullar amyloidosis was observed in 26 cases associated with visceral gout, often (14 cases) without amyloid deposits in other organs. Amyloid was confirmed in 78 out of 230 (26%) liver biopsies. The species distribution was significant different from the necropsy-results with 66.6% amyloid positive Saker falcon samples. However, biopsies were only taken from birds suspicious for any hepatopathy.

Conclusion: Amyloidosis is an increasing problem in hunting falcons in the UAE, affecting mainly pure Gyr falcons and Gyr hybrids. Since AA-amyloidosis was confirmed in these cases, an elevation of serum amyloid-A (SAA), due to chronic infectious disease, is most probably the main cause of the disease; however, an underlying genetic predisposition may also play a role.

EXPERIMENTAL MURID HERPESVIRUS 4 INFECTION: EFFECTS IN THE NATURAL HOST AND IN THE EXPERIMENTAL MODEL

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Introduction: Experimental murid herpesvirus 4 (MuHV-4) infection of laboratory mice is a well-established model system for gammaherpesvirus pathogenesis. Its effect on the natural host, the wood mouse (*Apodemus sylvaticus*), however, has not yet been examined in detail. This study describes the differences in the course of infection, pathological findings and response of wood mice and laboratory mice after experimental MuHV-4 infection.
Materials and Methods: BALB/c mice and wood mice were infected with MuHV-4 strain 68 (MHV-68) intranasally. Lungs and spleens were collected at days (d) 3, 5, 7, 10, 14, 21, 28 and 40 post infection (pi) and examined for the presence of infectious and latent virus by plaque and reactivation assays respectively. Viral DNA load was measured by real-time PCR and pathological changes studied by histology. The latter included the demonstration of viral antigen positive cells and latently-infected cells by "*in situ*" hybridisation for viral tRNA as well as the study of leukocyte subpopulations.
Results: Infection in both rodent species showed a similar time-course with peaks of infectious virus in lungs at d 7 pi and latently-infected cells in the spleen at d 14 pi However, in wood mice, the peak titre of infectious virus in lungs was three logs lower, and the reactive splenic leukocytosis seen in BALB/c mice was absent. Latent infection was established in both species in both the lungs and spleen. Histological studies showed that, in BALB/c mice, diffuse T cell- and macrophage-dominated infiltration, together with a transient period of epithelial necrosis and vasculitis were observed in the lung. In wood mice, however, multifocal granulomatous infiltrates and a severe, B cell-dominated perivascular and peribronchial mononuclear infiltration with B cell emigration predominated. Viral antigen was demonstrated in alveolar epithelial cells and, for a longer time period, in granulomatous infiltrates of wood mice, whereas viral tRNAs were first seen in pulmonary epithelial cells, but then, in wood mice, within B cell infiltrates. Spleens of BALB/c mice exhibited intense macrophage infiltration in the red pulp, together with an intense, but disorderly germinal centre reaction with numerous viral tRNA-positive cells and T cell hyperplasia, whereas wood mouse spleens mainly showed orderly development of secondary follicles containing few tRNA-positive cells.~~CNBr-digestion showed faster migration of some peptide fragments.~~
Conclusion: Our studies demonstrated major differences in the effect of MuHV-4 infection between natural and model host. In the first, a brief phase of viral replication is followed by an intense, B cell-dominated immune response. In the latter, however, MuHV-4 seems to induce a prolonged macrophage and T lymphocyte dominated response both in lung and lymphatic tissues, with higher amounts of latent virus. We

conclude that the wood mouse may be a more relevant system to study the host-pathogen relationships of a gammaherpesvirus.

EXPRESSION OF TNF-α IN JOINT LESIONS OF PIGS

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Introduction: Lesions of the locomotive system are widespread in the Danish pig population and of major economical and ethical concern. In order to elucidate the relation between gross, microbiological, and histological/immunological findings, a study focusing on TNF-α expression in the synovial membrane and regional lymph nodes of animals with different joint lesions was performed.
Materials and Methods: Joints from 29 pigs aged 4 to 24 weeks were included in the study. The joint lesions were evaluated macroscopically and microbiologically. The animals were grouped into five groups according to the aetiology: group I – six animals with *Streptococcus suis* serotype 2 arthritis; group II – seven animals with *Mycoplasma hyosyoviae* arthritis; group III – eight animals with *Erysipelothrix rhusiopathiae* arthritis; group IV – five animals with arthroses; and group V – three animals without lesions. Samples from the synovial membrane, and the regional lymph nodes were fixed in Bouin solution and in 10% buffered formalin and examined histologically. The TNF-α expression was evaluated immunohistochemically using Biosource's monoclonal anti-human TNF-α antibody.
Results: Both within the synovial membranes and the regional lymph nodes, the expression of TNF-α reflected the grouping of joint lesions. The TNF-α expression was prominent in group I and observed both in neutrophils and macrophages located in the exudates and in the synovial membranes. In group II, the expression was less prominent and observed mainly in intravascular macrophages and in the synovial intima. In group III the expression was limited and predominantly observed intravascularly. Very few TNF-α positive cells were observed in groups IV and V. As in the synovial membrane, the expression of TNF-α in the lymph nodes was extensive in group I, and the positive cells (neutrophils and macrophages) were mainly observed in the sinus system. However, macrophages were often also seen perifollicularly. In group II the expression was more moderate and observed in macrophages apparently arranged in a random pattern, but with a tendency towards a perifollicular location. The expression in group III was marked and as in group I, the positive cells were mainly neutrophils. Only a few to a moderate number of cells expressed TNF-α in groups IV and V, and most was expressed by macrophages in the sinus system.
Conclusion: As the expression of TNF-α is reflecting the destruction of cartilage in the different types of infection, it is likely, as also shown in experimental studies in rats (Vet Path, 2004, 41: 235-243), that TNF-α has a major role for the outcome of arthritis in pigs. Moreover, as this proinflammatory cytokine was only expressed to a limited degree in the non-infectious joint lesions, i.e. arthroses, the development of these may not be influenced by TNF-α.

SPONTANEOUS ATOPIC-LIKE DERMATITIS WITH IgE HYPERPRODUCTION IN BEIGE RATS

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Introduction: Atopic dermatitis is a common skin disease characterized by chronic recurrent eczematous lesions, but its exact etiology and mechanism are unclear. Beige rats (DA bg/bg), a mutant model of Chediak-Higashi syndrome, develop skin lesions characterized by pruritus, excoriation, erosion and alopecia (Ozaki et al. 1997). We describe the characters of skin lesions of beige rat to evaluate its possible usefulness as a model of atopic dermatitis.
Materials and Methods: Beige rats at 4, 8, 13, 16, 26 and 52 weeks of age were used. Histological analysis of the skin was performed along both the measurement of plasma IgE and cytokines levels. Th1 and Th2 cytokines and RANTES mRNA expression of skin and lymph node were evaluated. Passive cutaneous anaphylaxis reaction and maximization tests were performed.
Results: Skin lesions begin to develop with increased serum IgE levels and the expression of IL-4 mRNA in the lymph node and skin. Histologically, skin lesions are characterized by acanthosis, ulceration and inflammatory cell infiltration in the dermis. Inflammatory cells consist of CD3+, CD4+, ED1+, ED2+ and I-A+ mononuclear cells, eosinophils, degranulated mast cells and neutrophils accompanying IL-4, γ-interferon and RANTES mRNA expressions of the skin. Inflammatory cells are chronically reduced with decreased expressions of IL-4, γ-interferon and RANTES mRNA. In addition, the rats show a high sensitivity to passive cutaneous anaphylaxis reactions and maximization tests.
Conclusion: Our results show that the skin lesions of Beige rats are morphologically similar to atopic dermatitis, being characterized by mixed responses to Th1 and Th2. It is suggested that type I allergy and high-contact sensitivity may initiate and accelerate the dermatitis of Beige rats.

MORPHOLOGICAL LESIONS OF PARAVERTEBRAL MUSCLES IN RABBIT AS THE EFFECT OF SHORT-TERM EXPERIMENTAL ELECTROSTIMULATION

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Introduction: Lateral Electrical Surface Stimulation (LESS) is used in clinical practice to treat idiopathic scoliosis (IS) in children and adolescents. One of the treatment of IS is using 9 h/day the lateral electrical surface stimulation (LESS) which – unfortunately results in side-effects. It consists of applying LESS for 2 h daily. In view of negative effects of long-timed and positive of short-timed (st) LESS, studies were undertaken in order to determine the morphological effect of LESS-st on the paravertebral muscles.

Materials and Methods: Ten male, pure-bred (New Zealand White), aged approximately 3.5 months, weighed between 2000-2200 g and clinically healthy rabbits were used in this study. The animals were kept indoors in the room with controlled temperature (18°C) and humidity (70%). The experiment was conducted with the use of an electrical stimulator (SCOL-2, Elmech, Warsaw, Poland). The rabbits were randomly assigned to one of the two groups (each n=5): group I, where the rabbits were treated for 2 hours/day and group II (control) – without electrostimulation. Sections of right and left *musculus longissimus dorsi* (MLD): *musculus longissimus thoracis* (MLT) and *m. iliocostalis thoracis* (MIT) were microscopically (haematoxylin and eosin stain) and ultrastructurally examined (TEM: Opton 900 PC). The material was taken from areas targeted with electrostimulation on the right side and analogous areas on the left side of the spine.

Results: LESS-st occasionally caused unfavourable morphological disorders of small intensification within the area of *musculus longissimus dorsi* in the form of regressive lesions (mainly atrophy of fragments of muscle fibres and of cross striation, granular degeneration and disturbances in the course of the Z line). However, there were frequently observed positive effects in paravertebral muscles: muscle fibre hypertrophy, the rise in their congestion and increase in the number of mitochondria and their enlargement, and large clusters of glycogen. In the examined material of the left side of the rabbits from group II (not stimulated) morphological lesions were observed similar to those observed on the right side. The lesions visible here were of a vestigial character and they affected very small fragments of fibres.

Conclusion: It should be noted that short-term LESS causes sporadic low intensity disturbances only. Morphological pattern indicates that the muscle tissue structure of the muscles stabilizing the spine had strengthened.

INVESTIGATION OF THE EFFECT OF LIGNANS ON MURINE MAMMARY GLAND DIFFERENTIATION IN TG.NK MICE

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Introduction: Breast cancer is the most common form of cancer among women in the Western world. Phytoestrogens as lignans and isoflavonoids are suggested to protect against mammary cancer due to their estrogenic activities. Lignans are produced by intestinal flora from precursors primarily found in flaxseed and to a lesser degree in whole grain cereals, berries and nuts. Lignans like enterolactone and enterodiol have weak estrogenic activities. Murine mammary cancer development starts in the undifferentiated structures of the mammary gland, so-called terminal end buds. Enhanced differentiation of the proliferative terminal end buds into the more mature alveolar buds is considered to make the mammary gland less susceptible to cancer development. The aim of the present study was to investigate if lignans from flaxseed can stimulate mammary gland differentiation in an animal model predisposed to mammary tumourigenesis.

Material and Methods: The model used was MMTV/c-neu transgenic mouse strain (TG.NK) overexpressing the c-neu oncogene homologue of human erbB-2 oncogene. TG.NK mice received diets added flaxseed in doses adjusted to ~~mimick~~mimic 0.3, 1, or 3 times the daily human intake of lignans from weaning at the 4th week of age for 6 consecutive weeks. In order to investigate the effects of lignans on mammary gland development

10 animals per group were sacrificed at the age of 6 and 10 weeks, respectively. Whole mounts were prepared from the 4th mammary gland for differentiation analysis. Briefly, the gland was fixed in Carnoys Fixativ followed by staining with alume carmine. Whole mounts were analysed for differentiation by determining size, branching pattern and the number of terminal end buds, terminal ducts and alveolar buds per gland.

Results: Analysis of whole mounts revealed, that flaxseed exposure did not affect the differentiation pattern of the mammary gland. An increased proliferation in terminal structures of the 4th mammary gland resulting in big bold-like indefinable structures- was observed in ~~approximately~~ 50% of 10 weeks old mice in all experimental groups. The number of changes per animal was not statistically significantly increased in mice exposed to diets containing flaxseed (1.6-2.6 per animal) compared to the controls (1.0 per animal).

Conclusion: The results indicate that short time exposure to human relevant doses of flaxseed did not significantly affect mammary gland differentiation in transgenic TG.NK mice under current experimental conditions.

RELEASE OF INTERLEUKIN-1β INTO UTERINE BLOOD FROM INFLAMMATORY CHANGED SWINE UTERUS

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Introduction: Based on investigates from last decade revealed that uterus inflammatory in animals but particularly in cattles induce changes on hypothalamus-pituitary-adrenals-ovarian axis. Ascertained that levels of GnRH, LH, FSH, GH, PRL and TSH decrease and concentrations of CRH, ACTH, glycocorticoides and prostaglandins significant increase. Besides was shown that cytokines mediators of inflammatory state exert modulatory influence on secretion hypothalamic and pituitary hormones. One may suppose that in uterine inflammatory pro-inflammatory cytokines cause alterations of hormonal parameters. The purpose of this work was to estimate concentration of IL-1β of isotope J^{125} labeled in uterine and ovarian venous blood and tissues of these organs.

Materials and Methods: Polish Large White gilts (n=4) of similar age (7-8 months) and body mass (100-110 kg) with two controlled subsequent estrous cycles were used. The animals were divided into two groups: control (n=2) and treated (n=2). The day 3 after ovulation into uterine horns saline (20 ml) in control group and suspension of *Escherichia coli* (10^9 bakteria in 1 ml) in treated group were infused. After 8 days from uterine infusions swine were slaughtered and uteruses collected for farther investigations which was dependence getting ready "*ex vivo*" preparations. Each preparation included: uterine horn, ovary, uterine tube and half broad ligament of the uterus. Then the uterine vessels (venous and arterial) were cannulated and preparation own blood was perfusioned. After control blood samples collected from uterine and ovarian veins IL-1β of isotope J^{125} labeled into the uterine horn was administered. Succeeding blood samples (12) during one hour with frequency about 5 min. were collected. Plasma concentration of IL-1β expressed in CPM by gamma counter was analyzed.

Results: Results obtained submitted statistical analyze and the Bonferroni and area under curve tests were used. Values area under curve is shown in the table:

Specification	Ovarian blood	Uterine blood
Control group	105.4	91.0
Experimental 1	408.8	797.5
Experimental 2	408.4	625.5

Values area under curve indicate that release of IL-1β into uterine end ovarian blood in treated gilts was significant higher than control swine. Differences expressed in percentage were as follow: for ovarian 387.47-387.85 and uterine blood 687.36-876.37. In ovarian and uterine tissues between examined groups significant differences no observed. One may suppose that IL-1β released from inflammatory uterus no reach to ovary in the local pathway in countercurrent transfer manner.

Conclusions: It was found that in the swine IL-1β is release into blood outflow from inflammatory changed uterus. Amount released of IL-1β expressed in CPM/ml as compared with the control group was 4-fold higher in ovarian and 7and 9-fold in uterine blood.

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL EVALUATION OF THE SUBCUTANEOUS TISSUE REACTION CAUSED BY GLUCOSE SENSORS IMPLANTED IN PIGS

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Introduction: In the last couple of decades the first generation of systems capable of monitoring glucose levels continuously has been under development. Especially, the minimal invasive techniques with direct measurements of glucose in interstitial fluid have recently shown consistent and reliable results in humans and different animals. In contrast to the well described functionality of these sensors little is known about the subcutaneous tissue reaction they cause. The aim of the present work was to evaluate the subcutaneous reaction caused by sensors implanted from 1 h to 7 days by histology and immunohistochemistry.
Materials and Methods: In the subcutis on the back of pigs (Landrace, Yorkshire, Duroc) fabricated glucose sensors were implanted with a 18 G inserter needle for 1 h, 2 h, 1 day, 3 days, and 7 days. For comparison of tissue reactions a commercial glucose sensor (MiniMed Continuous Glucose Monitoring System (CGMS), Medtronic MiniMed, Los Angeles, California, USA) was implanted for 3 and 7 days. After euthanasia tissue around the sensors was sampled and processed for histological and immunohistochemical examination in order to evaluate the tissue reaction. Immunostaining was performed for the detection of fibrinogen and macrophages.
Results: No to minimal haemorrhage and fibrin deposition were observed 1 h, 2 h, and 1 day following implantation of sensors. Only a few inflammatory cells dominated by neutrophils were observed in the subcutis near the sensor from 2 h up to 1 day after implantation. The cellular infiltration and fibrin deposition was intensified 3 days after implantation. Moreover, the fibrin was occasionally intermingled with basophilic layers of Feulgen-positive DNA-residues apparently originating from invading cells, i.e. mainly neutrophils. Hemorrhage together with oedema was often observed at the margin of the sensor cavity, and beyond the zone of fibrin and basophilic material a mixed infiltration, mainly made up by neutrophils and macrophages was seen.
Fibrin deposition together with infiltration of neutrophils was reduced 7 days after implantation. At this stage the inflammatory reaction was mainly characterized by macrophages, epitheloid cells, giant cells, and granulation tissue.
Conclusion: This study clearly shows the dynamics of the subcutaneous inflammatory reaction caused by a commercial (CGMS) and fabricated glucose sensors. The tissue reaction is ranging from an acute focal fibrinous/suppurative dermatitis (up to day 3) to chronic fibrinous and granulating foreign body dermatitis on day 7. Further experiments are in progress for clarification whether the tissue reaction will affect the quality of sensor measurements.

OSTEOGENESIS IMPERFECTA IN A YOUNG ADULT CAT

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Introduction: Osteogenesis imperfecta (OI) is a genetically and clinically heterogenous disorder of bone and connective tissue principally characterised by ~~osteoporosis,~~ fragile bones and associated with osteoporosis, hyperextensible joints and dentinogenesis imperfecta. OI has been long recognized in humans ~~(in which it is probably the most common inherited connective tissue disorder)and~~ but has also been described in ~~Charolais, Friesian and Holstein~~ cattle, sheep and various breeds of dog. In humans and dogs, OI has been shown to be due to mutations in the COL1A1 and COL1A2 genes that encode the α1 and α2 collagen chains of type I collagen, respectively. OI-like syndromes have been ~~clinically and histologically~~ described in cats but have not been characterized fully. This study attempts to characterize the lesions and underlying alterations to collagen in a~~not biochemically studied.~~ young adult cat with clinically suspected OI.

Materials and Methods: A post-mortem examination was performed and radiographs taken of a 14 months old cat ~~presented~~ with clinically suspected OI. The animal had suffered from multiple pathological fractures leading to lameness, dyschezia and constipation. ~~Samples were taken and fixed in 10% buffered formalin, bones were decalcified in EDTA, routinely embedded in paraffin and sections taken for microscopic examination~~Organ, bone and teeth sections were examined histologically. Skin type I collagen was solubilised using pepsin and cyanogen bromide (CNBr) digestion and analysed by ~~Collagen characteristics were investigated by pepsin and Cyanogen Bromide (CNBr) digestion of skin biopsies followed by~~ SDS-polyacrylamide gel electrophoresis ~~and staining of gels with Commassie Blue~~.

Results: Major gross and histological alterations were restricted to the skeletal system. ~~Grossly, recent~~ Recent fractures and bony call~~o~~uses indicative of old fractures were found at multiple sites. Radiography of long bones suggested osteopenia. Histologically, disorderly arrangement of articular and epiphyseal cartilage was observed and the primary spongiosa at the growth plate was sparse. Furthermore, there was evidence for persistence of woven bone and retarded formation of mature bone. Analysis of skin type I collagen ~~by pepsin digestion~~ showed that migration of α1 and α2 chains and their ratio (2:1) was comparable to that of normal cat skin. However, differences were observed in the pattern of CNBr peptides obtained.~~CNBr digestion showed faster migration of some peptide fragments.~~

Conclusion: Gross and histological findings confirmed the clinical and radiographical evidence of OI in this cat. At 14 month of age this animal was unusually old, compared to other veterinary cases in the literature. The histological features are similar to those described for non-lethal forms of OI in humans and support the biochemical observations that there may be a mutation in the COL1A1 or COL1A2 genes. PCR and sequencing will be attempted to identify the mutation specifically.

ESSENTIAL ROLE OF THE MURINE PHOSPHATIDYLSERINE RECEPTOR (PTDSR) IN EMBRYONIC ORGAN DIFFERENTIATION

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Introduction: Apoptotic cells translocate an otherwise internal membrane lipid component, phosphatidylserine, to their cell surface, signaling the apoptotic event to surrounding cells. Phagocytosis of apoptotic cells is fundamental to normal tissue development, cellular homeostasis and the resolution of inflammation. The phosphatidylserine receptor (Ptdsr) has been implicated *in vitro* to be a pivotal mediator of apoptotic cell clearance and anti-inflammatory signaling. Here, Ptdsr-deficient mice were generated to explore putative *in vivo* functions of Ptdsr during embryogenesis.
Materials and Methods: Ptdsr-deficient mice were generated by homologous recombination in murine embryonic stem cells in the C57BL/6J background. The Ptdsr exons 1 and 2 were replaced by a loxP-flanked neomycin selection cassette. The hypothesis of a critical role of Ptdsr for apoptotic cell clearance was tested on the formalin fixed, paraffin embedded murine organs. Ptdsr-/- mice were completely serially sectioned, and haematoxylin and eosin stained tissue sections were examined as well as immunohistochemical staining for the activated caspase-3 and F 4/80 antigens.
Results: While wild type and heterozygous (Ptdsr+/-) mice were viable and showed no obvious abnormalities, homozygous (Ptdsr-/-) mice died perinatally with multiple gross organ defects of variable severity. In particular, Ptdsr-/- pups had growth retardation, anaemia, subcutaneous oedema, uni- or bilateral anophthalmia and various skull deformations. Detailed histological analyses revealed delayed bronchoalveolar, glomerular and intestinal differentiation as well as a disturbed haematopoetic, cardiac and cerebral differentiation. Comprising a novel lesion in mice, anophthalmia in Ptdsr-/- mice was associated with the induction of ectopic eye structures in the paranasal sinuses. The number and distribution pattern of activated caspase-3- and F 4/80-positive cells were identical in wild type mice as compared to Ptdsr+/- and Ptdsr-/- animals at any embryonic stage.
Conclusions: Targeted inactivation of the Ptdsr gene revealed that Ptdsr is crucial for the differentiation of several organs during development, but not for apoptotic cell clearance, arguing for a novel essential role of Ptdsr during embryogenesis: 1) absence of Ptdsr causes perinatal lethality that is associated with multiple severe organ defects including uni- or bilateral anophthalmia and delayed or disturbed differentiation in the lung, kidneys, intestine, liver, heart and brain; 2) an impairment in removal of apoptotic cells was not detected *in vivo*.

PATHOGENICITY OF AN ISOLATE OF BOVINE VIRUS DIARRHEA VIRUS 2 (BVDV-2) FROM NORTHERN GERMANY

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Introduction: The majority of BVDV strains in North America belong to BVDV-2. They include highly virulent strains. BVDV-2 has also been isolated in Germany with low frequency. The aim of this study was to investigate the pathogenicity of a BVDV-2 isolate from Northern Germany.

Materials and Methods: Ten 8-11 months old cattle of dairy breed were inoculated with a BVDV-2 isolate from a dairy farm in Northern Germany where several calves had died with severe hemorrhages. Four calves were seronegative(sn) and 6 had neutralizing anti-bodies (nabs) to BVDV. Each animal received 1 ml containing at least 105.7 TCID of BVDV into each nostril and 2 ml intramuscularly. Animals were observed daily for clinical signs, blood was collected at days 2, 5, 7/8 and 12 post inoculation (pi). Two sn calves and 3 seropositive (sp) calves were necropsied at day 7/8 pi and 2 sn calves and 1 sp calf at day 13 pi. Two sp calves were used for clinical observations, only. In tissue sections, presence of BVDV antigen and lesions were compared.

Results: After inoculation mild to moderate signs of respiratory disease were observed in the sn calves. Similar, but milder symptomes were observed in the sp animals. Elevated body temperature with maxima of 40.5°C or higher were seen in both groups, but with shorter duration in sp animals. A decrease of peripheral blood lymphocytes was observed in both groups, but more pronounced in sn animals. Virus was reisolated between days 2 and 9 from all sn and between days 2 and 7 from three of six sp animals. In sn animals nabs were seen at day 12 pi, in sp animals a marked increase of nabs was already seen at day 8 pi. In both groups titres to BVDV-2 were higher than those to BVDV-1. In the sn animals necropsied at day 7 pi, BVDV antigen was detected in lymphoid follicles of tonsils, lymph nodes and Peyer`s patches, in thymic cortex and in epithelial cells of tonsils and Peyer´s patches. Depletion of lymphoid tissues was mild in one and moderate to severe in the second calf. At day 13 pi, most viral antigen had been cleared, but there was severe depletion of the Peyer´s patches and thymus. In one animal, viral antigen was present in vascular walls of associated with lymphohistiocytic, segmentally necrotizing arteritis. In the sp animals, viral antigen was present in lymphoid follicles of tonsils, lymph nodes, Peyer`s patches and in the thymus associated with variable degrees of depletion at these sites at day 8 pi. At day 13 pi, neither viral antigen nor lesions were seen.

Conclusions: Clinical findings, viral distribution and lesions caused by the BVDV-2 isolate from Northern Germany are comparable to the findings in BVDV-2 strains of low virulence in North America. Despite the mild clinical signs, there is a marked depletion of lymphoid tissues. As late consequence of infection, arteritis was observed in several tissues of one animal. Seropositive animals were not protected against infection, but it was milder and viral clearance and recovery were faster.

CLINICAL AND PATHOLOGICAL STUDY OF PIGS EXPERIMENTALLY INOCULATED WITH CSFV GLENTORF STRAIN

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Introduction: Classical swine fever (CSF), a highly infectious disease of pigs, has still a great economic impact on pig production in many countries. In several CSF outbreaks observed recently in Europe the disease was recognized too late mainly due to non-specific clinical signs. Such courses of CSF were caused by viruses that were suspected to be of low virulence. The purpose of the study was to evaluate clinical and pathological signs caused by the low/moderately virulent strain Glentorf of CSF virus.
Materials and Methods: In the study 9 pigs (Large White x Landrace) were used. Group I animals (5 pigs, age 7 weeks) were intranasally inoculated with $10^{6.5}$ $TCID_{50}$ of Glentorf strain and group II animals (4 pigs, age 9 weeks) were intranasally infected with 10^{3} $TCID_{50}$ of the same CSF virus strain. After inoculation clinical signs and body temperature were recorded daily during the whole experiment. After death or euthanasia of pigs pathological changes were recorded. Samples form different organs were taken and fixed in 10% buffered formalin for histopathological studies and immunohistochemical detection of glycoprotein E2 of CSFV.
Results: Animals in group I became febrile from 3-5 day post inoculation (dpi) and all but one displayed the acute form of infection. Four pigs died between 9 and 16 dpi and at necropsy pathological changes typical for acute form of CSF were found. One animal survived showing only growth retardation and was culled at 52 dpi. At necropsy, no pathological changes typical of CSFV infection were found. Animals of group II became febrile from 4-6 dpi and fever lasted for 4.75 days in average. Only 2 pigs displayed a mild clinical signs of infection. All the animals were painlessly slaughtered at 28 dpi and at necropsy no pathological signs of CSF were found. Histopathological findings were typical of CSFV infection in group I and E2 was highly distributed mainly in lymphoid tissues. Group II showed only light histopathological alterations and the presence of E2 was rarely evident.
Conclusion: The intensity of clinical signs, histopathological findings and distribution of E2 glycoprotein of CSF seems to be more dependent on infectious doses of the Glentorf strain than on the age of the inoculated pigs.

MORPHOLOGICAL AND PHENOTYPICAL FEATURES OF FELINE VACCINATION SITE-ASSOCIATED FIBROSARCOMA CELL LINES

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Introduction: Feline fibrosarcomas are the most common skin tumours in cats and often arise at vaccination sites. It is now believed that inflammation plays an important role in the development of these tumours, but the exact mechanisms of tumourigenesis are still unknown. Aim of this study was to obtain a permanent resource of cells for further studies, to characterise the cell lines microscopically, ultrastructurally, by chromosomal analysis and to compare their features to those of the original tumours.
Materials and Methods: Five permanent cell lines were established from a total of 26 tumours histologically classified as feline fibrosarcomas which were taken into culture by mechanical dissection and explantation and grown in Dulbecco Minimal Essential Medium (DMEM) high glucose with 10% foetal calf serum and antibiotics. Cell lines were studied for their *in vitro* morphology by light as well as scanning and transmission electron microscopy. They were examined for FeLV, for the production of collagen (AZAN stain) and for the expression of actin, vimentin, desmin, cytokeratin and tartrate resistent acid phosphatase (TRAP). Chromosomes were analysed after cell-cycle arrest with colcemide.
Results: All cell lines proved FeLV-negative. They differed in morphology and growth rates, but shared the main characteristics: cells were pleomorphic, polygonal or spindle shaped, and arranged in long bundles and fascicles when forming a monolayer. With confluency, contact inhibition was lost. Collagen production was detected. All cells expressed vimentin and actin; few cells stained positive for cytokeratin, but desmin expression was not observed. Multinucleated giant cells were found in variable numbers both in cultures as well as in the original tumours. In culture, these giant cells were generally TRAP-negative, although three original tumours contained TRAP-positive giant cells. Chromosomal analysis revealed huge numerical aberrations, ranging from 28 to 113 per nucleus. Only few cells contained a normal diploid set of chromosomes. In one cell line, chromosome A1 continuously displayed an achromatic region, thereby representing a marker chromosome. In another cell line, microchromosomes were frequently seen.
Conclusion: The study confirms that neoplastic cells in feline fibrosarcomas can grow without any further environmental stimulation. *In vitro*, cells mainly display features of fibroblasts and possibly myofibroblasts. The lack of TRAP expression in giant cells indicates that differentiation towards osteoclasts does not occur in culture, suggesting that environmental stimulation (i.e. by tumour infiltrating lymphocytes) is essential for this. Chromosome analysis revealed extreme karyotypic heterogeneity with no alterations specific for all feline fibrosarcomas.

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

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Introduction: Abscess disease in sheep is a process caused by *Staphylococcus aureus anaerobious* that affects 3 to 8 months old animals and that produces abscesses normally located around the neck that can contain important quantities of purulent material. Abscess disease has not been completely studied and many aspects of the process remain unknown.

Materials, Methods and Results: In this work, we have studied two ovine farms affected by the process. These farms select 2 to 3 months old lambs from local flocks for selection purposes when they are 8 to 12 months old. However, the process also affects many other commercial farms. Up to 65% of the animals were affected in the farms studied. The animals spontaneously developed abscesses in the subcutaneous tissue of the neck, normally near the glottis but also reaching the base of the ear. Depending on the case, one or more abscesses could be detected. However, abscesses could also be seen in other subcutaneous regions such as the forelimbs or even in internal organs such as the lung. Abscesses could reach a remarkable size and always contained variable quantities of a dense to semi liquid yellowish-green material that was surrounded by a thick fibrous tissue. Lymph nodes showed marked chronic lymphadenitis and were always located near the fibrotic capsule of the abscess. Microscopically, the purulent material was formed by a vast majority of neutrophils but also macrophages, epitheliod cells, lymphocytes and plasma cell were seen. A thick fibrotic capsule was always the external limit of the abscess. Colonies of rounded bacteria could be seen in all cases, especially near the capsule. They were normally surrounded by Splendore-Hoeppli material and by an intense inflammatory reaction. Microbiological studies demonstrated the presence of *Staphylococcus aureus* in pure cultures in all cases. Autovaccines were prepared from the isolates and their use in the affected farms reduced the prevalence of the disease in a variable manner, the appearance of new cases only being around 15%.

Conclusion: Abscess disease of sheep is an important process that affects young animals and that needs further research in order to understand its epidemiology and pathogenesis. The differential diagnosis with the macroscopically-similar lesions caused by *Corynebacterium pseudotuberculosis*, the etiological agent of caseous lymphadenitis will be underlined. Autovaccines are of help against the disease although they can not completely avoid the appearance of new cases. The possibility of the redefinition of this disease as a botryomycosis will be discussed.

References: De la Fuente et al. (**1985**) *Staphylococcus aureus subsp. anaerobious subsp. nov.*, the causal agent of abscess disease of sheep. Int J System Bacteriol 35: 99-102.

NEW STRATEGIES IN TUMOUR THERAPY USING THE SEMLIKI FOREST VIRUS VECTOR

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Introduction: The aim of this project is to evaluate the potential of new approaches to tumour gene therapy using the Semliki Forest virus (SFV) vector. The angiogenesis dependency of tumour growth has led to increased interest in anti-angiogenesis therapies. With this in mind it was decided to test the efficacy of a recombinant SFV vaccine expressing Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2/Flk-1), an antigen up-regulated on tumour endothelium, in the treatment of experimentally induced tumours in mice.

Materials and Methods: RNA was isolated from mid-gestation mouse embryos (approx. day 14) and the gene for VEGFR-2 was isolated using RT-PCR. VEGFR-2 is composed of 1348 amino acids that are coded for by 4100 nucleotide bases. Primers were designed to RT-PCR the whole receptor but the products when sent for sequencing showed many mutations and amino acid changes. It was then decided to RT-PCR only the extracellular and transmembrane domains of the receptor and the RT-PCR product were reduced in size from 4.1 kb to 2.4 kb. The smaller product (Flk-1) produced a more accurate result with two conservative base changes when sequencing analysis was performed. The smaller product was then cloned into the SFV vector using Xho1/Spe1 restriction sites and BHK cells electroporated with SFV-Flk-1 RNA demonstrated strong positive immunofluoresence for Flk-1. SCC VII cells, a cell line derived from a squamous cell carcinoma (SCC) that arose spontaneously in a C3H/HeJ mouse and a recognised model of head and neck SCC in man, were obtained from Dr. Ruth Modzelewski, University of Pittsburgh. SCC VII cells and CT26 colon carcinoma cells were cultured and tumours were induced in C3H/HeJ and BALB/c mice, respectively; following subcutaneous inoculation. Mice were inoculated at weekly intervals up to five times prior to the induction of tumours with rSFV particles encoding the VEGFR-2 gene. Tumour growth in rSFV-treated and control mice was measured and tumours were sampled for histological and immunohistochemical examination.

Results: Preliminary results showed a statistically significant reduction in tumour growth in mice treated with the rSFV-VEGFR-2 compared with controls. Treated and control tumours had a similar histological appearance. Morphometric analyses of intra-tumoural microvascular density and cell proliferative activity are in progress.

Discussion: Our findings indicate that peripheral immunological tolerance against an antigen expressed on tumour vascular endothelium can be broken using the SFV vector. The reduction in tumour growth seen in mice treated with the vector suggests that the vector has significant potential in cancer immunotherapy.

DYNAMICS OF INFLAMMATORY REACTION CAUSED BY SUBCUTANEOUS ADMINISTRATION OF VACCINES IN CATS ASSESSMENT OF THE POTENTIAL ONCOGENIC RISK

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Introduction: Vaccination is a complex aggression, due to the effects of mechanical, physico-chemical and biological factors, independently from the possible role of viral inactivation agents, cell culture factors, etc. In order to assess the implication of such an aggression in the development of the so-called "fibrosarcoma complex" in cat, the basic tissue reactions developing at the site of vaccine injection were investigated and the inflammatory reaction dynamics between day 2 and day 21 was reconstituted, using different types of multivalent adjuvanted or monovalent non adjuvanted vaccines.
Materials and Methods: Twenty five European cats, aged 7-10 months, were subcutaneously injected at three different sites, 10 of them with a non-adjuvanted monovalent canarypox vectored FeLV vaccine and 3 x 5 cats with respectively three different commercial adjuvanted vaccines. Skin biopsies were performed after anaesthesia and surgical preparation at 2 days (site 1), 7 days (site 2) and 21 days (site 3) post injection and formaldehyde fixed. Microscopic examinations were performed on 3-5 µm sections, following paraffin inclusion and haematoxylin and eosin staining.
Results: Initial lesions occurring with the adjuvanted vaccines showed either the presence of numerous vacuolar macrophages and lipophagic granulomas, serious cytosteatonecrosis or intense and widely spread calcifications, whereas lesions initiated by the non-adjuvanted vaccine were characterized by the presence of very few vacuolar macrophages and absence of necrosis and calcification. Late lesions gave evidence of the inflammatory healing reaction of tissues, both for the adjuvanted and non-adjuvanted vaccines. It circumscribes a lumen resulting from collagen dilaceration and/or necrosis. Constitutive cells, from lumen towards periphery, include non-differentiated large, basophilic mesenchymal cells ("bordering" cells), as well as more mature histiofibroblastic and lymphoid cells. Lesions obtained with adjuvanted vaccines were more consistent, roughly centimetric in size, showing an outer region of the reactive granuloma which may prove fibroblastic, whereas lesions initiated by the non-adjuvanted vaccine were inconsistent, roughly of millimetric size and had sporadic fibroblastic content, sometimes displaying a strictly hyperplasic lymphoid reaction.
Conclusion: The inconsistency and limited extent of the inflammatory lesion (sometimes limited to a massive lymphoid infiltration) caused by the injection of the non-adjuvanted vaccine seems unlikely to be the cause of a feline fibrosarcoma (except if fibroblasts would appear after day 21). On the other hand, adjuvanted vaccines induce consistent lesions of larger size with the presence of a peripheral fibroblastic population. The latter could be the cause of dissecting septal panniculitis developing from the epicentre, which many pathologists consider as the potential cause for some fibrosarcoma subtypes.

ULTRASTRUCTURAL FINDINGS ASSOCIATED WITH PORCINE DERMATITIS NEPHROPATHY SYNDROME (PDNS)

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Introduction: Data are presented from 21 pigs with characteristic pathological and histological lesions associated with Porcine Dermatitis Nephropathy Syndrome (PDNS). These histomorphological findings are ultrastructurally verified.
Materials and Methods: During 2001 and 2004, complete necropsy was performed in a total of 21 pigs with PDNS. The animals were of both sexes and aged 3 to 6 months. Tissue samples were fixed by 7% buffered formaldehyde, embedded in paraffin and stained by haematoxylin and eosin and Giemsa. For ultrastructural examination the samples were fixed in glutaraldehyde and embedded in epoxy resin in line with standard laboratory procedures.
Results: The affected pigs showed severe necrotic skin lesions that were partly confluent, enlarged haemorrhagic lymph nodes and enlarged pale and soft kidneys. Most significant histological features were necrotic skin lesions, exudative glomerulonephritis, interstitial nephritis and necrotising systemic vasculitis. Ultrastructurally, an accumulation of electron dens material was detectable in glomerular mesangium. Tubular epithelial cells contained intracytoplamic crystals. Using PCR, 20 animals were tested positively for porcin circovirus type 2 (PCV-2) and 5 for porcine reproductive and respiratory syndrome virus (PRRSV).
Conclusion: The pathogenesis of the condition is unknown. The histological appearance of the lesions and the immunopathological observation by other authors suggest that type III hypersensitivity is a possible etiology. The present poster describes the histopathological and ultrastructurally findings.

PROLIFERATIVE ACTIVITY AND DNA PLOIDY IN MELANOMAS IN DOGS

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Introduction: Melanomas are tumours developing from melanocytes – cells originating from the primordial neural tube, which frequency in dogs is estimated for 4-7% of all tumours. Our investigations showed that during 9 years (1993-2002) melanomas comprised 11.6% of all skin neoplasms. The aim of the work was the determination of proliferative activity and DNA ploidy in malignant and benign melanomas in dogs.
Materials and Methods: The investigations were performed on 54 melanomas collected surgically from dogs. Segments were fixed in 8% buffered formalin. Paraffin sections were stained by the following methods: haematoxylin and eosin, Masson-Fontana, Feulgen – for the evaluation of DNA ploidy and mitotic indexes as mean number of mitotic figures by scanning 10 fields – 0.1743 mm^2 at x 400. Proliferative activity was also determined by of the Ki67 expression (MiB1 and En-Vision System DAKO).
Results: Most of tumours originated from German shepherds, Schnauzers, Dachshund and Mongrels. It was noted in the survey investigation that 3 out of 54 examined neoplasms were benign and 51 malignant tumours. These melanomas were of epithelioid type (18), fusocellulare (16), and mixed tumours (20). Due to the various biological character of melanomas, they were all divided into 3 groups: melanomas from various skin areas (25), melanomas from the area of digitals (16), and melanomas of the mucous membrane (oral and nasal cavities); (13). Out of 54 investigated melanomas 8 were the amelanotic melanomas. The evaluation of the proliferative activity using antibodies against Ki67 showed a correlation with mitotic indexes. The investigation of the DNA ploidy revealed the occurrence in melanomas of hypoploidia as well as hyperploidia which testifies to the tumour malignancy. A more considerable degree of aneuploidy was observed in melanomas from the digital than from any other skin area. Often a considerable aneuploidy occurs (over 8 DNA Index) in amelanotic melanomas in which no hypoploidy was observed. Histomorphological evaluation of malignancy and the evaluation of ploidy agreed in 88.8%.
Conclusion: Summing up, the evaluation of the DNA ploidia may be a marker of the melanoma malignancy.

ONE CASE OF EQUINE MALIGNANT SCHWANNOMA IN THE UPPER EYELID: MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL DIAGNOSIS

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Introduction: Benign schwannoma referred to as the haired eyelid is reported in the WHO classification of domestic animal ocular tumours, and cutaneous equine Peripheral Nerve Sheath Tumours (PNSTs) tend to occur more frequently on the eyelid, as do squamous cell carcinomas, melanomas and sarcoid lesions. Therefore, the diagnosis of eyelid PNSTs in the horse has always been based on morphology rather than immunohistochemical patterns.
Materials and Methods: We examined a newly formed mass localized in the left upper eyelid of an 8 years old, male trotter, submitted to surgery. Five mm sections from the excised wax embedded mass were stained with haematoxylin and eosin (HE), periodic acid Schiff (PAS) and alcian blue (pH=1) methods. S-100 protein, glial fibrillary acidic protein (GFAP), vimentin, smooth muscle antigen (SMA) and myoglobin, synaptophysin, pancytokeratins, neuron-specific enolase (NSE), laminin and Ki67 protein were tested to identify the cell origin and the mitotic index of the tumour.
Results: The bilobated nodular mass was 7 x 5 cm in size, and developed in the subcutaneous site infiltrating the adjacent muscular fascicles. It was firm with white, shiny and smooth outer and cut surfaces. During surgery there was no any anatomical correlation or continuity with other structures. Microscopically, the tumour was characterized by compact areas of spindle-shaped neoplastic cells and loosely textured areas. The closely packed tumour cells showed large ovoid nuclei, while the loosely arranged neoplastic cells were markedly spindle in shape with small elongated tapered, sometimes blunt-ended, nuclei and a finely fibrillary cytoplasm. The neoplastic cells were arranged in interwoven sheets and whorls lacking a central recognizable vessel structures. Occasional elongated nuclear palisades were observed, simulating Antoni type A patterns. A number of mitotic figures were seen. The neoplastic cells immuno-reacted to S-100 and vimentin in both the compact and loosely textured areas, but were completely negative for SMA and myoglobin, excluding an eyelid muscle fibre origin of this tumour. Likewise, the tumour showed no synaptophysin-reaction, nor pancytokeratins- and GFAP-reaction. Laminin was diffusely expressed in a pericellular linear or granular pattern, indicating a constant presence of basal membrane-like structures that fibromatous component of peripheral nerve sheath does not express. A marked and diffuse NSE-reaction suggested a neuroectodermal origin of this tumour. The mitotic index, investigated by Ki67, was high.
Conclusion: Morphological and immunohistochemical findings observed in this neoplastic tissue on the upper eyelid were consistent with a malignant schwannoma.

CEREBRAL GRANULAR CELL TUMOUR IN A CAT

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Introduction: Cerebral granular cell tumour of the Central Nervous System (CNS) has been reported in the rat, ferret and dog to be of meningeal cell derivation. In human oncology, cerebral granular cell tumours are considered to be derived from specialized pituicytes, Schwann cells or astrocytes. Intracranial granular cell tumours have never been previously reported in the cat.
Materials and Methods: A cerebral intraventricular tumour from a 6 years old, shorthaired breed, female cat, affected by tremors and seizures, was submitted to histological examination performed by haematoxylin and eosin (HE), periodic acid Schiff (PAS) and Gabe Martoja trichromic stainings. Immunohistochemistry tests were done on S-100 protein, glial fibrillary acidic protein (GFAP), vimentin, synaptophysin, pancytokeratins, neuron-specific enolase (NSE), laminin, lysozime and Ki67 protein to identify the cell origin and the mitotic index of the tumour.
Results: The newly-formed intracerebral tissue appeared to be composed of large cells arranged in sheets characterized by abundant, pale, eosinophilic cytoplasm with distinct intracytoplasmic granules. These granules were distinctly-variably diastase-resistant PAS-positive. Numerous foci of calcification and cholesterinic patterns were observed in the tumour. The tumour appeared to be circumscribed by meningial or ependymal layer and infiltrating the corpus callosus above the third cerebral ventricle. Granular neoplastic cells were diffusely and strongly vimentin-positive. GFAP distinguished ependymal layer and scattered ependymal cells from choroid epithelium involved in the tumour. The granules appeared slightly positive to S-100 protein. A marked interstitial immunoreactivity to GFAP, S-100, NSE and lysozime was present. The neoplastic cells expressed no cytokeratins or synaptophysin. Laminin immuno-labelling was limited to blood vessel basal membranes. The mitotic index was low.
Conclusion: Histological examination showed typical findings of meningial tumours such as psammoma body-like figures and cholesterinic degenerations. Based on the negative staining for GFAP expression, the tumour was not considered of astrocytic derivation. The uniformly positive staining for vimentin, along with the focally positive reaction to S-100, supported the meningial origin. The complete loss of synaptophisin immunolabelling excluded any neural and epithelial neuroendocrine neoplasm. GFAP, S-100, lysozime and NSE interstitial immunoreactivity suggested the presence of a mixed non neoplastic reactive cell population including ependymal and subependymal cells, as well as meningial elements. To our knowledge, this is the first report of a cerebral granular cell tumour in the cat.

HISTOPATHOLOGY OF A BOVINE CUTANEOUS HEMANGIOMA AND SOME ASPECTS OF ITS ETIOLOGY

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Introduction: Haemangioma is a benign tumour of endothelial cells of blood vessels. Common is dogs, but rare in other domestic animals. It is dermal or subcutaneous tumour occurring anywhere on the body. In cattle, haemangioma usually occur in mature animals. There is evidence that in some light skinned, shorthaired dog breeds, haemangioma may be caused by prolonged exposure to sunlight. Haemangioma are generally slow growing and do not recur after complete surgical removal.
This paper describes a case of cutaneous haemangioma in a cow including its histopathology and successful treatment. In addition this case report showed that prolonged exposure to sunlight is an important factor and there is a hereditary predisposition, for occurrence of this tumour in the cow.
Materials and Methods: In June 2001, a skin biopsy specimen from a 5 years old Holestein native crossbreed cow was submitted to the department of pathology, Faculty of Veterinary Medicine, University of Tehran.
Results: The animal had a non-responsive lesion within the light skinned over the medial aspect of the left hock, with recurrent haemorrhage after licking. The lesion was a single sessile mass, red and black in colour, and ovoid in shape, with an uneven, ulcereated surface, approximately 4 x 2.5 cm in diameter, within non-pigmented skin. Histopathological examination revealed that the tumour was composed of numerous vascular spaces lined by a single layer of well differentiated endothelial cells with plump nuclei. These variably sized interconnecting channels were separated by varying amounts of connective tissue stroma. Many of these channels were collapsed or empty, some contained blood and others, particularly near the surface, were thrombosed. The animal was treated with a combination of surgical excision of the lesion with an adequate margin of surrounding tissue, and cryosurgery, using a double freeze-thaw cycle. During the next six months, the cow was visited several times; the wound was observed to have healed favourably and the cosmetic result was excellent.
Conclusion: The history, no recurrence after surgical excision, gross pathology, and histopathologic findings were typical of haemangioma. According to the owner, another daughter of its dam with a different sire (half-sib) had been culled at five years of age because of similar lesions, which had occurred on both hindlimbs. This suggests that a hereditary predisposition may play a role in the etiology of haemangioma; however, environmental factors may also have an influence. The cow in this study was housed at high altitude in an environment where there were high levels of sunshine.

ANGUILLA ANGUILLA L. GENOTOXIC AND LIVER BIOTRANSFORMATION RESPONSES TO ABIETIC ACID EXPOSURE

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Introduction: Pulp and paper mill effluents include a variety of compounds that are highly toxic to aquatic organisms. Softwood is resinous, containing resin acids (RAs) in their tissue, part of which are released to the effluent during pulping and bleaching processes. Abietic (AA) and dehydroabietic acid are among the most abundant RAs in those effluents.

Material and Methods: Adult eels (*Anguilla anguilla* L.) were exposed during 8, 16, 24 and 72 hours exposure to 0, 0.1, 0.3, 0.9 and 2.7 μM abietic acid (AA). Genotoxicity was measured as erythrocytic nuclear abnormalities (ENA), as well as DNA strand breaks in blood and liver. Liver cytochrome P450 (P450) content, liver ethoxyresorufin-O-deethylase (EROD) and glutathione-S-transferase (GST) activities were determined as biotransformation biomarkers. Liver alanine amino transferase (ALT) activity was also measured as an indication of tissue damage.

Results: Low AA concentrations, such as 0.1, 0.3 μM AA have a delayed *A. anguilla* L. liver EROD activity induction, whereas the higher AA concentration has also a delayed effect probably due to a consequence of tissue liver high inhibitory concentration. Eel's liver GST activity results demonstrated that only low AA concentrations promoted liver GST increase, whereas high AA concentrations, such as 0.9 and 2.7 μM, did not alter it. Our results concerning liver ALT activity indicated a liver damage induced by high AA concentrations, such as 2.7 and 0.9 μM AA. Our eels ENA result analysis reveal that AA is a weak ENA inducer in *A. anguilla* L. Blood DNA integrity results, suggest that low AA concentrations promoted late blood DNA integrity decrease, nevertheless high AA concentrations were early blood genotoxic inducers compared to low AA doses. According to our present research results concerning the eel's liver DNA damage, all AA exposure concentrations decreased liver DNA integrity.

Conclusions: AA is more genotoxic to liver than to blood in *A. anguilla* L., as far as DNA strand breaks is concerned. However, AA is a weak ENA inducer, since only the highest AA exposure concentration, had a delayed ENA increase. Eels liver EROD inducing potency was revealed at 24 hours for all AA exposure concentrations, despite its highly increase at 0.9 μM AA during the whole experiment. The relation with high hepatic phase I biotransformation and genotoxicity is difficult to establish in *A. anguilla* L. exposed to AA, since low liver EROD activities seem capable to promote early liver DNA strand breaks increase, whereas ENAs were only observed after long exposures to the highest AA concentration.

References: Maria et al. (**2004**) Ecotoxicology and Environmental Safety 58: 202-210.

BIOMARKER RESPONSES IN A POLLUTED RIVER: EFFECTS OF PULP MILL CONTAMINANTS ON CAGED *ANGUILLA ANGUILLA* L.

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Introduction: Industrial effluents of wood processing are highly complex mixtures of organic substances, toxic or otherwise harmful to fish and other aquatic organisms. Of these, chlorinated and non-chlorinated small-molecular weight fractions of pulp and paper mill effluents, the so-called resin acids (RAs) and chlorophenolics (CPS), are important. Biochemical, physiological and structural effects in fish exposed to these mixtures has been documented during the past 20 years.
Materials and Methods: Caged *Anguilla anguilla* L. (silver eel) were exposed in situ during 8 and 48 hours to the pulp mill contaminants of Vouga River, at different distances (left bank: site 1 – 50 m; right bank: site 2 – 100 and site 3 – 2000 m) from deactivated pulp and paper mill sewage outlet as well as to clean water under laboratory conditions (control). Eels liver biotransformation (phase I) was measured as ethoxyresorufin-O-deethylase (EROD) activity, cytochrome P450 (P450), and glutathione-S-transferase activity (GST) (phase II). Genotoxic responses were determined as blood and liver DNA strand breaks as well as erythrocytic nuclear abnormalities (ENA).
Results: Pulp mill contaminants of Vouga River failed to significant increase liver P450 content and GST activity. Nevertheless, liver EROD activity was significantly increased after 8 hours exposure at site 3 (2000 m). A significant increase in ENA frequency was observed after 8 and 48 hours exposure at site 2 (100 m), as well as at site 3 after 48 hours exposure to pulp mill contaminants of Vouga. Genotoxicity measured as DNA integrity decrease was found in blood after 48 hours exposure at site 2, whereas in liver it was observed after 8 hours exposure at site 2, as well as at site 1 (50 m) after 48 hours exposure to pulp mill contaminants.
Conclusion: This study reveal that Vouga River contaminants contain genotoxic and/or progenotoxic compounds, which are able to induce early genotoxicity in eel's liver cells and a delayed genotoxicity in blood as DNA strand breaks at site 2 (100 m) and site 1 (50 m). Citogenetics effects (ENA frequency) were observed at site 2 during all experimental conditions. Liver EROD activity increase was observed before ENA increase at site 3 (2000 m).
References: Maria et al. (**2004**) Fresenius Environmental Bulletin 13 (4): 317-325.

CLINICOPATHOLOGIC FEATURES OF A TUBAL ADENOMA IN A BITCH

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Introduction: Primary tumours of the uterine tube are extremely rare in domestic animals. The few cases reported in literature have mostly been found in the bitch as adenomas and adenocarcinomas deriving from the fimbriated end of the uterine tube. A case of tubal leiomyoma and some lipomas of the mesosalpinx have also been described.

Materials and Methods: A 10 years old mixed breed bitch was referred to a practice veterinarian for an abdominal enlargement, which had increased in the previous two months. Ascites was diagnosed and abundant reddish fluid was aspirated. As the general condition did not improve, the animal was examined by the Veterinary Teaching Hospital of the University of Messina. Transabdominal palpation and ultrasound examination revealed the presence of a large mass, occupying the whole abdominal cavity, with an evident unilocular cystic structure. At laparotomy, the mass apparently originated from the right ovarian bursa. Ovariohysterectomy was performed after aspiration of 3.5 liters of serosanguinous fluid from the mass and the genital organs and the neoplasm were processed for gross, histopathologic and immunohistochemical examination.

Results: The excised organs included both the ovaries with *corpora lutea*, and a slightly thickened uterus with cystic endometrial hyperplasia. The mass (30 cm in diameter) totally replaced the right ovarian bursa and presented a reddish smooth outer surface and, on section, a thick wall from which several papilliferous proliferations projected toward the cystic lumen. At light microscopy, the cystic wall showed the following recognizable layers: serosa, subserosa, muscularis, submucosa and mucosa. The last ones were organized in complex branched papilliferous folds of fibrovascular and smooth muscle tissue lined by columnar epithelium, including tall ciliated cells and nonciliated secretory cells. Low grade nuclear atypia and no mitotic figures were seen in epithelial cells, with focal nuclear crowding. Immunohistochemically, the epithelium showed a moderate to strong immunoreaction for vimentin, cytokeratins (8/18, AE1/AE3, 34BE12), as the stroma expressed strong immunoreaction for vimentin and alpha smooth muscle actin antigens.

Conclusion: A diagnosis of tubal adenoma based on the most recent international histological classification of genital tumours in domestic animals (WHO) was made. The severity of the clinical picture was unique and due to the considerable enlargement of the neoplasm and to the ascites. The gross and microscopic features suggested origin of the tumour from the ampullar portion of the right uterine tube. Although the histology alone clearly indicated the diagnosis, immunohistochemistry confirmed it and must be regarded as a reliable technique for characterizing undifferentiated forms or for distinguishing tubal adenomas from epithelial or mixed epithelial and mesenchymal tumours of ovarian/uterine origin.

IMMUNOHISTOCHEMICAL EXPRESSION OF ENDOTHELIN-1, VEGF AND VEGF-R1 IN MAMMARY TUMOURS IN THE DOG AND THEIR ROLE IN NEOPLASTIC ANGIOGENESIS

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Introduction: Angiogenesis is a fundamental process for tumour growth and the formation of metastases. The endothelin-1 (ET-1), a member of a family of mammalian vasoactive peptides, is expressed by endothelial cells and by many neoplastic cells. ET-1, binding the ET_A receptor, acts as mitogen for tumours cells, endothelial cells and vascular smooth muscle cells. In addition, ET-1 may indirectely enhance endothelial cell proliferation through stimulation of vascular endothelial growth factor (VEGF) production by other cells type. VEGF-R1, also known as Flt-1, as binding VEGF, is upregulated in tumours tissues and proliferating endothelium. The aim of this study was to verify the immunohiatochemical expression of ET-1, VEGF and VEGF-R1 antigens in dog mammary tumours to evaluate the specific nuclear stain and to clarify whether the overexpression correlates with tumourigenesis.
Materials and Methods: A total of 30 canine mammary tumours were classified according to standard diagnostic criteria by WHO (5 simple adenomas, 12 simple carcinomas tubular type, 6 simple carcinomas solid type and 7 complex carcinomas). For immunohistochemical investigations, 4 μm tick sections, after microwaves antigen retrieval, were incubated with: anti-endothelin-1 mouse Mab (Sigma, S. Louis, USA), anti-VEGF rabbit Pab, anti-Flt-1 rabbit Pab (Santa Cruz Biotech, USA) and anti-cytokeratin mouse Mab (DAKO, USA). This reaction was detected using an ABC-peroxidase method (Vector Lab., Burlingame, USA). The antigen expressions were performed examining 10 representative tissue areas (x 40): the percentage of positive cells and the intensity of staining were evaluated.
Results: Specific staining of ET-1, VEGF and VEGF-R1 was present in all endothelial cells. The immunohistochemical expression of ET-1 was widespride and intense in tumour cells positive to cytokeratin, most in carcinomas than in adenomas. In complex carcinomas the specific stain of myoepithelial cells were weaker than of epithelial ones. In all tumours, the percentage of positive cells to VEGF and VEGF-R1 was always lower than ET-1 expression while the intensity was strong in both. There was correlation in positive expression of ET-1, VEGF and Flt-1 with histologic grade and tumour size.
Conclusion: The present study is a demonstration of ET-1, VEGF and VEGF-R1 overexpression in canine mammary tumours as well as in human tumours. The immunoreactivity is significantly increased in carcinomas compared with adenomas and in the epithelial cells compared with myoepithelial cells. This suggests a correlation between ET-1, VEGF and Flt-1 expression and tumour grade, neoplastic progression and metastatisation. The immunoreactivity of these markers cannot be standardised till today because of the small number of cases analysed; other investigations will be necessary to provide a suitable method of screening in routinely surgical pathology. This study will be continued evaluating also the correlation between primary breast tumours and metastatic neoplasms.

NEUROPATHOLOGICAL STUDY OF THE BRAIN OF AN ALBINO GORILLA

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Introduction: Snowflake was an albino aged primate (about 39 years old) which remained practically its whole life in captivity in the Zoological Park of Barcelona. The scientific and social interests created over this gorilla promoted its detailed study.

Materials and Methods: Thanks to the agreement between the ZOO and the Animal Tissue Bank of Catalunya (BTAC, http://quiro.uab.es/btac), the brain, and other tissues, of the albino gorilla Snowflake, were collected processed and studied before making it available for the scientific community. One half of the brain was frozen at -80ºC and the other was formalin fixed and paraffin embedded. Histopathological and inmunohistochemical studies were carried out over several areas of the paraffin embedded brain.

Results: The preliminary results of the gorilla's brain studies show generalized injuries such as spongiosis, vascular fibrosis, intraneuronal accumulation of lipofucsin and neuromelanin, corpora amilacea formation, and other aging lesions. Specific local lesions were observed in the *globus pallidus* (GP) such as calcification of blood vessel wall and numerous spheroids associated to iron deposits. In the *substantia nigra* (SN), the iron deposition was less important and it was associated to a high density of corpora amilacea.

Through immunohistochemical techniques we have characterized the spheroids content and we have detected amiloid material accumulation in the wall of some blood vessels (amiloid angiopathy).

Conclusion: All the neurodegenerative changes observed in this study are associated to the normal process of aging in primates. However, the pallido-nigral degeneration found in Snowflake, differs from that observed in preceding studies of aged primates.

ANIMAL TISSUE BANK OF CATALUNYA

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The Animal Tissue Bank of Catalunya (Banc de Teixits Animals de Catalunya, BTAC) is a non for profit entity which collects, processes, diagnoses, stores and distributes animal nervous tissue samples to the scientific community. BTAC offers samples of a great variety of species and a wide range of ages. Animal tissues come from, the Animal Pathology Service of the Universitat Autònoma de Barcelona (UAB), the Priocat Lab (The Catalunya Reference Laboratory for Animal Prion Diseases), and the Barcelona Zoological Park. Non pathologic tissues and pathologic specimens, including Transmissible Spongiform Encephalopaties (TSEs), are available.

The material collected is principally nervous tissue but in some instances samples from other tissues are also included. BTAC performs well established protocols for sample processing which can be modified upon the applicant requirements; routinely, a half of the sample is frozen at -80ºC and the other half is formalin fixed and paraffin embedded.

All samples included in the bank are submitted to a quality control check in order to assure the optimal conditions of the processed tissue for research purposes. Furthermore, a team of specialized pathologists carries out a thorough anatomopathological examination to characterise the samples. These and other data about the tissue (i.e. samples origin, clinical information, processing details, etc) are also included in our database.

BTAC is open to be coordinated with other animal and human tissue banks. Actually develops, apart from its own research lines, collaborations with other groups and, in fact, the bank is open and willing to establish new research collaborations.

The bank samples are available upon request and after the approval of such request by the BTAC Scientific Committee.

BTAC is placed in the Campus of the UAB. More information is available in the web site http://quiro.uab.es/btac.

The bank creation was supported by the funding project; reference SAF2001-4772-E, of the Spanish Technology and Science Minister.

CONGENITAL TREMOR IN PIGLETS: CLINICO-PATHOLOGICAL FEATURES AND AETIOLOGICAL INVESTIGATIONS

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Introduction: Congenital tremor (CT) is a neonatal central nervous system (CNS) disorder of swine, causally linked to a number of different agents (viruses, toxic compounds, host's genetic factors) and classified into distinct disease types. The most frequent one is A2 type, putatively ascribed to PCV2 infection. In spite of the above distinction, all CT types are characterized by rhythmic, intentional limb and head tremors , as well as by brain and/or spinal cord hypomyelination.

Materials and Methods: A spontaneous CT episode in a litter from a little, rural type herd in Southern Italy is reported here. Both breeders were Large White x Landrace hybrids. No CT signs had been observed in the previous litter obtained from the same breeders. The entire litter showed intense, rhythmic, whole-body intentional tremors, typically increasing with excitement and ceasing during rest, ataxia and rigidity of the hindlimbs. Symptoms completely disappeared in all piglets at around 2 months of age. A 6 days old male piglet was euthanized and submitted to post-mortem examination. Tissue samples from CNS and major organs were fixed in 10% buffered formalin and embedded in paraffin. 5 μm thick sections were stained by haematoxylin and eosin, while 10 μm thick sections, cut at different CNS levels, were stained by Luxol Fast Blue (LFB). Specific immuno- histochemical investigations for PCV2 were carried out on a wide range of tissues, with blood samples being also collected and serologically tested for hog cholera virus (HCV), bovine diarrhoea virus, border disease virus, Aujeszky's disease virus, porcine parvovirus (PPV) and porcine reproductive and respiratory syndrome virus. Finally, brain, liver and gastric content specimens were submitted to toxicological investigations for organophosphates, organochlorines, heavy metals and aflatoxins.

Results and Discussion: Macroscopically, no gross lesions were found, whereas histological CNS examination revealed a moderate to severe, generally symmetrical hypomyelination, with lesions apparently being more prominent in cerebellar and spinal cord white matter. At this level, in LFB-stained sections, more or less numerous and variably sized "holes", often exhibiting at their time a symmetrical distribution, were observed. Immunohistochemically, no specific labelling for PCV2 could be detected, with serological examinations being negative except for PPV, probably due to colostrum-derived antibodies. Finally, toxicological investigations turned out to be negative for all the concerned chemicals.

Conclusions: Anamnestic, clinical, pathological, serological and toxicological investigations allowed us to classify the disease episode under study as a type A2 CT case. Nevertheless, we are still unable to identify both the putative responsible viral agent and the primary source of infection, since no pig had been introduced, short before, in the little and "segregated" herd under study. Furthermore, our laboratory results confirm that the aetiological relationship between PCV2 and type A2 CT is far from being elucidated, though it cannot be completely ruled out at this stage.

USE OF CYTOKERATIN STAINING FOR THE DETECTION OF LYMPH NODE MICROMETASTASIS IN CANINE MAMMARY MALIGNANT TUMOURS

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Introduction: The status of axillary lymph nodes is the single most important prognostic factor in human breast cancer. In the dog, there is ongoing debate on the clinical value of regional (axillary/inguinal) node metastasis, although the WHO TNM classification of canine mammary tumours uses the nodal status as one of the variables for the clinical staging. To date, there are no data regarding the relevance of occult lymph node micrometastasis in the outcome of dogs with mammary malignant tumours. The use of immunostaining is a valuable tool for the detection of either small clusters or isolated cancer cells in lymph nodes where routine evaluation of haematoxylin and eosin (HE) stained slides is negative or doubtful for the presence of these cells.
Materials and Methods: Sixty mammary malignant tumours and 131 local and regional lymph nodes were surgically removed from 40 female dogs. Both the neoplasms and lymph nodes were microscopically evaluated after routine processing and staining with HE. Serial slides of the lymph nodes were also processed and stained for immunohistochemistry (IHC) using anti-pancytokeratin (ck) antibodies AE1:AE3 (Zymed Laboratories) and ck14 (clone LL002-Serotec Laboratories), and microscopically evaluated for the presence of stained cells. Evaluation of IHC stained slides was performed without knowledge of HE results.
Results: Overall, results between HE and IHC were concordant in 50 (83%) tumours (42 negative, 8 positive). Ten (19%) of 52 tumours that were considered as not invading lymph nodes on HE slides, were positive in IHC. Of these 10 cases, only in one were cells identified with both antibodies, the others stained only with AE1:AE3. In two IHC positive cases, metastatic cells appeared isolated, while in all others there were clusters of tumour cells, some of them showing different degrees of organization, either in different nodes or within the same node.
Conclusions: Detection of lymph node metastasis upgrades the clinical stage of mammary malignant tumours with potential consequences in the prognosis and/or need for adjuvant therapy. Routine evaluation of HE stained lymph node slides alone may underestimate the presence of micrometastasis. The use of imunohistochemistry is a valuable tool for the detection of both isolated and clusters of neoplastic cells in regional lymph nodes, allowing a better understanding of the metastatic routes of canine mammary tumours. In human patients, there is not yet consensus on the definition of micrometastasis nor in to witch extent do they should be used to upgrade clinical stage of patients with breast cancer.

ANASARCA IN ENGLISH BULLDOG PUPPIES: PATHOLOGICAL FINDINGS AND ETIOPATHOGENIC CONSIDERATIONS

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Introduction: Anasarca is a condition of fetal hydropsy able to cause obstetric injuries both in animals and humans. It consists of a diffuse oedematous status of the subcutaneous connective tissue, with or without production of transudate in serosal cavities. The pathogenesis of the condition is not entirely understood.
Materials and Methods: A pregnant 3.5 years old female English bulldog was admitted to the Veterinary Teaching Hospital of the University of Messina, 62 days after mating. Clinical examination showed anorexia, moderate depression, dyspnoea, abdominal enlargement and vulvar swelling. Ultrasonographic examination revealed the death of two oversized foetuses. Six puppies were removed by caesarean section. Three of them were very weak and died within two hours, whereas the lastone is still live and alive. Complete necropsies of four puppies were performed. Samples of different tissues and organs were collected, fixed in 10% buffered formalin and paraffin wax embedded. Histological sections were stained with haematoxylin and eosin.
Results: The puppies showed variable degrees of size increasing and diffuse anasarca. The skin was thickened and a yellowish fluid spoured at skinning. The same fluid filled the serosal cavities. The majority of body organs were unremarkable, except the hearts, which were enlarged and pale. The most important histopathological lesions were located in the skin (diffuse dermic oedema), heart (myocarditis), spleen and thymus (hyperplasia) of all subjects.
Discussion: The observed findings were similar to those already reported in literature in dogs and other animal species. In dogs, English bulldogs present the higher incidence of this status in comparison with other canine breeds. In dogs, the anasarca is also known as “water pups” and a similar condition has been described in humans. In bovines and ovines, the pathogenesis seems to be related to an autosomal recessive gene, whereas in other animals it has not been verified, even if the high predisposition in some breed of dogs indicates a genetic defect. Among the different pathogenic hypotheses, minute virus of canines (MCV) has been shown to cause transplacental infections with ccasional findings of anasarca or myocarditis in some pups. This evenience could be considered also in our cases, where the occurrence of gross and histopathological inflammatory lesions particularly in the heart and lymphoid tissues of the puppies suggested a probable viral infection as the cause of the condition.

AFIP ONLINE VETERINARY PATHOLOGY TRAINING PROGRAM

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The Department of Veterinary Pathology, Armed Forces Institute of Pathology, has implemented three online resources in veterinary pathology. 1) The Veterinary Systemic Pathology Resource, funded from a Department of Education, Fund for the Improvement of Post-secondary Education grant, utilizes the same eleven-organ system training archive as the AFIP residency program. The database can be viewed as unknowns or sorted by organ system, animal species, and disease category for over 675 entities in numerous species. Linked sequential images with legends are provided to simulate examination of the tissue at the microscope. Lower magnification images contain hyperlinked "hotspots" which delineate the viewable area contained in the next higher magnification. Each case manuscript is reviewed and updated every three years. 2) The Registry of Toxicologic Pathology web conference provides toxicologic pathologists with an anonymous forum for the exchange of ideas and information concerning toxicologic pathology research and related issues. There are nine conferences annually, with four cases per conference. Registered participants in the conference include more than 45 international corporate and government agencies, greater than 675 individual users, and nine US veterinary schools. Four hours of continuing education credit is offered per conference under the AAVSB of RACE approval program. 3) The Department of Veterinary Pathology provides the Wednesday Slide Conference on the World-Wide-Web to veterinary pathologists worldwide in an effort to enable everyone to participate in this valuable forum. The Wednesday Slide Conference is in its 51st year, has 135 international participants and is considered an integral part of many international veterinary training programs. This yearly conference is held on each Wednesday for 25 weeks from September through May for residents and board-certified veterinary pathologists. All three of the resources are available on the World Wide Web and are a valuable resource to veterinary pathology training programs.

INTERACTION OF UV LIGHT WITH HUMAN PAPILLOMAVIRUS (HPV) 20 AND HPV27 E6/E7 PROTEINS IN TRANSGENIC MICE

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Introduction: Papillomavirus infections and UV light have been associated with the development of non-melanoma skin cancer. The mechanism through which the cutaneous papillomaviruses and UV irradiation interact with cellular factors to induce malignant tumours is unclear. We generated a transgenic mouse model to analyse the role of E6 and E7 proteins of cutaneous Human Papillomavirus (HPV) 20 and HPV27 under the influence of UV irradiation. HPV20 has been detected in malignant cutaneous tumours, whereas HPV27 is most often present in benign warts.
Materials and Methods: Two lines of hairless SKH-hr 1 mice were generated expressing the E6 and E7 proteins of HPV20 and HPV27 as transgenes under transcriptional control of the keratin 10 promotor. The mice were exposed to chronic UV-B irradiation (3 times per week for 15 weeks) starting at 8 weeks of age. Skin samples were taken at weeks 7, 11, 15 during UV-treatment and every 5 weeks during a following observation period of 20 weeks.
Samples were analysed by haematoxylin and eosin staining and by immunohistochemistry for various proteins.
Results: The irradiated transgenic mice developed papillomas and squamous cell carcinomas earlier and with a higher incidence than the wildtype control group. The proliferative potential of the skin during and after UV-treatment was increased measured by BrdU-incorporation and keratin 6 expressions. The expression levels of involucrin and loricrin, the major proteins of the cornified cellular envelope, were increased and stayed up-regulated after finishing UV-irradiation in the HPV20 transgenic mice. The p53 expression after UV-treatment was reduced in both groups of transgenic mice. The p53 family member, p63, seemed to be abrogated in irradiated and non-irradiated HPV20 transgenic mice.
Conclusion: Comparing the results obtained from the HPV20 versus HPV27 transgenic mice and the non-transgenic control group, regular skin differentiation seemed to be influenced in combination with UV-irradiation in the HPV20 transgenic group.
These models seem to be appropriate for further *in vivo* and *in vitro* studies for elucidating the responsible cellular mechanisms.

RESEARCH THE T LYMPHOCYTES SUBSETS IN THE PERIPHERAL BLOOD, PORTAL VEIN BLOOD AND HEPATIC VEIN BLOOD IN THE DOGS – PRELIMINARY REPORT

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Introduction: The liver is the largest solid organ in the animal body, receiving large amounts of circulating blood through its dual blood supply: the hepatic artery and the portal vein. Since little is known about lymphocyte subpopulation in the normal canine liver compared with those of blood. Most of the large numbers of lymphocytes present in the liver are T cells, expressing the αβ T cell antigen receptor (TCR αβ).

Material and Methods: Ten dogs (from 5 months to 15 years old; 6 males and 4 females). The stained samples of blood were run on the flow cytometer and analyzed (BD FACStark Flow Cytometer). Whole blood was added to the reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to leucocyte surface antigens. Mark antibody CD21+, CD8+, CD4+ and CD8+:CD4+ in peripheral blood, portal vein blood and hepatic vein blood.

Results: In peripheral blood CD21+ was 6.17±7.05, CD8+ – 22.20±35.3, CD4+ – 48.4±39.7, CD8+:CD4+ – 24.13±13.5; portal vein blood CD21+ was 11.0±8.4, CD8+ – 17.04±34.12, CD4+ – 44.15±37.25, CD8+:CD4+ – 24.32±16.8; hepatic vein blood CD21+ was 6.07±8.25, CD8+ – 15.26±34.40, CD4+ – 44.66±35.20, CD8+:CD4+ – 30.7±23.86.

Conclusion: Differences of quantity among T cells in the portal vein blood and hepatic vein blood says that cells are stopped in the liver. Further studies are required to better understand the possible correlation between peripheral and liver-resident lymphocytes.

INFECTION OF CATTLE WITH *NEOSPORA CANINUM* IN EARLY AND LATE GESTATION – DIFFERENCES IN PARASITE DISTRIBUTION AND LESIONS IN THE FOETUS AND PLACENTA

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Introduction: *Neospora caninum* is an apicomplexan parasite, which is an important cause of bovine abortion throughout the world. It is not known why *N. caninum* infected cattle abort, but it has been hypothesised that the parasite could be causing death by multiplying uncontrollably in an immunologically immature foetus. This hypothesis is supported by the observation that infection early in gestation leads to foetal death whereas infection later in gestation results in the birth of live, although congenitally infected calves. This report describes the lesions and distribution of parasites in the placenta and foetus of pregnant cattle experimentally infected with *N. caninum*.

Materials and Methods: Six cows were experimentally challenged with *N. caninum* at 70 days gestation, a time point known to induce foetal death (group I) and six cows challenged at 210 days gestation, a time point known not to affect the pregnancy, but leading to persistent infection of the foetus (group II). Cows were euthanized three weeks after challenge and samples of endometrium and placenta, and all principle foetal tissues were examined for the presence of parasites and associated pathological changes by histology, including immunohistology, and electron microscopy.

Results: In group I, multifocal epithelial necrosis with the presence of multiple intra- and extra-cellular *N. caninum* tachyzoites was observed in the placentomes, without any evidence of an inflammatory reaction. In the foetus, tachyzoites were found in most organs, but were most frequent within the liver, where they were found intra- and extra-cellularly, often associated with necrosis. In group II, findings were minimal. Sparse clusters of tachyzoites, with an associated inflammatory reaction were detected, mainly restricted to the CNS.

Conclusion: Our preliminary findings suggest that the parasite persists for longer as an actively multiplying form in the foetus and the placenta in early pregnancy. At the time of foetal death, there were numerous tachyzoites and associated necrosis in the placenta and foetal organs. In the older foetus, three weeks after infection, fewer tachyzoites were seen. These observations suggest that the parasite is able to multiply uncontrollably in an immunologically immature foetus, and, by causing necrosis of tissues, could be contributing to death of the foetus.

COMPARISON OF CONFIRMATORY AND RAPID TESTS FOR SCRAPIE DIAGNOSIS

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Introduction: The ability of different rapid tests for detecting PrP^{sc} in CNS and LRS tissues in sheep, even before the onset of clinical symptoms in some scrapie cases, has been previously assessed; however, its comparison with confirmatory tests are scarce in the literature. The main objective of this study was to compare the sensitivity for PrP^{sc} detection of two currently applied rapid tests (Western blot (WB) and Check-LIA, Prionics®) using CNS as well as LRS samples corresponding to clinical and preclinical field cases from naturally infected animals.
Materials and Methods: Thirty four scrapie positive sheep of Rasa Aragonesa breed and ARQ/ARQ genotype were included in this study. Twenty seven animals presented clinical signs of the disease. The following tissues were collected: the whole brain, tonsils and the retropharyngeal lymph node (RPLN). The half portion of each of them were immediately fixed (in formalin 10%) for histopathological processing and the remaining sample stored at -70°C for rapid tests developing.
Results: All samples were analysed by three tests: IHC, WB and LIA. All three could detect all positive cases even when PrP^{sc} accumulation in CNS (preclinical animals) was not observed, although IHC showed a higher sensitivity. PrP^{sc} presence in the CNS was confirmed by IHC in all 34 sheep studied except in three preclinical animals which were considered scrapie positive cases by LRS IHC. Meanwhile, in two sheep at the terminal stage LRS IHC showed negative results but PrP^{sc} accumulation was evidenced by the analysis of the brain.
Conclusion: Therefore, the most remarkable conclusion that can be drawn from all these results presented is that all animals involved in the study could be diagnosed, regardless the test applied (rapid as well as confirmatory assays) but only in case that CNS and LRS (tonsil/RPLN) analysis were considered.

GASTROINTESTINAL ROUND CELL TUMOURS IN CATS AND DOGS: A RETROSPECTIVE AND COMPARATIVE ANALYSIS

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Introduction: Alimentary lymphoma (AL) in cats is the most common type of gastrointestinal (GI) neoplasm and also the most frequent form of lymphoma. On the contrary, in dogs primary GI tumours are mostly epithelial in origin, and AL accounts for approximately only 5-7% of all canine lymphomas. Other primary GI round cell tumours are rare in both species, almost exclusively represented by plasma cell and mast cell tumours (in dogs and cats, respectively). The purpose of this study was to classify round cell tumours of the GI tract in dogs and cats, to determine their immunophenotype and to compare the results between the two species.
Materials and Methods: Haematoxylin and eosin stained sections from formalin fixed paraffin embedded samples of GI tumours archived in our Department from 12 year period (1992-2004) have been reviewed, and round cell tumours extrapolated. In the selected 82 cases (68 cats, 18 dogs), CD3 and CD79 immunohistochemistry was performed, and these were classified histologically according to the recent WHO classification system for the hematopoietic tumours.
Results: In our series of 109 feline GI neoplasms, round cell tumours accounted for 62.3% (30.4% epithelial, 7.3% mesenchymal tumours), and mainly consisted of AL (65/68, 96%) located in the intestine in 59 out of 65 cases. The only other primary non-lymphoid neoplasms were two malignant histiocytomas and one mast cell tumour. In the 96 canine GI neoplasms, round cell tumours accounted for 19% (59.3% epithelial, 21.9% mesenchymal tumours), all represented by AL (17 out of 18 cases located in the intestine).
Most of feline GI lymphomas showed a CD3+ T cell phenotype, while a clear prevalence was not observed in dogs. In both dogs and cats, T cell lymphomas were mainly represented by intestinal T cell lymphoma, although some cases of peripheral T cell lymphoma and angiotropic lymphoma were also present. As regard the B cell lymphomas, B large cell lymphomas (diffuse large cell lymphomas and large cell immunoblastic lymphoma) were the most frequent in dogs and cats, followed by follicular (centre cell and MALT lymphoma).
Conclusion: Our results showed in the feline GI neoplasms the uppermost prevalence of round cell tumours, whereas in the dog epithelial tumours were most common. Within round cell tumours, almost all were GI lymphomas in both species. The absence of prevalence of T out of B cell lymphomas in the dog and the marked prevalence of T cell lymphomas in the cat observed in our study are not aligned with the most recent literature data. These preliminary results lay the basis to further studies including a wider number of cases.

PATHOLOGICAL AND PATOHISTOLOGICAL FINDINGS IN PIGS AFFECTED WITH PORCINE PLEUROPNEUMONIA

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Introduction: Porcine pleuropneumonia is infectious disease of fattening pigs occurring worldwide, caused by *Actinobacillus pleuropneumoniae* (APP). Once established in a herd, animals can become chronically infected, sometime with signs of acute or even fatal pleuropneumonia, usually manifested after changes in ambient conditions. Clinical and pathological symptoms vary, depending of course of disease, imunological status of animals and secondary infections.
Materials and Methods: Materials for investigation were 70 affected lungs of dead animals and carcasses (collected at farms and slaughterhouses), with origin of 4 pig farms in Republic of Macedonia. In investigation were used classical diagnostic methods, such as necropsy and histopathology, followed by isolation of etiological agent at two different media (chocolate agar with added PolyVitex and blood agar with inoculated *Staphylococcus*).
Results: The presence of APP was detected only in one of 4 investigated farms. Pathological changes in dead animals were observed unilateral (in 4 animals) and bilateral (also in 4 animals), located in all lung's lobes. In slaughtered animals, the changes were observed mostly in diaphragmatic lobes. The most frequent pathological findings were necrotic-haemoragical changes of lung tissue and fibrous pleuritis with adhesion on both pleuras. Histological findings observed, were infiltration by lymphohistiocite cells located interalveolary and perybronchialy, thickened interalveolar septa and strong, diffuse interalveolar and interlobular septal oedema. Following this, were noted perivascular stenoses in blood vessels. In microbiological investigation, twenty five isolates were obtained on both used media.
Discussion: APP was detected not just in fattening, but also in weaned category of pigs and the acute form of disease was most frequent in fattening pigs with 40-50 kg body weight. Pathological findings were manifested unilateral and bilateral in all lobes, with severe pathological lesions. In some cases in development of this lesions, beside *A. pleuropneumoniae*, influence have and other agents (bacterial or viral).

GIANT CELL-RICH OSTEOSARCOMA IN THE CALVARIUM OF A CAT: STUDIES ABOUT MULTINUCLEATED GIANT CELLS ORIGIN

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Introduction: A 13 years old spayed domestic cat was presented for wobbling in four limbs, generalized ataxia and partial visual deficit. Physical examination revealed diffuse and severe muscular atrophy, poor groomed haircoat and with the neurological examination the lesion was localized in the left fore brain. An inflammatory, neoplastic or vascular problem was suspected. Computerized Tomography demonstrated a 1.5 cm diameter mass located around the left temporal calvarium. Histologically the neoformation was classified as osteosarcoma, giant cell variant.
The aim of this study was to determine the multinucleated giant cells origin.
Materials and Methods: Formalin fixed, paraffin embedded tissue from the neoformation was submitted to our laboratory, classified and graded according to WHO diagnostic criteria. Other staining methods employed were Masson trichromic stain and Tartrase Resistant Acid Phosphatase (TRAP) stain. Immunohistochemical staining was performed using rabbit anti-bovine S-100, mouse anti-swine vimentin, mouse anti-human cytokeratin, and anti-mouse MHC II (Class II Major Histocompatibility Complex). Electron microscopy was performed for multinucleated giant cells.
Results: TRAP staining exhibited diffuse and strongly cytoplasmatic stain uptake in multinucleated giant cells. No cellular staining was observed in polygonal cells in stroma. Immunohistochemical staining for vimentin exhibited diffuse and moderately cytoplasmatic stain uptake in giant cells and faintly in polygonal cells; no observable cellular staining against cytokeratin and S-100. Giant cells failed to stain with MHC II; instead many polygonal cells dipped in stromal tissue appeared moderately MHC II-positive. Ultrastructurally all giant cells contained moderate short profiles of rough endoplasmatic reticulum (RER) and no lysosomes were observed.
Conclusion: Multinucleated giant cells showed some osteoclasts-like features, as the TRAP-positive stain, the IHC pattern (vimentin-positive and S-100 and cytokeratin – negative stain) and the osteolytic behavior, but also some features typical of osteoblastic cells as the negativity to MHC II reaction and EM results. Therefore, as in literature, there is no evidence of a clear and univocal clarification for the origin of these giant cells; in our opinion the best explanation to these two different ways could stay in the poor differentiation of multinucleated giant cells.

ANTI-HUMAN SERUM AMYLOID P COMPONENT (SAP) ANTIBODY FOR THE IMMUNOHISTOCHEMICAL DIAGNOSIS OF AMYLOIDOSIS IN RUMINANTS

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Introduction: Amyloidosis represents a heterogenous group of diseases that have in common the deposition of fibrils composed of proteins of β-pleated sheet structure. Amyloid consists primarily of the amyloid fibrils, of which more than 20 types have been identified in man, but also of the amyloid P component (AP). This component, identical to the serum counterpart (SAP), is found in all types of human amyloid, and may constitute up to 15% of the deposits. In this study we compared the amyloid-specific Congo red stain with an immunohistochemical protocol using an anti-human SAP antibody, for the diagnosis of amyloidosis in formalin fixed animal tissue samples.

Materials and Methods: Formalin fixed tissue samples with amyloid deposits, kept as paraffin wax blocks, were retrieved from the archives of the Department of Veterinary Pathobiology, Laboratory of Pathology. Four serial sections were cut from each block, and the sections stained with haematoxylin and eosin and Congo red according to standard procedures, and stained immunohistochemically with and an anti-human SAP antibody and normal antibodies (controls). Tissues from 14 different animals with amyloidosis and from four cows without amyloidosis (controls) were investigated. The amyloidosis-affected animals comprised seven cows, one yak (*Bos grunniens*) and one sheep with AA-amyloidosis; one dog with an IAPP-amyloid producing pancreatic endocrine tumour; two cats with AIAPP-amyloidosis of the islets of Langerhans; one cat with AA-amyloidosis; and one cat with an amyloid-producing odontogenic tumour.

R**esults:** Intense immunostaining of amyloid was seen in each of the nine ruminants, using a protocol without any antigen retrieval. The method seemed more sensitive in these animals than the Congo red stain, but was unable to detect amyloid in the dog and the four cats regardless of the use of various antigen retrieval methods.

Conclusion: The use of the immunohistochemical protocol applying antibodies raised against human SAP, and without antigen retrieval, seems to be a promising method for diagnosing amyloidosis in ruminants. The method seems more sensitive than the histochemical Congo red method. However this stain specifically identifies the β-pleated sheet structure of the deposits, and thus amyloid and amyloidosis, and immunohistochemistry should always be co-evaluated with histochemistry.

NEURAL DISORDERS IN PIGS ASSOCIATED TO LEAD INTOXICATION

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Introduction: Lead is considered a usual cause of intoxication in cattle, being related to pastures in areas where wastes of motor oil, batteries or paint containers are found. However, lead poisoning in pigs is extremely rare due to the natural resistance to the metal attributed to these animals.
Materials and Methods: Hind limbs paralysis and poliuria was observed among the boars and sows of a semi-extensive operation of Iberian breed pigs in south-eastern Spain. The duration of the process was 2 months, affecting 100% of adult animals. Necropsy findings of 3 animals (1 boar and 2 sows) were unspecific.
Results: The histopathological study showed the presence of an intense tubulonephrosis with occasional acidophilic intranuclear and intracytoplasmic inclusion bodies in the tubular epithelial cells. Similar inclusion bodies were found in a high number of hepatocytes. Wallerian's degeneration of axons was observed in the sciatic and femoral nerves, as well as neuron degeneration in the grey matter of the spinal cord. The renal and nervous lesions were considered indicative of lead intoxication. This extent was confirmed by ultrastructural examination, which revealed the existence of the characteristic inclusion bodies corresponding to lead-protein complexes in the nuclei and cytoplasm of hepatocytes and tubular epithelial cells. Additionally, the toxicological analysis by atomic absorption spectrometry of renal and hepatic samples showed high levels of lead in both organs.
Conclusion: Despite natural resistance of pig to lead poisoning it may occur with a different form that ruminants and horses.
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IN VITRO ANGUILLA ANGUILLA L. GILL AND LIVER MICROSOMAL EROD ACTIVITY UNDER DIFFERENT THIOL CONCENTRATIONS

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Introduction: Thiol compounds such as L-cysteine (Cys), N-acetyl L-cysteine (NAC) and reduced glutathione (GSH) have shown the ability to minimize the *in vitro* inhibitory action of some heavy metals (Hg^{2+}, Cu^{2+}, Zn^{2+}) on liver microsomal EROD activity. However, little is known concerning the *in vitro* effects of high concentrations of these thiols on EROD activity.

Materials and Methods: One 500 g adult European eel (*Anguilla anguilla* L.) caught at the Aveiro Lagoon was acclimated to laboratory conditions for one week prior to experimentation in a 70 l aquarium under the conditions: 20°C temperature under a natural photoperiod, in aerated (dissolved oxygen 7.6±0.3 mg/l), filtered, dechlorinated and recirculating, tap water, with pH 7.2±0.4. The eel was exposed (4 mg/kg *i.p.* injection) to β-naphthoflavone (BNF) during 24 hours in order to induce a high EROD activity. Liver microsomes were exposed in a cuvette to the concentration range 0.5-10 mM GSH, Cys and NAC, whereas gill microsomes were exposed to 0.5-3 mM thiol.

Results: Liver microsomes exposure to 0.5 mM GSH induced a 10% increase in EROD activity when compared to control values. However, liver microsomal exposure to 2, 3, 4, 5, 6 and 10 mM GSH caused a 23, 36, 52, 58, 85 and 100% inhibition, respectively. Cys 0.5 mM induced a 24% liver microsomal EROD activity increase whereas an exposure to higher Cys concentrations caused unstable oscillatory enzyme activities with a significant decrease at 2, 4 and 6 mM Cys exposure. A 16% liver microsomal EROD activity increase was observed after exposure to 0.5 mM NAC. However, NAC induced a significant liver microsomal EROD activity decrease 19, 22, 34, 49, 72, 84 and 100% after exposure to respectively 1, 2, 3, 4, 5, 6 and 10 mM. Gill microsomal EROD activity has shown to be very sensitive to the thiolic compounds tested as it was significantly inhibited after exposure to GSH, Cys and NAC 0.5 mM.

Conclusion: This study demonstrated that increasing thiol concentration may have consequences on EROD activity. Moreover, for the same thiol concentrations, microsomal EROD activity was found to be more sensitive in gill than in liver.

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VIMENTIN – THE MARKER IN DOGS KIDNEY INJURY

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Introduction: The glomerular injury reciprocally may reflect in various tubular pathological changes with decreased blood supply. Vimentin expression was very strong in glomerular structures and endothelium and smooth muscles of the blood vessels wall in the healthy kidney. The intensity of this expression is also considered as the marker of the health status and functional ability of the kidney.
The aim of this work was to perform the histopathological evaluation of dogs kidneys, with special attention to vimentin immunocytochemical expression.
Materials and Methods: The kidneys obtained from 50 dogs with nephropathy were examined in this study. All specimens were fixed in 10% neutral buffered formalin and paraffin embedded. The kidney sections were stained haematoxylin and eosin, vimentin expression was visualized by using monoclonal antibodies clone V9 (Novocastra).
Results: It is important to notice that the decrease in vimentin expression were seen in the blood vessels cells earlier then development of the remarkable histopathological changes were visible. The vimentin expression appeared in altered tubular cells, but was decreased in glomerular structures, proportionally to their signs of the injury.
Conclusion: The changes in kidneys vimentin expression is a very good marker of its early injury and is characterized by the decrease seen in glomerules cells and blood vessels, but is appearing in tubules epithelium cells.

SALT POISONING IN SHEEP

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Introduction: Salt poisoning is a common central nervous system disease in livestock. Animals are tolerant of high dietary salt levels if they have concomitant access to fresh water.
Material and Methods: Eight out of 70 sheep aged between 3 and 8 months had been affected. The cases were studied by histopathological examination.
Results: Six of ill sheep were dead. In two referred male cases to clinic, the clinical signs were obvious: Tem, HR and RR were normal, aimless wandering, central blindness, ataxia and circling toward left and falling, tremor and convulsion were observed. Both of them were treated with thiamin, 10 mg/kg b.w. every 3 hours intravenously for 5 times, but there was not any recovery sign. Necropsy findings were: eosinophilic meningoencephalitis, eosinophilic perivascular cuffing (PVC), laminar cerebrocortical necrosis (CCN), disseminated intravascular coagulation (DIC) and cerebral oedema, with softening and flattening of the gyri accompanied by acute gastroenteritis. In history these sheep received salt for 4% on diet ratio although they have restricted situation on water consumption.
Discussion: Perivascular infiltration of eosinophils and eosinophilic meningoencephalitis are not a constant finding in histopathological examination in ruminants because most affected ruminants indicate perivascular mononuclear cells in sodium chloride toxicity.

MHC CLASS II EXPRESSION IN EQUINE SARCOID

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Introduction: Sarcoid is probably the most common cutaneuos tumour of the horse. No predilected age, sex or breed has been observed, even if Arabs and thoroughbred within seven years of age seem to be more affected. It has been hypothesized that sarcoid can be caused by bovine papillomavirus. While the mode of transmission of infection has not been elucidated, viral gene expression, in particular of E5, may contribute to virus persistence and disease pathogenesis by down-regulating MHC class I expression (Chambers et al. 2003). Aim of the present study is to investigate the MHC class II expression in order to detect the immunogenic response induced by equine sarcoid.
Materials and Methods: Sixteen equine sarcoids have been surgical removed, on large basis of excision, including portion of normal skin from 16 subjects within seven years of age with no sex or breed predilection. Formalin fixed paraffin embedded sections were stained for histological routine methods and for immunohistochemistry MHC class II (HLA-DR Clone TAL 1B5, 1:25, Dako) using Dako LSAB2 HRP kit – DAB (Dako) system.
Results: At histological examination epidermal hyperplasia and hyperkeratosis was observed in all specimens as well as rete peg formation. In the dermis whorls of spindle-shaped or fusiform proliferating fibroblast were recorded. Fibroblast close to dermal-epidermal junction showed a perpendicular orientation. Minimal lymphocytic infiltrations in tumour were detected. Immuhistochemistry investigation showed many cells, MHC class II immunopositive, with dendritic shape in sarcoid tissues but not in skin outside neoplasia.
Conclusions: In equine sarcoid MHC class I expression down-regulation has been demonstrated (Chambers et al. 2003). In our investigation MHC class II is expressed, in all sarcoid examined, in relevant number of cells with dendritic shape in sarcoidal tissue but not outside the neoplasia. The minimal presence of lymphocytic infiltrates associated with MHC class II immunopositive cells could support an immunogenic theory for equine sarcoid. Works are in progress for immunophenotyping the lymphocytic sub-population of these infiltrates.

EXPRESSION OF MATRIX METALLOPROTEINASES AND THEIR INHIBITORS IN THE BRAINS OF AGED DOGS AND HUMANS – A COMPARATIVE STUDY

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Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder being the major form of dementia in the human elderly. Microscopically, AD is characterized by β-amyloid deposits in brain parenchyma (plaques) and often in brain vessels (congophilic angiopathy) as well as intracellular hyperphosphorylated τ-proteins forming neurofibrillary tangles. Plaques in dog brains show morphological similarities to diffuse plaques of Alzheimer patients and, therefore, might represent an early stage of plaque evolution. Matrix metalloproteinases (MMPs) are zinc-dependent enzymes that are able to cleave molecules of the extracellular matrix. Lately, some MMPs and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs), have been reported to be up-regulated in the brains of Alzheimer patients. In order to study the role of MMPs and TIMPs in plaque evolution and pathogenesis both human and canine brains have been investigated comparatively.

Materials and Methods: Paraffin sections of formalin fixed brain tissue of five young dogs (2-3 years), five old dogs without plaques (9-14 years) and 10 old dogs with plaques (10-18 years) as well as five brains from Alzheimer patients have been investigated. β-amyloid deposits were detected by immunohistology and silver staining using the Campbell-Switzer method. For immunohistological investigations both monoclonal antibodies (directed against β-amyloid, MMP-2 and -11, TIMP-3 and synaptophysin) and polyclonal antibodies (directed against GFAP, MMP-1, -3, -7, -9, -12, -13, -14 and TIMP-1 and -2) were used employing the ABC method. To confirm a possible association of MMP- and TIMP-signals with plaques, double-labelling was performed.

Results: MMP-3, MMP-9 and MMP-13 showed plaque-associated staining in the brains of Alzheimer patients. Immunoreactivity was present in activated microglia (MMP-13), neurons and predominantly in diffuse plaques (MMP-3, -9 and -13). In the brains of old dogs with plaques the expression of MMP-3, MMP-9 and MMP-13 showed no plaque association. However, age-dependent differences in the expression patterns of several MMPs and TIMPs in the brains of young dogs and old dogs with and without plaques were detected.

Conclusion: Expression of certain MMPs and TIMPs is associated with plaques in the brains of Alzheimer patients, whereas there was no plaque-associated up-regulation of these MMPs and TIMPs in the brains of old dogs, indicating a species-specific plaque pathogenesis.

MOLECULAR MARKERS OF THERAPEUTIC EFFICACY IN CANINE CUTANEOUS T CELL LYMPHOMA (CTCL) AND ANAPLASTIC LARGE CELL LYMPHOMA (ALCL)

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Introduction: The objective of this study is to review and correlate results of clinical, histological and immunohistochemical features of canine CTCL and ALCL, according to the World Health Organization (WHO) Classification of 2002. The intended goal is to define subsequently the criteria for the diagnosis of these distinct clinicopathologic entities, and to determine the prevalence of molecular markers of therapeutic efficacy.
Material and Methods: Skin samples of seven cases of canine CTCL and ALCL have been reviewed by histological preparations utilizing haematoxylin and eosin, immunohistochemistry for CD3, CD79α, CD18 and retinoid acid receptors for RAR and RXR isomers. Results of these examinations were correlated with available clinical data.
Results: The diagnoses included: CTCL 4 and ALCL 3. All cases were CD3+ CD79α-, CD18-, all were RXRα+, 6 were RARα+, 6 RXRγ+, 5 RARβ+ and 4 RXRβ+. The presence of RARγ was not detected in any of the cases. T cell lineage was CD3 positive, whereas negativity for CD79α and CD18 excluded B cell lineage and histiocytic origin of the neoplastic cells.
Conclusions: Differentiation of the various types of canine CTCL and ALCL requires careful histological and cytological review, immunohistochemical results, as well as correlation with clinical data. The presence of retinoid receptors suggests that, as in humans, the use of specific ligands may be effective in the treatment of canine CTCL and ALCL. Further updating of the WHO System is required to better define the characteristic features of canine CTCL and ALCL to enable more accurate diagnosis and prognosis and thus effective treatment.

INTRACRANIAL TUBERCULOSIS IN A WILD SPANISH DEER (*CERVUS ELAPHUS*)

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Introduction: The presence of inflammatory lesions in cranial bones in deer has been reported rarely. The present study describes a case of intracranial tuberculosis in a wild Spanish deer *(Cervus elaphus)* from a Natural Park in the south of Spain (Hornachuelos, Córdoba, Spain).
Materials and Methods: A 3 years old deer with clear signs of blindness, weakness and poor body conditions was slaughtered and sent for necropsy. Samples were processed routinely for histopathological examination (haematoxylin and eosin, Gram and Ziehl-Neelsen staining).
Results: Gross findings revealed the presence of large retropharyngeal, mediastinic and mandibular lymph nodes and clearly thickening of the cribriform plate of ethmoid bone that comprised the rostral portion of brain and the optic chiasma. Similar lesions were observed in lung and liver. All the lesions showed a necrotic material like a yellowish cheesy mass. Microscopical study showed the characteristic tuberculous granuloma with the presence of a cellular infiltrate with numerous macrophages, epithelioid and giant cells bordering central caseation necrosis and calcification, surrounded by a connective tissue band and neutrophils. Perivascular cells infiltrate and gliosis were observed at central nervous system. The presence of Gram-positive and acid-fast stained bacilli contained within epithelioid and giant cells was used to diagnose the case as tuberculosis.
Conclusion: The bone destruction and regenerative osteophyte formation observed in the intracranial tuberculous granuloma, corresponding with tuberculosis in the skeleton, could be the origin of the blindness. This type of tuberculosis is most frequent in spongy and highly vascular bones and rarely has been describe in the cranium flat bones of ruminants, particularly deers. These lesions in bones occur mainly in young animals and are associated with a haematogenous spread of mycobacteria of immunologically compromised hosts. For differential diagnosis, these lesions could not be confused with the lesions appeared in actinomycosis, which are the most common cause of brain abscesses in adult male deers.

A STUDY CONCERNING THE VALUE OF GAMMA-GLUTAMYLE TRANSFERASE IN DIAGNOSIS OF EXPERIMENTALLY INDUCED ACUTE RENAL FAILURE

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Introduction: Acute renal failure is a reversible syndrome, if the disease is diagnosed in the early stages and also managed properly.
Materials and Methods: We experimentally induced acute renal failure in six dogs, which had normal renal function by administering gentamycin (8 mg/kg/q 12 h, *i.m*., administered daily). Then blood and urine samples were collected daily for 16 day and BUN, serum creatinine, urine creatinine and urinary γ-glutamyletransferase were measured. Necropsy was done after death of dogs and apiece of kidney kept in formalin buffers solution 10% and referred to pathology laboratory.
Results: Measurment of urinary γ-glutamyletransferase was a more sensitive and reliable method of assessing acute tubular damage induced by gentamycin, than determination of serum BUN and creatinine. In histopathological study with haematoxylin and eosin staining, acute renal failure is diagnosed.
Conclusion: The early stages of acute renal failure can be recognized by the use of these tests and by applying proper treatments, irreversible morphologic damages may be prevented.

CELL TYPE IDENTIFICATION AND CORRESPONDING NOMENCLATURE IN THE ROUTINARY DIAGNOSIS OF MIXED CANINE MAMMARY TUMOURS; A COLLABORATIVE STUDY AMONG VETERINARY PATHOLOGISTS FROM SEVERAL INSTITUTIONS; EVALUATION OF THE APPLICABILITY OF THE 1999 WHO'S CLASSIFICATION

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Introduction: During the last 40 years, several histological classifications of canine mammary tumours (CMT) have been proposed but none has been unanimously accepted. Nowadays, the most widely used, authoritative resources for the classification of tumours are the World Health Organization (WHO) classifications. The latest WHO International Classification of Mammary Tumours of the dog and the cat (Misdorp et al. 1999) combines histogenetic and descriptive morphologic criteria, incorporating histological prognostic features that have been associated with increasing malignancy. However, doubts have arisen concerning its capability to identify some of the CMT with more than one tissue component (epithelial, myoepithelial, mesenchymal). The purpose of this study was twofold: first, to compare the name routinarily given to histological subtypes of mixed CMT by Spanish and Portuguese veterinary pathologists; and second, to evaluate the level of applicability, acceptance and usefulness of the WHO International Classification of Mammary Tumours of the dog (Misdorp et al. 1999).
Materials and Methods: Eighteen cases of routinely diagnosed CMT with epithelial/myoepithelial and mesenchymal components were selected from the archives of the Veterinary Pathology Laboratories of the Veterinary Schools of Madrid and Cordoba. After a call for participation, haematoxylin and eosin stained tissue sections, together with a table for evaluation, were mailed to 12 Spanish (8 public Faculties of Veterinary Medicine, 1 Animal Health Institute, and 2 private Veterinary Pathology laboratories), and 3 Portuguese (2 public Faculties of Veterinary Medicine, and 1 Research Institute) Institutions. In addition, 5 veterinary pathologists voluntarily participated in the study as experts. The individual diagnoses of the two authors were also included. Cell-type specific and proliferation tumour markers were used to analyze further the results obtained in haematoxylin and eosin stained tissue sections concerning the identification of different tissue components and their benign or malignant nature, respectively.
Results: The degree of concordance among participants in the study concerning the type of neoplastic component present in a given tumour was low. The myoepithelial-cell type component was the most difficult to identify. Most tumours were given 3 or more different names. Those mixed tumours with the highest heterogenicity in nomenclature were those with both benign and malignant neoplastic components. Most of the participants did not find a place for such tumours in the latest WHO's Classification.

MONOCYTOSIS IS A HEMATOLOGICAL FINDING OF INITIAL BOVINE ENZOOTIC HEMATURIA

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Introduction: Bovine Enzootic Haematuria (BEH) is a syndrome characterized by persistent haematuria, anemia and haemorrhages due to the existence of urinary bladder tumours. The disease is naturally induced by the chronic ingestion of bracken fern (*Pteridium spp*.) after a period of two or three years. There are few studies concerning clinical pathology of cows with BEH and most of them are referred to experimental disease in cows. The objective of this work was to describe analytical changes in blood and urine, finding parameters with special diagnostic value.

Materials and Methods: This study was carried out in two livestock with known BEH: livestock A located in the south-west of Spain (Caceres, Extremadura) with grazing local breed cows, and livestock B located in the North of Madrid (Los Molinos) with Frisian cows grazing freely only during the day light. Physical exam, haematology, serum biochemistry and urinalysis were performed in 66 cows and 13 calves (livestock A) and 53 cows (livestock B). Statistical study was performed in the Statistical Centre of the Complutense University.

Results and Discussion: Multivariate statistical analyses established three classes or groups of cows with statistically significant similar analytical characteristics, regarding the studied variables and increasing severity of the disease. In class 1, the statistical procedure included animals that significantly had normal values in many variables studied. This group was characterized by a marked monocytosis ($p=0.004$). In class 2, animals with mild, moderate or severe alterations in numerous variables were grouped. These animals had moderate monocytosis ($p=0.012$). In class 3, animals with many altered determinations were selected. Normal blood values of monocytes ($p=0.006$) were found in this class. Monocytosis was also detected in 30.8% of the calves (1-1.5 years of age, livestock A). Most of the analysed parameters had never been studied in this disease previously. One of the most striking altered parameters was the level of monocytes that was significantly increased in animals with initial BEH and in calves grazing bracken fern without other clinical alterations. Monocytosis occurs with chronic inflammation but monocyte count is not highly responsive in large animals. Monocytosis could be attributed to an initial response to bracken fern consumption since marked monocytosis has been demonstrated in rats fed with ptaquiloside, a potent carcinogenetic component of bracken fern.

Conclusion: Monocytosis is an initial haematological marker of Bovine Enzootic Haematuria.

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TWO MAIN TYPES OF THYROID GLAND ALTERATIONS IN HORSES TRANSPORTED FROM EASTERN EUROPE TO SOUTH ITALY (BARI)

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Introduction: In this study, we considered 156 thyroid glands, isolated from slaughtered eastern european horses, with the aim to evaluate the effects of transportation mediated-stress on the hypothalamus-pituitary-adrenal-axis (HPAA). We focussed our attention on the thyroid gland as a putative target organ following hyperactivation of the HPAA.

Materials and Methods: Macroscopically, all glands were increased in both size and weight. Thereafter organs were immediately fixed in buffered formalin and stained with the following methods: haematoxylin and eosin (HE), periodic acid Schiff (PAS), Van Gieson.

Results: Here, we provide some examples of hystological findings on the thyroid glands observed. Actually, two types of alterations were evident among the animals studied:

1. a morphological aspect consistent with a condition of hyperactivation of the thyroid gland;
2. a morphological aspect consistent with an Hashimoto-like disease.

The salient features of the first histological aspect are the following:

a. hyperplasia of the parenchyma,
b. follicles of various size with irregular contours and partly atrophic,
c. vacuolization of colloid,
d. perivasal fibrosis which occupies the lumen, also involving follicles which appear deformed,
e. mononuclear cell infiltrates.

With special reference to the Hashimoto-like disease appearance, we observed follicles of various sizes with solid clusters of epitelioid cells in the absence of a follicular cavity (follicular adenomatosis). In some fields, there was a marked interfollicular and perilymphatic fibrosis in the presence of a mononuclear cell infiltrate.

Conclusion: The described different alterations of the thyroid gland, in animals apparently healthy, merit to be better evaluated in order to understand their pathogenesis and systemic effects.

DEPRESSION OF CELLULAR IMMUNITY IN TROTTERS: REDUCTION OF MIF ACTIVITY AFTER RACE

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Introduction: There is evidence that prolonged and/or intensive physical training can affect the immune response, in humans and animals. In particular, in athletes physical and emotional stress can activate the hypothalamus pituitary adrenal axis (HPAA) with release of corticosteroids that, in turn, suppress the immune response.
Materials and Methods: In nine trotters, before and after race, 40 ml of blood were collected from the jugular vein and heparinized. Lympho-monocytes (2 x 10^6 cells), isolated on a discontinuous gradient of ficoll-hypaque, were stimulated with 1 µg/ml of phytohaemoagglutinin (PHA) and incubated for 1 h at 37°C and 5% CO_2. Unstimulated and stimulated supernatants were added to autologous monocytes (10 x 10^6) in agarose wells and incubated overnight. Percentage of inhibition was expressed by comparing the migration area of monocytes in the experimental wells with that in the control wells. Migration areas were read by an inverted microscope.
Results: The present research shows that, after race, migration inhibiting factor (MIF) activity contained in stimulated supernatants was reduced in 7/9 animals.
In table percentages of migration inhibition are illustrated.

MIF	Before race	After race
1 – horse	14%	20%
2 – horse	18%	23%
3 – horse	60%	47%
4 – horse	36%	29%
5 – horse	55%	41%
6 – horse	35%	29%
7 – horse	54%	30%
8 – horse	43%	38%
9 – horse	46%	36%

Conclusion: The reduced MIF activity after race indicates that a transitory depression of the cellular immunity may lead to infectious processes in trotters mostly involving the respiratory system.

AN OUTBREAK OF PURULENT NASOMAXILLAR AND MANDIBULAR OSTEOMIELITIS IN A FLOCK SHEEP CAUSED BY *PSEUDOMONAS AERUGINOSA*

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Introduction: In this communication we describe an outbreak of severe necrotic-purulent nasomaxillar and mandibular osteomielitis in a flock sheep caused by *Pseudomonas aeruginosa*. This microorganism species are sporadic and oportunistic pathogens of animals being a very important ubiquitous organism of rapid growth with pH close to neutral and the organic matter in solution, at a temperature of more than 20ºC and a good supply of dissolved oxigen. In the same way the predisposing factors and infection origin were analysed.
Material and Methods: The affected farm is in south-west of Valencia and had a large flock of 450 crossbreed ewes that grazed in a typical mediterranean area. Between May 2003 and February 2004, in accordance with sheep farmer, close to 12% of the ewes, irrespective of age, developed a severe deformation of the mandibular and maxillar bones. The animals afected showed initially anorexia, depression, postration, fever, the next phase was characteristed by a swelling of the nasomaxillar and mandibular area and a purulent nasal discharge, which finally become caquectic with a poor body condition, hair loss and death in a period no longer than two months. The mortality rate was 80% of affected animals. Eight animals were necropsied and histopathological studies were carried out.
Results: Gross lesions were confined to cheek and deep zones affecting the nasomaxilar and mandibular bones. The initial lesion is a small ulcer of the gingiva which spreads rapidly and may involve deep tissues. It consist of a dirty necrotic purulent abscess surrounded by a zone of acute inflammation inside the alveolar teeth cavity leading to a deformed bone structure. The submandibular and retropharingeal lymph nodes were always enlarged, congestive with a severe oedema. Swabs of the affected tissue were collected from all animals and *Pseudomonas aeruginosa* was isolated in heavy, pure culture from all abscesses. A susceptibility antibiotic profile was carried out that was sensitive to ciprofloxacin, and gentamicin and resistant to clavulanic acid/amoxyciclin, cefoxitin, sulphametoxazol and trimethoprim, cloramphenicol and doxycicline.
Discussion: The epidemiological aspects and predisposing factors are being studied and may be a nonspecific and associated mucosal trauma and debility that facilitates the development of the infection and the disease. Finally, several autors have related that spirochetal organisms are frequently found deep in the lesions within the tissue section stained with Warthin-Starry silver stain. The etiologic role of these organisms in this condition will be discussed in the near future as at this moment as we are still waiting on decisive results.

DISTRIBUTION AND CHEMICAL CODING OF INTRAMURAL NEURONS IN THE PORCINE ILEUM DURING PROLIFERATIVE ENTEROPATHY (PPE)

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Introduction: Innervation of the mammalian gastrointestinal tract has been the subject of many studies, which revealed that mammalian viscera are unique with respect to the arrangement of their nervous supply. In contrast to many other organs, the stomach and gut are primarily innervated by numerous intrinsic (enteric) neurons found within intramural ganglia, as well as by extrinsic autonomic and afferent nerve cells. As enteric neurons are now widely accepted to be particularly highly adaptive in their response to various pathological processes including inflammation, it appears to be of interest to study the chemical coding of neurons in the intramural ganglia of the ileum wall undergoing the PPE evoked by *Lawsonia intracellularis*.

Materials and Methods: The study was performed on 6 juvenile pigs of the Large White Polish breed. The animals were housed and treated in accordance with the rules approved by the local Ethical Commission. The pigs were divided into the control (C, n=3) group and group consist of pigs with clinically diagnosed *Lawsonia intracellularis* infection (PPE, n=3). All the animals were sacrificed with an overdose of sodium pentobarbital (Vetbutal®, Biowet, Poland, 90 mg/kg b.w.) and perfused transcardially with 4% buffered paraformaldehyde (pH 7.4). The ileums were cut out its samples were post-fixed by immersion in the same fixative for several hours and finally they were stored in 18% sucrose until sectioning. The tissue were cut into 10 μm thick cryostat serial transversal sections and then processed for double-labelling immunofluorescence using combinations of antisera raised in different species and directed towards PGP 9.5 and VIP, SP, CGRP, SOM, NPY and GAL.

Results: In the pig, enteric neurons are found in ganglia located within three intramural plexuses: inner submucous (ISP), outer submucous (OSP) and myenteric (MP). Immunohistochemistry revealed up-regulation of neurons containing GAL, SOM and CGRP in the PPE group. GAL-positive neurons in the ISP, OSP, MP of the PPE group amounted to 67.2%, 35.6%, 28.07% and in the C group amounted to 23.5%, 22.1%, 18.6%, respectively. SOM- positive neurons in the ISP, OSP, MP of the PPE group amounted to 46.6%, 36.3%, 20.8% and in the C group amounted to 17.4%, 3.1%, 7.7%, respectively. CGRP-positive neurons in the ISP, OSP, MP of the PPE group amounted to 53.8%, 41.6%, 25.7% and in the C group amounted to 7.3%, 17.1%, 15.6%, respectively. It should be stressed that no NPY-positive neurons were observed in the intramural ganglia of both animal groups.

Conclusion: The present results show that peptides may have an important role in function of porcine enteric nerve pathways not only under physiological but also pathological condition, when the nervous system is "stressed", challenged or afflicted by disease (such as during PPE disease). However, the exact physiological relevance of adaptive changes observed remains to be elucidated in detail.

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THE DISTRIBUTION AND CHEMICAL CODING OF NEURONS IN PREVERTEBRAL GANGLIA SUPPLYING THE URINARY BLADDER TRIGONE IN THE PIG

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Introduction: The function of the urinary bladder is to store urine and, at appropriate intervals, to evacuate it. The innervation of urogenital organs has been found to originate from three sets of peripheral nerves: sacral parasymphatetic (pelvic nerves), thoracolumbar symphatetic (hypogastric nerves and prevertebral ganglia – PVG), and sacral somatic (primarily the pudendal nerves) (for references see Pidsudko 2004). Since our knowledge on the distribution and chemical coding of the urinary bladder trigone-projecting neurons (UBT-PN) in the pig is very limited, we have combined retrograde tracing and double-immunolabelling to elucidate the exact localisation and neurochemical features of UBT-PN neurons involved in this neural pathway.
Materials and Methods: The study was performed on 5 juvenile pigs of the Large White Polish breed. The animals were housed and treated in accordance with the rules approved by the local Ethical Commission. In the experimental animals, the fluorescent retrograde neuronal tracer fast blue (FB) was injected into both the left and right side of the urinary bladder trigone during laparatomy performed under pentobarbital anesthesia. After a survival period of three weeks the pigs were reanaesthetised and transcardially perfused with 4% buffered paraformaldehyde. The collected prevertebral ganglia (PVG) (the coeliac and superior mesenteric ganglion complex (C-SMG), as well as the ovarian (OG), aortico-renal (ARG) and adrenal (ADG) ganglia) were post-fixed by immersion in the same fixative for several hours and finally they were stored in 18% sucrose until sectioning. The left and right PVG were cut into 10 μm thick cryostat serial sections. All the sections containing retrogradely labelled neurons were processed for double-labelling immunofluorescence with antibodies listed in Table 1 (Pol J Vet Sci, 2004, 7(3) suppl: 97-99).
Results: The porcine PVG was found to contain many FB-positive (FB+) neurons projecting to the urinary bladder trigone which were distributed bilaterally, i.e. within both the left and right ganglia. The PVG complexes contained 1181±120 (mean±SE) of FB+ neurons. The majority (about 90% of all FB+ neurons) were localized mainly in OG. There were 921±98 in OG, 216±19 in ARG and 44±3 in ADG of FB+ perikarya considering both the left and right PVG. Immunohistochemistry revealed that the vast majority of the UBT-PN was TH/DBH-immunoreactive (IR) (88%). A prominent proportion of FB+/TH-IR neurons contained also immunoreactivity to NPY (18%) and smaller numbers were SOM- (5%) or GAL-IR (0.6%).
Conclusion: This study has revealed a relatively large population of differently coded PVG UBT-PN, which they are probably, involved in the complexity of the urinary bladder neural pathways.
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INFECTIOUS BURSAL DISEASE IN A FLOCK OF VILLAGE CHICKENS – CASE REPORT

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Introduction: First cases of Infectious Bursal Disease caused by very virulent IBDV (vvIBDV) were recorded in Serbia in 1992, and initiated the introduction of vaccines of intermediate pathogenicity in poultry flocks that provided efficient protection from the disease. However, village chickens were not vaccinated and remain as reservoir of infection ever since. Pathology caused by vvIBDV was most prominent in layer hens when disease occurs in fully susceptible 4-8 weeks old chickens. On the other hand, bleeding in the muscle was also observed in layer flocks that did not experience morbidity and the mortality that was on the technology acceptable level. Concerning broiler chickens in the beginning there was some confusion among veterinarians made by findings of haemorrhage in the proventriculus, or the only pathological changes were swollen kidneys and frequently deposits of the urate. Sometimes even these changes on kidney were absent so histopathology of bursa needed to be examined. In this paper, we show one such case from small village flock of 50 broiler chickens that was not vaccinated and experienced 10% mortality at 5 weeks of age.

Materials and Methods: The post-mortem examination was conducted on 3/5 broiler chickens 5 weeks of age with the history of sudden death, from a flock of 50 broiler chickens. After the necropsy, samples of bursa were collected for histopathology (haematoxylin and eosin stain) and bursal sections were scored for pathology lesions. Agar gel immunodiffusion test was run on small pieces of bursa tissue against positive anti-IBDV sera.

Results: Upon pathology examination, in all cases we could not find haemorrhages in thigh and pectoral muscles, kidneys appear normal. One bursa was slightly swollen but an incision revealed its normal appearance, without bleeding or fibrinous exudates. Second and third bursa was of quite normal appearance and of normal size without any visible changes. AGP test, run as described, was positive. On histopathology examination first and second bursa were almost entirely depleted from lymphocytes in cortex and medulla and proliferation of connective tissue was observed that is very typical for IBDV infection. Bursa score was 4 in two bursas and third bursa had no lesions.

Conclusion: In this particular case we found typical IBDV lesions in two bursa samples upon histopathology examination and in spite of the fact that gross pathology was poor.

HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL FINDINGS AND DOCUMENTATION OF TRANSMISSION IN RATS AND MICE EXPERIMENTALLY INFECTED WITH ENCEPHALOMYOCARDITIS VIRUS (EMCV)

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Introduction: The aim of this study was to document rat-to-rat and mouse-to-mouse transmission of EMCV, to evaluate the histopathological changes and study the distribution of EMCV antigen in several organs of experimentally infected rats and mice. An insight into the pathogenesis of EMCV infection in rodents and their epidemiologic role in the dissemination of the virus was attempted.

Materials and Methods: Ten Wistar rats (8 weeks old) and five BALB/c mice (8 weeks old) were inoculated with a Greek myocardial EMCV strain (oronasaly, $0.5 \times 10^{4.5}$ $TCID_{50}$ dose per animal). A Belgian myocardial EMCV strain was used in another ten rats and five mice (oronasaly, $0.5 \times 10^{4.5}$ $TCID_{50}$ dose per animal). Two days later each inoculated rat or mouse was coupled with a contact rat or mouse respectively. Two rats and two mice and their couples served as uninfected controls. Two mice died (6 and 32 days post inoculation (dpi) and days post couplet (dpc), respectively). All other animals were euthanized 11-62 dpi or dpc. After death or euthanasia, samples of the several organs were processed for virus isolation and histopathological examination. Viral antigen was demonstrated by the ABC method using the monoclonal antibody 4F3.

Results: All contact rats and mice were infected and the virus was isolated from their faeces and several organs. Rat-to-rat and mouse-to-mouse transmission was slow and the infected rats and mice excrete the virus in faeces for a long period. The main histopathological changes observed in both inoculated and contact infected animals were focal interstitial pancreatitis, degeneration and necrosis of pancreatic acinar cells, depletion of thymus and Payer's patches and interstitial pneumonia. Additionally, only in the two mice that died multifocal interstitial myocarditis was observed. In rats, EMCV antigen was detected in the cytoplasm of cardiac muscle cells, pancreatic acinar cells, epithelial cells of the liver and in macrophages of the spleen, lung and thymus. In mice, EMCV antigen was detected in the pancreatic acinar cells, in the macrophages of the lung and thymus and in the cytoplasm of cardiac muscle cells from three animals (two that died and one of their couples).

Discussion: Both EMCV strains appear to be cardiotropic in rats, yet they do not produce myocarditis in this species. On the contrary, in mice, they rarely affect the myocardium but when they do, they cause fatal myocarditis. Additionally both strains seem to be pancreotropic in rats and mice. The frequent presence of EMCV in macrophages suggests that macrophages play a role in viral replication and dissemination of the virus. Our results strongly support the role of rodents in the epidemiology of EMCV infections on pig farms either as reservoir hosts or as transmitters of the virus to the pigs.

GENE-EXPRESSION OF MATRIX-METALLOPROTEINASES AND THEIR INHIBITORS IN DH82 CELLS PERSISTENTLY INFECTED WITH CDV

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Introduction: Matrix-metalloproteinases (MMPs) are a family of zinc containing endopeptidases that have the potential to cleave protein and proteoglycan components of the extracellular matrix. The activity of MMPs is under the tight control of tissue inhibitors of matrix metalloproteinases (TIMPs). MMPs play an essential role in normal and pathophysiological processes like wound healing, metastasis of tumours, opening of the blood brain barrier and demyelination. Canine distemper virus (CDV) is a morbillivirus belonging to the *Paramyxoviridae* family with the potential to induce demyelinating leukoencephalitis (DL). Following CDV infection, there is an up-regulation of the major histocompatibility complex class II (MHC II) in DL brains. MHC II is present on various cell types including microglia/macrophages. The aim of this study was to investigate the impact of CDV infection on MMP and TIMP expression in macrophages using a canine macrophage tumour cell line.
Materials and Methods: DH82 cells are a canine macrophage-monocytic cell line isolated from a dog with malignant histiocytosis. Persistent CDV-infection was established with the Onderstepoort strain. Quantitative polymerase chain reactions (qPCRs) were created for MMP-2, MMP-9, MMP-13, MMP-14, TIMP-1, TIMP-2, GAPDH and CDV. For quantitation tenfold serial dilutions of agarose-gel purified PCR products ranging from 10^2 to 10^8 copies per sample were used to generate standard curves for estimation of copy numbers. Gene expressions were analysed using the Sybr Green I dye except for MMP-9 (Taq-Man Chemistry). In addition MMP-2 and MMP-9 zymography was performed to demonstrate their activity.
Results: All MMPs and TIMPs examined were produced in measurable amounts in both infected and non-infected DH82 cells. The highest degree of MMP and TIMP expression was observed in non-infected DH82 cells. In CDV infected DH82 cells MMP-9, MMP-13, MMP-14 and TIMP-2 decreased to 30% and 42% compared to non-infected DH82 cells. More surprisingly the amount of MMP-2 in CDV infected DH82 cells decreased to 6% compared to non-infected DH82 cells. The expression of TIMP-1 in CDV infected DH82 cells was 84% compared to non-infected DH82 cells. Zymographically, these results were confirmed as the activity of MMP-2 and MMP-9 was reduced in infected DH82 cells.
Conclusion: In summary, MMP expression was strikingly down-regulated in CDV infected tumour cells. It may be speculated that CDV infection interferes with synthesis or activation of MMPs. The significance of these findings with respect to myelin loss in nervous distemper needs to be investigated in further studies.

INCIDENCE OF AN INTENSE *CALIGUS MINIMUS*, *C. PAGETI*, *C. MUGILIS* AND *C. APODUS* INFECTION IN LAGOON CULTURED SEA BASS (*DICENTRARCHUS LABRAX* L.) IN GREECE

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Introduction: Caligid infestations have been reported in cultured marine fish in the Mediterranean region. To date, no pathology has been attributed to *C. minimus* in sea bass and existing data concerns only epidemiology and biological information mainly in wild sea bass. This investigation concerns a case of severe pathology and mortality in cultured sea bass due exclusively to *Caligidae* infections.
Materials and Methods: During the years 1999-2001, 420 sea bass (150-300 g, 1+-2+ year class) reared in Eratino lagoon (North Greece) were examined during a survey for metazoan parasites of sea bass. Fourteen samples each consisting of 30 fish were randomly selected at monthly intervals. Collected fish underwent microbiological, parasitological, macroscopic and histological examination.
Results: Fish showed slow swimming at the surface of the water, were lethargic and cachectic. External ulcers located in the head region were apparent even underneath the water of the lagoon at colder months. On gross examination of infected fish either pin-point or dispersed ulcerative skin lesions on the head as well as around and inside the buccal area more over hemorrhages were seen. The tongue was severely ulcerated and osseous tissue was obvious even macroscopically. Four *Caligidae* species were found namely: *C. minimus*, *C. pageti*, *C. mugilis* and *C. apodus* and the gill trematode parasite *Serranicotyle labracis* was present in small numbers but this was not associated with external lesions or mortalities, even when its prevalence was high. The pathology was mainly attributed to *C. minimus* and to a lesser degree to *C. mugilis* infestations. The integument where the parasites were located showed ulceration with marked inflammatory, mainly mononuclear, cellular infiltration of the dermis as a result of the attachment and feeding activity of the parasites. A marked reactive epidermal hyperplasia was observed at those areas as well as at the periphery of ulcerated lesions. Furthermore, many epidermal cells around the damaged area showed signs of necrosis, vacuolar degeneration of basal cells was prominent and epidermis was also characterized by diffuse areas of spongiosis. A characteristic ulceration, which was even deeper at the tip of the tongue, in many fish revealing granulation and osseous tissue was seen. In many cases increased fibroplasia was noticed within dermal collagenous connective tissue.
Conclusion: The results of this study showed that *C. minimus* can cause severe pathology in cultured sea bass especially during winter months. This is a very unusual preliminary report of enhanced pathology of two *Caligidae* species infecting a new host *D. labrax*. Our results confirm the occurrence of emerging parasites in the domesticated species and point out the risk related to the free exchange of parasitic infections between different fish species when restricted in small areas such as lagoons and cages.

APOPTOSIS IN LYMPHOID ORGANS OF PIGS NATURALLY INFECTED BY PORCINE CIRCOVIRUS TYPE 2 (PCV2)

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Introduction: Post-weaning multisystemic wasting syndrome (PMWS) is a disease of pigs associated to porcine circovirus type 2 (PCV2) infection. Main histopathologic findings include histiocytic infiltration and lymphocyte depletion of lymphoid tissues. Lymphocyte depletion can be induced in viral infections throughout apoptosis.
Materials and Methods: Twenty one 2-3.5 months old conventional pigs with growth retardation, selected from high-health status farms that were suffering PMWS outbreaks. All these pigs were serologically positive to PCV2. Five conventional healthy pigs were selected from another farm, free from PMWS, and seronegative to the most common swine pathogens; these pigs were negative to PCV2 by IPMA and PCR in serum. Spleen, thymus, tonsil, ileum and superficial inguinal lymph node were collected from each pig and fixed in 10% neutral buffered formalin; after processed for histopathologic examination, in situ hybridization to detect PCV2 genome and immunohistochemistry to detect cleaved-caspase-3 (CCasp3). Pigs were classified into three lesional stages considering the overall tendency of lymphoid depletion grade and the amount of PCV2 genome in lymphoid tissues. Lesional stages S1, S2 and S3. Apoptotic cells were quantified using an automated program. TaqMan© Real Time PCR was performed to quantify PCV2 genome load in serum samples.
Results: Control pigs: Positive labelling was mainly detected in the nuclei of lymphocyte-like cells. The apoptotic labelling pattern using CCasp3 antibody observed was the one described for normal lymphoid tissue. Overall, this stage characterized by high rates of apoptosis. S1 pigs: The cell types labelled in this lesional stage were mainly the same found in the control tissues. In tonsil and Peyer's patches, immunolabelling distribution was similar in all animals and identical to control pigs. Peyer's patches, spleen, lymph node and thymus, but not for tonsil. S2 pigs: The labelled cells were of the same type found in S1; overall, apoptotic rates were significantly lower than those of the control group. Moreover, apoptotic rates for tonsil and Peyer's patches were significantly lower compared with the ones from S1 pigs. In general, S2 lymphoid lesions were mainly associated with low rates of apoptosis. S3 pigs: The cell types labelled and the pattern of labelling distribution was as in S2. Compared with S2, significant lower rates were only found for Peyer's patches. Overall, in most lymphoid tissues S3 lesions were mainly associated with low rates of apoptosis.
Conclusion: Apoptotic rates in lymphoid tissues were inversely correlated with the viral load in serum and severity of lesions. Our results indicate that apoptosis is not a remarkable feature in PMWS lymphoid lesions development.

CO-EXPRESSION OF E CADHERIN α-CATENIN AND β-CATENIN IN CANINE MAMMARY TUMOURS

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Introduction: The aim of the present paper was to evaluate the co-expressions of E cadherin, α- and β-catenin, which play a crucial role in the organization and manteinance of epithelial tissue architecture and whose decrease or loss is involved in the loss of tumour differentiation and in the development of invasive phenotype, in a series of canine mammary tumours and to correlate them with the histological grade of neoplasm and with the expression of APC protein which regulates β-catenin catabolism and contributes to the β-catenin correct distribution on the cell membrane.
Materials and Methods: Two-color immunofluorescence method was applied on 20 samples of canine mammary tumours (8 benign and 12 malignant) coupling the antibodies as follows: α-catenin/β-catenin; α-catenin/E cadherin; β-catenin/E cadherin and β-catenin /APC at 1:20 dilutions. A confocal laser scanning microscope was used to evaluate the slides. The distribution of E cadherin and catenins was scored as: M (membranous) when the positivity was localized at the intercellular borders of neoplastic cells; H (heterogeneous) when the immunolabelling was localized at the intercellular borders of some neoplastic cells intermingled with negative cells; C (cytoplasmic) when the immunolabelling was distributed throught the cytoplasm.
Results: In benign and more differentiated malignant tumours a membranous co-expression of E cadherin and catenins in all neoplastic cells was observed. In less differentiated malignant tumours a decrease or loss of E cadherin was associated to a disrupted expression of catenins. Neoplastic cells with a β-catenin cytoplasmic positivity showed a superimposable E cadherin expression or resulted negative. A colocalization of APC protein and β-catenin was observed in benign and malignant tumours. Neoplastic cells in which the APC protein was absent showed a correct membranous β-catenin expression. Only in two cases of malignant tumours APC negative cells showed a citoplasmic β-catenin expression.
Conclusion: The decrease, loss or cytoplasmic E cadherin localization correlated with disrutption of catenins in malignant tumours confirms that the correct localization of E cadherin is catenin dipendent and its loss contributes to the acquisition of less diffrentiated malignant phenotypes. β-catenin cytoplasmic accumulation may be linked to disruption of its catabolysm, due to mutations of APC protein, which can not degrade β-catenin as demonstrated in human and canine colorectal tumours. However, our results about APC and β-catenin co-localization suggest that this mechanism is little involved in canine mammary tumours, in which molecular alterations of the adhesive proteins could play a primary role.

CASE REPORT OF SARCOID IN PREPUCE IN DONKEY IN IRAN

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Introduction: Equine sarcoid is the most commonly diagnosed tumour in horses and it accounts for up to 33% of all reported equine tumours. Sarcoids may be called the fibroblast. The mechanism of this uncontrolled growth of fibroblasts is unknown, but a virus (papillomavirus) has been implicated. There is no sex or breed predilection, however, age may play a role as the majority of sarcoids (~ 70%) occur in horses that younger than 4 years old. The most common sites of the occurrence are the head, ears, limbs, and ventral abdomen. The 4 major forms of sarcoids observed are the flat (occult), verrucous (warty), fibroblastic (proud flesh) and mixed form. The flat sarcoid appears as a circular area of hair loss and is limited to the superficial layer of the skin. These lesions usually remain static or regress spontaneously with time. The verrucous sarcoid, as the name implies, appears warty or cauliflower-like. It may grow, remain static, regress with time, or change into a fibroblastic sarcoid. Trauma to the lesion may enhance this change. The fibroblastic sarcoid is the most aggressive type and has the appearance of a true neoplasm. The surface is often ulcerated, has the tendency to bleed when the horse rubs or bumps this area, and often will become infected. The only way to find out if the mass is a tumour, and if so, what type of tumour, is by doing a biopsy.
Materials and Methods: In February 2004 a four years old donkey was presented to veterinary clinic of Tabriz Islamic Azad University. It weighted 250 kg. Temperature, heart rate and respiratory rate were regular. It had the firm solitary circumscribed mass with small hemorrhagic area in right region of superficial layer of penis in prepuce cutaneous. The animal was treated with Actylpromasin 0.05 mg/kg (*i.v.*), Rampun 0.5 mg/kg (*i.v.*) for sedative and aditionally anaesthetised with Lidocaine 6cc (0.02 solution) infiltrations in order to enable pulling hind limb to the upper position for the surgery ,we took a piece of mass and the sample was referred to the laboratory of pathology. The colour of mass was white and the diameter of tumour was D1:60.4 mm and D2:54.7 mm. Haematoxylin and eosin staining was used.
Results: Histopathologically, the fibrocytes and fibroblasts were found. The cells had no mitotic figures, and polymorphic figures were very few. The haphazardly figure were not found in the cells. A few delicate structures of angiogenesis were noted.
Conclusion: Biopsy is the only way to find out if the mass is a tumour, and if so, what type of tumour it is. This is an easy procedure that can be performed on a sedated equine, which is locally anesthetised. Most of the cells diagnosed were fibrocystic. Gross pathology coarser, warty or cauliflower-like lesions were not found in surface of tumour like mass and the fibroblastic sarcoid was diagnosed.

THE SPECIALTY EXAM OF THE EUROPEAN COLLEGE OF VETERINARY PATHOLOGISTS (ECVP)

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2005 ECVP Exam Committee: http://bris.ac.uk/pathandmicro/eurovet/ecvpmain.html

The European College of Veterinary Pathologists (ECVP) is an international organisation that promotes high standards and excellence in all fields of Veterinary Pathology. To become members, candidates are requested to sit a speciality exam held once a year in February. The exam represents part of the process for establishing standardized European training programs and the acquisition of a uniform group of qualified specialists across Europe. The compilation and administration of the exam is the responsibility of the ECVP Examination Committee (EC) appointed by the ECVP Council. The EC consists of 10 members, including one chairperson, that serve for 5 years, and 2 proctors recruited for 1 year. The selection of an EC member/proctor is based on ECVP membership and the need to have a representation of different areas of expertise on the EC. The ECVP exam is composed of 5 sections. Histopathology comprises 1 cytologic and 18 histologic slides and 1 electron micrograph (20 questions). Gross Pathology encompasses 60 macroscopic images. The theoretical part is composed of General Pathology (60 questions), Veterinary Pathology (90 questions) and Comprehensive Pathology (5 questions). The majority of questions are selected from books and journals listed in the official ECVP reading list. Each exam section is overseen by a section leader (SL) who receives contributions from the other EC members and creates a draft that is reviewed by a selected group of EC members. Based on reviewer comments, SLs prepare a second draft that is reassessed by all members in a general meeting. The finalized exam is balanced with respect to species, organ system, etiology, mechanisms of disease, classical and new diseases and other recent developments. At the exam venue, EC members are responsible for ensuring that facilities are managed appropriately. At the beginning of the exam, candidates draw a number under the confidential supervision of the ECVP President. All candidates are identified by their number throughout the exam ensuring anonymous marking by the EC members. The passing score for each section is 60% and all sections must be passed to become an ECVP diplomat. Candidates may carry over approved sections if they have passed 2 or more parts and can repeat the failed sections twice within the next 4 years. After Council's approval candidates' names are disclosed and the EC chairperson and the ECVP secretary send the results by email and regular mail. The preparation of the ECVP exam has developed into a logically organized algorithm. This algorithm has been accomplished by the effort of past and present EC members who have provided European veterinary pathologists with a standardized, impartial exam. The ECVP exam is internationally recognised. ECVP and ACVP diplomats by exam can now act as sponsors or supervisors for applicants to either College. Information for trainees, sponsors and candidates is available at www.bris.ac.uk/Depwww.bris.ac.uk/Depts/PathAndMicro/EuroVet/ecvpmain.htm.

P-GLYCOPROTEIN (PGP) IMMUNODETECTION IN CANINE CUTANEOUS MAST CELL TUMOUR AND CORRELATION WITH THE HISTOLOGICAL GRADING SYSTEM

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Introduction: Canine cutaneous mast cell tumour (MCT) is a malignant neoplasm which frequently requires a combination of surgical and chemotherapeutical treatment approach. However, one of the most frustrating aspects of MCT treatment is the development of multidrug resistance (MDR) due to the expression of MDR1 gene codifing P-glycoprotein (Pgp). The aim of this study is to determine and describe the pattern of Pgp expression (PgpE) and to verify the relationship between PgpE and histological grade. Furthermore, preliminary follow up data are then compared to PgpE.
Materials and Methods: Formalin fixed, paraffin wax embedded tissue from surgical samples of 42 cases of canine mast cell tumour, submitted to our laboratory were classified and graded according to WHO diagnostic criteria. Immunohistochemical staining was performed using mouse monoclonal antibody C494 and as secondary antibody an EnVision+TM Peroxidase anti-mouse detection system. The positive reaction was evaluated as the percent of labelled cells in 10 areas hpf. As positive control, section from normal canine kidney and human hepatocellular carcinoma were used. The Spearman test to asses the significance or our investigation, the Mann-Whitney test to verify any statistical variations in Pgp expression and mast cell tumour of different grade and a survival analysis were performed.
Results: The 42 MCT cases were allocated into grade I (24), grade II (9) and grade III (9). Pgp positive reaction was found in 32/42 MCT with a diffuse staining in the cytoplasm and occasionally in the cell membrane. 15/24 grade I, 8/9 grade II, 9/9 grade III were Pgp positive. Results indicate a positive relation between PgpE and histologic grade of canine mast cell tumour ($r=0.58$, $p<0.001$). Statistical analysis showed significant differences in PgpE and histologic grade, especially between grade I vs III and II vs III, ($U=17.50$, $p<0.01$). Follow up studies revealed that only few cases underwent chemotherapy protocol, few died but the majority is still alive without any treatment. A survival analysis showed that both a higher grade and an higher positivity score for Pgp were associated with a shorter survival rate.
Conclusion: A positive relation exists between Pgp expression and histologic grade. Follow up data and survival analysis revealed that high grade and PgpE are correlated with a poor prognosis and a shorter survival time. On the basis of this preliminary study, we can consider Pgp expression as an indicator of worse prognosis but more extensive follow up study after chemotherapy protocol would be extremely useful to suggest a routine immunohistochemical evaluation for Pgp as a MDR marker.

MORPHOLOGICAL INVESTIGATIONS IN SECONDARY INTESTINAL LYMPHANGIECTASIA IN DOGS

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Introduction: Intestinal lymphangiectasia is characterized by obstruction of lymph drainage from the small intestine and lacteal dilation that distorts the villus architecture. In animals as in human beings it is essential to distinguish the primary idiopathic form from the secondary form which is caused by obstruction of lymph flow due to infiltration of the lymphatic system with inflammatory or neoplastic cells. In dogs, a large amount of inflammatory bowel disease (IBDs), are characterized by a moderate to severe lymphoplasmacytic infiltrate of the small intestinal mucosa. For clinicians, diagnosis of secondary intestinal lymphangiectasia may represent a problem due to the absence of specific clinical signs, but it is important for a correct nutritional therapy. To facilitate the interpretation of lymphatic vessel dilation in secondary forms, associated with chronic or chronic-active IBDs, we have carried out a systematic evaluation of high and wide and h/w ratio of chiliferous lymphatic vessels in a large population of dogs with a diagnosis of IBDs, in comparison with a control group.

Materials and Methods: The studied population consisted of 150 randomly selected dogs in which the diagnosis of lymphoplasmacytic enteritis had been established, belonging to a population of 1103 dogs, examined in a period of five years (1999-2004). Additionally, a group of 134 dogs without any signs referable to gastrointestinal problems were analysed endoscopically during other surgical procedures and histologic samples were collected as controls. Ten bioptic samples were taken from macroscopically affected (pathological groups) or normal (control group) areas of the proximal portion of the small intestinal mucosa per dog, fixed in 4% buffered formaldehyde, then embedded in paraffin in a longitudinal (5 specimens) and horizontal (5 specimens) manner. The histologic examination of haematoxylin and eosin stained sections included the assessment of the number of inflammatory cells (score 0-3) evaluated in each biopsy. Villous assessment comprehended height, width and the h/w average evaluation for the villi and villous chiliferous lymphatic vessels. The mean of the values obtained per biopsy were compared between control and pathological group by using Student's t test.

Results: The mean values of different parameters considered both in infiltrative lymphoplasmacytic enteritis and in controls were reported in table.

Group	Villi (mean values in μm)			Chyle ducts (mean values in μm)		
	height	width	h/w	height	width	h/w
IBDs	689.61	273.11	2.55	518.56	60.81	5.82
Controls	806.65	160.9	5.01	680.7	23.55	28.90

A good correlation was observed between degree of mucosal infiltration and lymphatic dilation and a correlation ($p<0.005$) was also observed between a diameter larger than 45.3 μm of chiliferous vessel and a mild to moderate loss of serological proteins and albumin, associated to body weight loss and persistent diarrhoea.

Conclusions: The comparison between the two groups indicates that in the course of IBD the height of the villi in the small intestinal tract remains unaltered, while on average the diameter of the villi and of chiliferous ducts is dramatically increased with respect to the control group. Based on our observations, we can speak of secondary lymphangectasia when the mean diameter of the chyle ducts is greater than 30 μm, even in the absence of manifest symptoms, while there is a strong relationship between a decrease in serum proteins and a mean cheliferous diameter of over 45.3 μm.

IMMUNOCHARACTERIZATION OF CANINE GRANULAR CELL TUMOUR ARISING FROM LEFT VOCAL CORD

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Introduction: Granular cell tumours (GCTs) are relatively uncommon tumours in domestic animals as in human beings (Dungworth 1993). Although the insurgence of GCTs associated by the presence of pseudoepitheliomatous squamous epithelial hyperplasia of the mucosa overlying the tumour have been frequently reported in vocal cords in human beings, this localization and feature is unusual in dog.
Materials and Methods: A 6 years old male collie was submitted to laryngoscopic examination that revealed severe hyperplasia of tonsil-lymphoid tissue at left side, bilateral edema of the larynx and a single, greyish-white nodular polyp involving the left vocal cord. The dog underwent direct laryngoscopy with biopsy for histologic examination. Samples of tumour and omolateral amygdale were fixed in either 10% buffered formalin or 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, and processed for histopathology, immunocitochemistry and transmission electron microscopy (TEM). Sections (3 μm) were stained with haematoxylin and eosin, and diastase–resistant periodic acid Schiff (PAS) to ascertain structural details. The mono and polyclonal antibodies used for immunohistochemistry, are summarized in Table 1.
Table1. Summary of antibodies used and results.

Primary antibody	Dilution	Source	Positivity
Glial fibrillary acidic protein (GFAP) (Mab)	1:500	Dako Co., Carpinteria, CA	sporadic
Porcine vimentin (Mab)	1:200	Dako Co., Carpinteria, CA	sporadic
Pancytokeratins (Pab)	1:400	Inmustain DPC	negative
α-Actin (Mab)	1:200	Sigma, St. Louis, MO	negative
Desmin (Pab)	1:400	BioGenex Lab, CA	negative
Neuron Specific Enolase (NSE) (Mab)	1:1000	Dako Co., Carpinteria, CA	sporadic
S-100 protein (Mab)	1:400	Novocastra Laboratories	strong
Synaptophisin (Mab)	1:200	Dako Co., Carpinteria, CA	negative
Rat chromogranin A (Pab)	1:100	Yanaihara Institute Inc., Japan	negative
CD68	1:100	Dako Co., Carpinteria, CA	strong
IV Collagen	1:50	Dako Co., Carpinteria, CA	strong
MIB-1	1:50	Innovex Biosciences, CA	sporadic

Results: Cytologically, the mass was characterized by the presence of well defined polygonal cells, with granular-eosinophilic cytoplasm and eccentric nucleus and these findings were confirmed by histopathologic and ultrastructural examination. The tumor was characterized by globoid to polygonal sheets of cells with foamy granular cytoplasm, which contained numerous PAS positives diastase-resistant granules, ultrastructurally represented by various-sized, heterogeneous lysosomes. However, these neoplastic cells accompanying epithelial hyperplasia of the larynx mucosa and the tonsil are showing an aspect that mimic an invasive squamous cell carcinoma.
Conclusions: Canine laryngeal oncocytomas and rhabdomyomas have been reported by different authors (Meuten et al. 1985, Pass et al. 1980) but, until now, laryngeal localisation of a GCT showing immunohistochemical findings that suggest the Schwann cells origin in association with pseudoepitheliomatous hyperplasia of laryngeal mucosa have not been previously described in this species.
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RENAL LIPOFUSCINOSIS IN DANISH SLAUGHTER CATTLE

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Introduction: Bovine renal lipofuscinosis (BRL) ("black kidneys") is an incidental finding in slaughter cattle. BRL has been known for more than a century, but the disorder has not been studied in detail. BRL is apparently not associated with clinical disease, and affected cattle are considered suitable for consumption. A study was performed to investigate the occurrence of BRL in Danish slaughter cattle.
Materials and Methods: The study was based on cattle admitted to four major Danish abattoirs during a 6 months period. Initially the gross morphology of BRL was characterised. Selected cases were also examined by histology and electron microscopy. Based on the individual identification numbers, the following data were obtained from a central database: date of slaughter, date of birth, sex, breed, sire, paternal and maternal grandsires, date of latest calving, course of latest calving, date of latest yield control, milk production (volume and weight of fat and protein) at latest yield control and during the latest 305 days, lactation number at slaughter, and weight of carcase at slaughter. The data were analysed by the Chi-square test or the General Linear Model procedures (SAS Institute, Cary, NC, USA).
Results: Out of 133939 bovines entering the abattoirs, 359 cases of BRL were recorded (0.27%). BRL was restricted to cattle of the Holstein or the Red Danish Dairy (RDD) breeds with breed prevalence of 0.21 % and 1.19 %, respectively and occurred significantly more frequent in Holsteins and the RDD breed than in other breeds ($p<0.0001$). The age of affected animals varied from 9 months to 10 years and 5 months (mean 5 years). The age of affected animals varied significantly from the age of other slaughter animals ($p<0.0001$). Very few animals less than three years were affected. On the other hand, a higher number of affected cattle were slaughtered in the age groups 4.5 to 6.5 years and 7.5 to 8.5 years. If cattle less than three years were omitted from the calculations, breed incidences were calculated to 2.51% in RDD breed and 0.44% in Holsteins.
The presence of BRL was significantly correlated to the sire and grand sires for both the Holstein and RDD breeds. Genealogical examination demonstrated that several sires having affected progeny and their grandsires often were genetically related and occurred in minor family groups.
Differences in production data were not found between BRL affected cattle and unaffected cattle aged at least 3 years.
Conclusion: The study demonstrates that BRL is a common disorder, which in Denmark is restricted to the Holstein and RDD breeds. Cattle aged 4.5 to 6.5 years or 7.5 to 8.5 years apparently have an increased risk of being culled. This aspect should be investigated in other geographic regions. The study strongly indicates that BRL is a genetic disorder, possibly with autosomal recessive inheritance.

CHANGES IN LYMPHOCYTE SUBSETS IN ACUTE INFECTION IN SWINE WITH FOOT AND MOUTH DISEASE VIRUS

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Introduction: Foot and mouth disease (FMD) is a highly contagious infection of cloven-hoofed mammals produced by a picornavirus that often causes rapidly spreading epizootics, especially among cattle, pigs and sheep. The disease is characterised by the formation of vesicles in the mouth and on the feet, causing pyrexia, inapetence, lameness and drop in production. Very little is known about the immune response during the acute phase of the disease. In this work we describe the changes observed in different lymphocyte populations during the first days of infection.
Materials and Methods: Large White x Landrace pigs 10 weeks old were inoculated intradermally in the coronary band with a high dose (10^5 PFUs) of FMD virus C-S8c1. Pigs were painlessly slaughtered in batches of 2 animals at 1-17 day post inoculation (dpi). Non inoculated and PBS-inoculated animals were used as controls. Several organs and blood samples were harvested for flow cytometric analyses of CD3+, CD4+, CD8+, CD45RA+ lymphocytes as well as monocyte-macrophages (SWC3+). Samples from lymphoid organs were taken, fixed in different solutions and embedded in paraffin wax. Immunohistochemical techniques were used to detect FMD viral antigens and surface markers of leukocyte subpopulations.
Results: Inoculated animals developed pyrexia from 1-2 dpi coinciding with viraemia and showed clinical signs such as inapetence, lameness and dyspnea. Vesicular lesions appeared from 2-3 dpi firstly in the inoculation site and lately in other localisation. Regional lymph nodes showed a progressive lymphoid depletion during the experiment, mostly related to T lymphocyte depletion. FMDV was observed mainly in mononuclear cells in the lymph nodes. Flow cytometry analysis of CD4+ and CD8+ showed a decrease in the number of T cells at 1-3 dpi in spleen and lymph nodes as well as in peripheral blood leukocytes. This decline in the number of cells occurred just before the onset of the viraemia peak, getting to normal values at 7 dpi when the viraemia was cleared.
Conclusion: These results suggest that not only skin lesions are present during FMDV infection, being an immunosuppression observed in the first stages of the disease, mainly affecting T lymphocytes.

STUDY OF HUMORAL IMMUNE RESPONSE DURING CLASSICAL SWINE FEVER (CSF) ON PARAFFIN EMBEDDED PIG TISSUES

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Introduction: The evolution of the cellular immune response in pigs infected with CSF virus could be associated with the quantitative changes and expression of cytokines by macrophages, which have been related with lymphocytes apoptosis. These changes may influence the delayed humoral immune response characteristic of the disease. The aim of this study was to characterize "*in situ*" the humoral immune response during CSF.
Materials and Methods: Thirty two Large White x Landrace pigs of 4 months old were inoculated intramuscularly with the virulent isolate "Alfort 187" and slaughtered in groups of 4 animals from 2 to 15 days post inoculation (dpi). Four animals were used as uninfected controls. Samples of spleen and thymus were fixed in 10% buffered formalin and Bouin's solution, and were routinely processed. For the immunohistochemical study, avidin-biotin-peroxidase complex method was used to immunolabelled activated B cells (λ-chains, IgM and IgG). Positive cells were counted and tested for significance ($p \leq 0.05$) by Student's t test.
Results: Despite the lymphoid depletion observed in B and T areas of spleen and thymus respectively, B lymphocytes and plasmatic cells λ-chains+ showed a significant increase in spleen from the initial stages of the disease, peaking at 14 dpi, whereas a slight increase of λ-chains+ cells was observed in thymus from 4 to 9 dpi. A significant increase of IgM+ cells was observed in thymus and spleen, peaking at 7 and 11 dpi respectively, while the presence of IgG+ cells was observed occasionally from 11 dpi onwards.
Discussion: The evolution of λ-chains+ cells throughout the disease was associated spatially and temporally with the presence of antigen positive cells. So, the evolution of monocytes-macrophages population and the cytokines levels released by them would make clear the interaction of these cell populations with B lymphocytes and could be involved in the proliferation and differentiation of B cells to plasmatic cells. The presence of immunoglobulins released by plasmatic cells would be related with the establishment of a typical humoral immune response, appearing an increase of IgM+ cells in early stages of the disease and the presence of a small number of IgG+ cells in late stages, according with the gamma-globulins serum levels of our study.
Acknowledgements: This work was supported financially by grants from DGESIC (PB98-1033).

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CYTOKINES EXPRESSION ON PARAFFIN-EMBEDDED TISSUES FROM CONVENTIONAL CALVES

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Introduction: Cytokines are produced by various cell types, chief among which are monocytes/macrophages (m-M∅s) cells. The aim of our study was to report on the fixatives and antigen retrieval procedure of choice to evaluate, through the employment of immunohistochemical staining methods, the labeling of bovine cells for different proinflammatory cytokines (TNFα, IL-1α and IL-6) in paraffin-embedded calve tissues.
Materials and Methods: Samples (spleen, thymus, retropharyngeal and mesenteric lymph nodes, tonsil, ileum, lung, liver and kidney) from five healthy animals of 4-6 months old were taken and fixed in 10% buffered formalin solution (24 hours) and Bouin's solution (8 hours) for structural and immunohistochemical studies. The avidin-biotin-peroxidase method in combination with different antigen unmasking techniques was used for the identification of cells expressing cytokines.
Results: m-M∅s were identified as the main cytokine-producing cells, although other cell types (mainly neutrophils) also stained positively. TNFα and IL-1α expression was located in the medulla and the mantle zone of follicles of lymph nodes, in the splenic red pulp, cortex and medulla of thymus and in the lamina propria of ileum. Occasionally, intraepithelial immunopositive-m-M∅s were observed in the crypts of Lieberkühn, as well as in lymphoid follicles of spleen and Payer's patches of ileum. A few IL-1α stained-cells appeared in the diffuse lymphoid tissue of tonsil, while occasional presence of intravascular and interstitial m-M∅ immunolabelled against TNFα was observed in lung. On the other hand, lymphocytes and m-M∅ were the main IL-6 producing cells, as well as some fibroblasts and endothelial cells. Focal presence of IL-6 positive cells was observed in the interfolicular area of lymph node cortex and thymic cortex, appearing occasionally immunostained cells in the splenic white pulp, tonsil and gut-associated lymphoid tissue. Additionally, some pulmonary m-M∅s were stained against IL-6. Specific immunoreaction against the studied cytokines was not observed in the rest of organs.
Conclusion: Bouin's solution was the best fixative for immunohistochemical detection of these chemical mediators, and the permeabilisation with Tween 20 the most suitable unmasking antigen method.
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HISTOLOGICAL STUDY OF THE CANINE MAMMARY GLAND DURING THE ESTROUS CYCLE

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Introduction: Mammary gland undergoes morphogenesis during all reproductive life of mammals. Mammary research has been driven by the cancer problem with less goal-oriented interest in basic biology. Mammary tumours are the most frequent neoplasm in female dog; however, little is known about the normal canine mammary gland. Here, we describe the histologic findings in canine female mammary gland during the oestrous cycle.
Materials and Methods: Thirty eight mammary glands free of macroscopic and microscopic lesions were studied. The stage of oestrous cycle was determined by vaginal cytology, histological examination of ovaries and uterus and the serum levels of progesterone and 17β-oestradiol. The 38 animals were placed into seven groups: 7 prepubertal, 6 proestrous (3 with $\leq$ 1 year), 5 oestrous, 5 early diestrous, 4 late diestrous, 5 early anestrous and 6 late anestrous.
Results: The histological findings were: in prepubertal and in the younger subgroup of proestrous, few ducts with several lateral projections of solid cellular bulbous epithelial structures (end buds); in the other subgroup of proestrous, the end buds were surrounded by a small amount of loose stroma and few lobuloalveolar units with marked cellular vacuolization and disorganization; in oestrous, ductal branches surrounded by a loose, oedematous concentric connective tissue forming a lobular pattern and lobules with vestigial secretion, vacuolated and picnotic cells; in early diestrous, a branching duct system surrounded by two kinds of connective tissue and evidences of mitotic activity in epithelial cells and fibroblasts of the periductal loose stroma; in late diestrous, great lobular development, all ducts and some alveoli distended with secretory material and others alveoli with narrow lumen with few or no secretion and a vacuolated myoepithelial layer; in early anestrous, increase number of ducts and alveoli with vacuolated and picnotic cells, evident thickness of the basement membranes and increase amount of interlobular and intralobular dense stroma; in late anestrous, small lobules with irregular outline, rare secretory alveoli, cellular epithelial vacuolization, thicker basement membranes and significantly amount of dense periductal connective tissue.
Discussion: The mammary gland in the prepubertal as well as in animals of the first proestrous was rudimentary with only few ductal structures surrounded by a poorly differentiated connective tissue. The first signs of development were observed in young animals in early diestrous consisting in ductal arborization associated with proliferation of the surrounding stroma and concomitant change in extracellular matrix. Interestingly, this histological image resembled feline fibroadenomatous change. Late diestrous was characterized by secretory differentiation and initial regressive changes. The regression phenomena were more evident in early anestrous and completely dominant in late anestrous. In proestrous of older animals, lobular remnants with some regressive signs were present and the stroma constituted the majority of the gland. In oestrous both regression and formation of lobular structures was observed.

LYMPHANGIOGENESIS IN MAMMARY TUMOURS OF THE CAT

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Introduction: Transport of tumour cells via lymphatics (Lcs) is the most common pathway of initial dissemination of many carcinomas. Notwithstanding the availability of lymphatic endothelium markers, the distinction between lymphatic and blood endothelium is difficult, because these markers do not always cross-react with animal tissues. Aim of this study is to test lymphangiogenesis in feline mammary gland tumours.
Materials and Methods: An immunohistochemical anti-laminin/anti-VEGFR3 double stain has been used to identify Lcs when negative for laminin. The Lcs were classified into positive and negative for VEGFR3 expression. The following samples have been tested: normal mammary glands (NMG) (10 cases), benign (BT) (10 cases) and malignant tumours (MT) (40 cases), these latter grouped by histological stage (stage 0 or non-infiltrating malignant tumour – 10 cases; stage I or infiltrating with stromal invasion – 10 cases; stage II or infiltrating with emboli in lymphatic or blood vessels and/or regional lymph node metastasis – 20 cases). IHC stained sections have been evaluated excluding blood vessels (showing positive laminin stain) and including lymph vessel (negative laminin stain), then furtherly subgrouped for presence and absence of VEGFR3. The count has been carried out on 10 intratumoural (intramammary in the NMG) and 20 extratumoural (extramammary in NMG) fields (0.789 square mm) for each section. The data obtained have been subjected to statistical analysis.
Results: In all groups (NMG, BT, MT) the content of Lcs in the extratumoural stroma (extramammary in the NMG) was significantly higher than in the intratumoural (intramammary) stroma: intratumoural vs extraumoural counts in tumours, Lcs with VEGFR3 $p<0.001$, total number of Lcs $p<0.001$; intramammary vs extramammary counts in NGM, Lcs without VEGFR3 $p<0.05$, Lcs with VEGFR3 $p<0.001$, total number of Lcs $p<0.01$. The count of Lcs with or without VEGFR3 expression did not show any significant difference in intratumoural and extratumoural stroma in both BT and MT compared respectively to the intramammary and extramammary counts in NMG. Comparing the three groups (NMG, BT, MT) no differences in the number of Lcs (with or without VEGFR3 expression) was recorded in the progression from NMG to MT. No variation was found in the number of Lcs (with or without VEGFR3 expression) in the three groups of carcinomas classified for histological stage.
Discussion and Conclusion: On the basis of our results, the number of Lcs seems not to be increased in malignant compared to benign tumours and NMG. The ability of mammary carcinomas to spread via the lymphatic pathway is known, and it seems to be not a consequence of a true increase of Lcs in intratumoural or extratumoural stroma, but a ligand/receptor interaction between neoplastic cells and lymphatic endothelium. A significantly higher number of Lcs is present in the extratumoural than in the intratumoural stroma, as known in human breast cancer. Extratumoural stroma, rich in Lcs, seems the major site of interaction between neoplastic cells and lymphatic endothelium.

COMPARATIVE HISTOPATHOLOGICAL DIAGNOSIS OF MELANOMA BY HAEMATOXYLIN AND EOSIN, SPECIAL STAINING AND IMMUNOHISTOCHEMICAL METHODS

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Introduction: Melanocytomas are common in dogs, horses and certain breeds of swine, less common in cats and cattle and rare in sheep and goats. Congenital and acquired melanocytomas in horses less than 2 years of age are relatively common but cattle develop melanocytomas infrequently. Congenital tumours and tumours in young animals have been reported in cattle.
Materials and Methods: During 1961-2002, 9 cases of melanomas have been diagnosed by haematoxylin and eosin staining method. From 9 cases, 6 cases were in horses and 3 cases in cattle. For confirmation of diagnosis, fontana masson silver and melanin bleaching methods and immunohistochemical staining for S-100 protein and HMB45 antigen were used. Four cases of squamous cell carcinoma (SCC) tumours (2 cases from horses and 2 cases from cattle) were used as controls.
Results: Majority of cases had intradermal histologic pattern. In 6 cases of 9 tumours, both S-100 protein and HMB45 staining were positive but in 3cases, there were doubtful results (maybe due to changing of Ag quality). In all of the 9 cases, the results of fontana masson silver and melanin bleaching methods were positive. In 4 cases of squamous cell carcinoma as controls, the results of 3 tests were negative.
Conclusion: Diagnosis of doubtful cases of melanoma tumours must be confirmed or reconfirmed by special staining and/or immunohistochemical methods. The cases of melanomas in this form in cattle are the first case report in Iran.

ISOLATION OF *BRANHAMELLA* IN FACIAL CELLULITIS IN CATTLE

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Introduction: *Branhamella* is a gram negative, glucose non-fermenting bacteria and subgenus of *Moraxella*. It may cause suppurative bronchitis, pneumonia, sinusitis and otitis media in humans. It has also been isolated from pneumonic lungs of calves.
Materials and Methods: Samples of involved tissues of bilateral facial skin of a Holstein heifer, 6.5 months pregnant, were cultured and checked by biochemical tests. The tissues were also studied by histopathological examination.
Results: Tissues of facial skin of the heifer were studied by histopathological examination. Macroscopically, the facial skin was swollen and edematous. Microscopic examination revealed cellulites by suppuration and exudation with neutrophil infiltration and fibrin deposition (purulent and fibrinonecrotic inflammation) in derm, hypoderm and intermuscular tissues. Bacteriological examination showed aerobic cocci in pairs, penicilline susceptible and glucose, growth on MacConkey agar, motility, urease, indole and gelatinase tests negative, but catalas, oxidase, nitrate tests and growth on blood agar were positive.
Conclusion: *Branhamella* was the only agent which was isolated from this kind of dermatitis. This bacterium has been isolated from pneumonic lungs of calves and is considered a commensal of the cojunctiva and upper respiratory tract in sheep, cattle and goats with low pathogenicity. The bacteriological characteristic of this isolated agent is very similar to *Branhamella ctarrhalis*.

THE INFLUENCE OF DEXAMETHASON ON THE OVARIAN AND UTERINE STRUCTURE AND ER, PR AND α-INHIBIN EXPRESSION IN PSEUDOPREGNANT RABBITS

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Introduction: Dexamethason is the popular corticosteroid hormone used in the therapy of human and animal diseases. The recent published data are indicating the close interaction between adrenal and ovarian steroids. The aim of this work was to perform histopathological and immunocytochemical investigation of ovaries and uteri of pseudopregnant rabbits exposed to therapeutic doses of dexamethason.
Materials and Methods: Laboratory rabbits obtained dexamethason *i.m*. in two therapeutic doses – 0.5 mg/kg b.w. and 1.5 mg/kg b.w. After 16 days they were mated by vasectomised male. After euthanasia ovaries and uterine samples were collected and stained haematoxylin and eosin, AB-PAS reaction, and by IPOX method for estrogen receptors, progesterone receptor and α-inhibin receptors.
Results: Histopathological changes in ovaries indicated dexamethason-dependent disturbances in oogenesis/folliculogenesis, as well as pronounced transformation in theca/interstitium cells. This transformation into cells capable to steroidogenesis were proved by well marked α-inhibin expression. In uterus dexamethason caused degenerative changes in endometrial epithelial cells, with significant decrease in their secretory activity, accompaning by loss of ER and PR nuclear expression. The changes observed in ovaries and uteri were increasing with dexamethason dose.
Conclusion: Dexamethason given to pseudopregnant rabbits were caused structural changes in the ovaries and uterus. The injury was the dose depended. The observed changes in the expression of immunocytochemical markers for examined organs were proved that dexamethason caused disturbances in cells metabolism of the ovary and uterus, which probably are developing due to hormonal imbalance.

CANINE MAST CELL TUMOURS: CORRELATION OF APOPTOSIS AND PROLIFERATION MARKERS WITH PROGNOSIS

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Introduction: Mast cell tumours (MCT) are the most commonly diagnosed skin tumours of dogs. The Patnaik histological grading scheme is the most frequently used method to predict the biological behaviour of an individual MCT. This grading scheme is very useful in predicting the behaviour of benign (grade I) and malignant forms (grade III) of the disease. However, it has much lower predictive value for the intermediate grade tumours (grade II) where up to 30% of these tumours can recur locally or metastasise. In this study, we carried out immunohistochemical and histochemical staining of a large number of canine MCT to determine whether the frequency of expression of the inhibitor of apoptosis, survivin, and that of a number of markers of cell proliferation (PCNA, Ki67 and AgNOR) can accurately predict canine MCT behaviour.

Materials and Methods: One hundred and thirty one cases of canine cutaneous MCT were identified from the pathology archives of AHT. Immunohistochemical staining of sections from the paraffin embedded tumour tissue from each case was carried out using antisera specific for Ki67, PCNA and survivin. Histochemical stains using the internationally standardized AgNOR staining protocol was performed. Digital microscopic images were captured for each section stained and positive and negative neoplastic cells counted. Statistical analysis was carried out to determine whether the histological markers could be used to predict survival.

Results: The mean survival was 565 days. The mean age of the dog was 8.3 years. Nineteen of 131 dogs had multiple cutaneous MCT. The Ki67 scores ranged from 0 to 22.2 (median 1.0). Mean AgNOR scores ranged from 1.13 to 4.1 (median 1.7). The PCNA scores ranged from 3.2 to 83 (median 48.6). 109 cases (83%) exhibited positive nuclear staining and 87 cases (6%) exhibited positive cytoplasmic staining for survivin. Cox regression models indicated that Ki67 score (Hazard Ratio 1.92; $p<0.001$) and mean AgNOR score (Hazard Ratio 2.57; $p<0.001$) were significantly associated with survival time for dogs with MCT. Cox regression analysis indicated that the binary Ki67 variable (cut off point Ki67 score of 1.8) was a significant predictor for survival in dogs with Grade II MCT. There was no significant association between survivin score or PCNA score and survival. Survival time was independent of the presence of multiple MCT mass lesions.

Conclusion: This study shows that Ki67 score is an independent prognostic marker for canine MCT and can split the grade II MCT into two groups (median survival time: 392 days – Ki67, score<1.8 vs 154 days – Ki67, score>1.8). Each individual MCT developing on a dog with multiple MCT masses, should be considered independently, with prognosis depending on the Patnaik grade and Ki67 score for each individual MCT mass. This is the first study to examine the expression of survivin as a potential prognostic marker for canine MCT.

CLONING OF THE CANINE HOMOLOGUE OF SURVIVIN

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Introduction: Survivin is a member of the inhibitors of apoptosis (IAP) family of proteins that are involved in protecting cells from pro-apoptotic signals delivered from inter- or intracellular sources. The IAP gene family is highly evolutionarily conserved and homologues have been identified in species as diverse as fish, fruit flies, yeast and nematodes. IAPs function as endogenous inhibitors of caspase-9 and caspase-3, although activation of other intracellullar signalling pathways may also be involved. In addition, survivin is involved in the maintenance of chromosomal stability at mitosis.

Survivin mRNA and protein are expressed widely in the human embryo, but only in selected stem cell compartments of the bone marrow and intestinal mucosa of adults. Survivin mRNA and protein are largely undetectable in the majority of normal differentiated human tissues; however, survivin has proved to be an almost universal tumour antigen, being over-expressed in the majority of human cancers. Furthermore, in some of these cancers, it has proved to be an independent prognostic marker. Tumours that exhibit high survivin expression, are often the most difficult to treat and can often being refractory to chemotherapy or radiotherapy.

Materials and Methods: Using the published human nucleotide sequence of survivin cDNA, homology searches were made of the publicly available raw canine genomic sequence data archives. Using the survivin exon homologues identified in this way, polymerase chain reaction (PCR) oligonucleotide primers were designed to amplify the individual exons from canine genomic DNA preparations. Oligonucleotide primers were also designed such that they spanned the entire coding sequence and were used in reverse transcription PCR (RT-PCR) experiments to amplify the full length coding sequence from mRNA isolated from biopsy specimens of canine nasal chondrosarcoma and thyroid carcinoma. Resulting amplicons were subcloned and sequenced.

Results: Four exons were identified from the canine genomic sequence database. These were amplified from canine genomic DNA preparations using the oligonucleotide PCR primers designed. There was 100% nucleotide sequence identity with the published raw canine genomic sequence data. The full coding region for canine survivin mRNA was amplified by RT-PCR from both neoplastic tissue preparations. The amplicon length was 592 base pairs. Within the cDNA sequence was an open reading frame of 426 base pairs. Sequence comparisons reveals 91% nucleotide sequence identity and 90% predicted amino acid sequence identity with human survivin.

Conclusion: The canine homologue of the IAP, survivin, is highly conserved between species, with extensive nucleotide and amino acid sequence homology with the human survivin sequence. Canine survivin has 4 exons within the genomic nucleotide sequence, with similar exon/intron boundaries to the human gene. Canine survivin mRNA can be detected in the neoplastic tissue of canine tumours.

AGE-RELATED SEQUENCE OF DIABETES-ASSOCIATED RENAL CHANGES IN GIPR^{dn} TRANSGENIC MICE

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Introduction: Transgenic mice, expressing a dominant negative glucose-dependent insulinotropic polypeptide receptor (GIPR^{dn}) have recently been shown to develop severe diabetes mellitus. The aim of the present study was to characterize the age-related sequence of diabetes-associated kidney lesions in GIPR^{dn} transgenic mice.

Materials and Methods: GIPR^{dn} transgenic animals were investigated at the age of 3, 8, 20 and 26 weeks. Age-matched non-transgenic littermates served as controls. Several clinico-chemical parameters were examined, including blood glucose, serum urea, creatinine, total protein, albumin, sodium, chloride and triglycerides. Urine protein analysis was performed, using SDS-PAGE. Stereological methods served to quantify diabetes-associated kidney changes. Glomerular lesions were determined qualitatively and semiquantitatively (glomerulosclerosis index).

Results: Blood glucose was significantly increased in GIPR^{dn} transgenic mice vs controls, at all time points examined. At the aged of 3 weeks none of the serum parameters examined differed from those of controls. At the aged of 8, 20 and 26 weeks triglycerides, urea and creatinine levels were increased in transgenic animals, whereas sodium, chloride, total protein, and albumin levels were decreased as compared to controls. As evidenced by SDS-PAGE, onset of albuminuria occurred at the aged of 8 weeks; at the aged of 26 weeks most transgenic animals showed pathological urine protein excretion. Renal dysfunction was accompanied by alterations in glomerular morphology. The predominant glomerular changes of GIPR^{dn} transgenic mice at the aged of 8 weeks consisted of mesangial expansion and matrix accumulation. Glomeruli of transgenic mice at the aged of 26 weeks exhibited mesangial expansion – with accumulation of the extracellular matrix proteins collagen type IV, laminin and fibronectin – hyalinosis, adhesions between the glomerular tuft and the capsule of Bowman, both collapse and distension of glomerular capillaries and sometimes cystic appearance of the capsule of Bowman. Tubulo-interstitial lesions included tubular atrophy, interstitial fibrosis and signs of proteinuria. The renal volume of transgenic mice was increased from week 8 onwards, as compared to controls. At the age of 26 weeks, renal enlargement was most pronounced. As assessed by quantitative stereological methods, glomerular hypertrophy first occurred in GIPR^{dn} transgenic mice at the aged of 8 weeks, reaching the most marked enlargement at the aged of 26 weeks vs younger counterparts. The glomerulosclerosis index was augmented in transgenic mice as compared to controls, from week 8 onwards. Glomerular lesions deteriorated up to 26 weeks of age.

Discussion: The present study shows that GIPR^{dn} transgenic animals develop progressive diabetes-associated kidney lesions. We conclude that GIPR^{dn} transgenic mice are a valuable model for studying diabetic kidney disease.

UNUSUAL DEGENERATIVE CHANGES IN THE BRAIN OF TNF-α-TRANSGENIC MICE INFECTED WITH THE NEUROTROPIC BORNA DISEASE VIRUS

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Introduction: TNF-α-transgenic and non-transgenic mice were infected experimentally with the neurotropic Borna Disease virus in order to analyse the effect between a virally-induced immune reaction and neuronal overexpression of TNF-α in the CNS.

Materials and Methods: TNF-α-transgenic and non-transgenic CBA7J x C57/Bl6 mice were infected neonatally with a mouse-adapted strain of Borna Disease virus (BDV). Mice were euthanized between day 7 and day 49. Brains were stained with haematoxylin and eosin and used for immunohistology for the detection of the viral nucleoprotein applying the monoclonal antibody Bo18. "*In situ*" hybridization was carried out to demonstrate TNF-α-specific mRNA. The transgenic construct was amplified by PCR.

Results: The BDV-infected mice showed no obvious clinical signs, except prefinal convulsion in few transgenic animals. The transgenic mice inoculated with BDV developed a nonpurulent meningoencephalitis, microglial activation and reactive astrogliosis. Additionally, mild to moderate vacuolation of the neuropil was present in the nucleus caudatus, cortex cerebri, mesencephalon, hippocampus, cerebellum and thalamus. Single cells adjacent to inflammatory infiltrates show degenerative changes resembling early forms of apoptotic cell death. In the non-transgenic BDV-infected animals strong inflammatory lesions, significant microglial activation and severe degenerative changes were absent. Virus antigen was present throughout the brain in a similar distribution in all infected mice groups. "*In situ*" hybridization revealed expression of TNF-α-mRNA in neurons predominantly in the hippocampus, cortex cerebri, thalamus and striatum of TNF-α-transgenic mice.

Conclusion: Interestingly, neuronal overexpression of TNF-α in combination with BDV-infection caused vacuolation and cellular degeneration in the mice brain in addition to the inflammatory lesions. Since these alterations were absent in non-transgenic BDV-infected mice, transgenic neuronal TNF-α-expression and the virally-induced immune response might be necessary for the induction of degenerative lesions in the mice brain.

AN OUTBREAK OF HERPESVIRUS INFECTION IN A COLONY OF MEDITERRANEAN TORTOISES: HISTOPATHOLOGICAL AND ULTRASTRUCTURAL FINDINGS

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Introduction: Respiratory diseases of chelonians are commonly present in captive tortoises both in aquatic and terrestrial species. Stomatitis-rhinitis disease associated with herpesvirus infection represents one of the most important respiratory conditions especially in Mediterranean tortoises. About 50 Mediterranean tortoises (*Testudo hermanni* and *Testudo graeca*) and 2 box turtles (*Terrapene Carolina*, recently introduced) from a private collection located in the centre of Italy showed clinical signs of stomatitis-rhinitis disease and died in a period of about 3 months. Two animals were investigated in order to confirm the presence of herpesvirus particles associated with the symptoms and to provide more information on the diffusion of this infection in Italy.
Materials and Methods: Two 8 years old, *Testudo hermanni*, dead immediately after hibernation were submitted for necropsy in different time. The veterinary referred a history of nasal discharge, open-mouthed breathing, conjunctivitis, presence of oral lesions (pseudomembranes) and weakness affecting almost all the 50 dead tortoises. Different tissues were collected and routinely processed for light microscopy (haematoxylin and eosin stained); moreover tracheal and brain samples were processed for electron microscopy.
Results: At the gross examination, two tortoises were cachectic, one showed signs of mucopurulent rhinitis and no caseous membranes were present in the oral cavity or on the tongue. Tortoise with rhinitis had bilateral pulmonary congestion and diffuse parenchymal consolidation. No lesions were seen in other organs. Both tortoises had a massive parasitic infestation of the colon and parasitology revealed the presence of male and female worms referable to *Spironoura concinnae*. Histologically both animals showed mild tracheitis associated with numerous eosinophilic intranuclear inclusion bodies especially in desquamated epithelial cells; one of them had severe bronchopneumoniae. Eosinophilic intranuclear inclusion bodies were also present in neurons and cerebral glia cells associated with moderate gliosis, neuronal necrosis and subependimal gliosis. One of the tortoises had severe and diffuse hepatocellular vacuolar degeneration with multifocal mild heterophilic and lymphoplasmacytic infiltrates. Ultrastructural examination revealed intranuclear viral particles that contain electron-lucent and electron-dense cores, approximately 100 nm in diameter, consistent with herpesvirus.
Conclusion: Herpesvirus induced stomatitis-rhinitis is an emerging disease of chelonians in Italy. Factors inducing stress, such as concomitant parasitic infestations, have been demonstrated to predispose the animal to the development of the disease. Although no definitive conclusions can be drawn, we hypothesise that the newly introduced turtles could have hosted the virus and then spread it to other animals. The diagnosis was made based on histopathological and ultrastructural findings.

SECOND OCCURRENCE OF MAMMARY DUCTULAR ADENOCARCINOMA IN A RHESUS MACAQUE

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Routine examination of a 27 years old female *Rhesus macaque* revealed a solitary, round mass 2.5 cm in diameter in the mammary region just lateral and superior to the left nipple. There was no palpable enlargement of axillary lymph nodes. Four years prior, this macaque had a 2.5 cm mammary ductular adenocarcinoma removed from the right breast. For this second occurrence, diagnostic workup included thoracic and abdominal radiographs, aspiration cytology of the mass, and blood screening. No masses or metastases were visible on radiographs. Aspiration cytology revealed clusters of epithelial cells with relatively uniform appearance. Blood screening revealed only mild elevations of BUN, ALT, and AST. Surgical excision of the mass was performed. The mass was confined to the subcutis and easily excised. There was a uniform tan appearance on cut section with little to no visible capsule. Histopathologic diagnosis was ductular adenocarcinoma. Five months later there were no palpable mammary masses and no axillary lymph node enlargement. Thoracic radiographs remain normal with no visible masses or metastases. To our knowledge, this is only the eleventh case of spontaneous mammary gland tumors to be reported in rhesus monkeys, and the first case of a single monkey with two separate occurrences of mammary neoplasia.

SOMATOSTATIN-IMMUNOREACTIVE NERVE STRUCTURES IN THE ILEUM AND LARGE INTESTINE OF PIGS UNDERGOING DYSENTERY

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Introduction: Immunohistochemical studies dealing with inflamed intestines, especially in case of acute inflammation undergoing with diarrhoea, are only fragmentary, so aim of the present study was to investigate immunohistochemical properties of nerve structures in the ileum and large intestine in pigs suffering from dysentery.
Materials and Methods: The study was performed on nine, 5 months old pigs divided into control group consisting of clinically healthy animals and experimental group consisting of pigs which were infected *per os* with *Brachyspira hyodysenteriae*. The diarrheic pigs were dehydrated, profoundly weak, gaunt and emaciated. All the animals were deeply anaesthetised and perfused transcardially with 4% paraformalehyde. Collected tissues included ileum, cecum, centripetal and centrifugal turns of the spiral colon and descending colon. For further studies the intestines from 3 individuals showing the most developed pathological changes was taken. The cryostat sections of the intestines were processed for double-labelling immunohistochemistry using antisera against protein gene product 9.5 (PGP 9.5) and somatostatin (SOM).
Results: Increased number of SOM-IR perykaria was encountered in the dysenteric pigs as compared to that in animals control in both myenteric plexus (MP) of all intestines studied, and in outer submucous plexus (OSP) of the cecum and centripetal turns. In OSP of ileum and centrifugal turns, the percentage of SOM-IR neurons did not change, whereas in OSP of descending colon the number of SOM-IR perycarya decreased. The inner submucous plexus (ISP) of all intestines studied contained decreased number of SOM-IR nerve cell bodies except ileum, where percentage of SOM-IR neurones was not changed. In all layers of intestines under investigation namely: muscular coat, plexuses (MP, OSP, ISP) and mucous membrane, the number of SOM-IR nerve fibres was lower in the dysenteric animals.
Conclusion: Decreased number of SOM-IR nerve fibres in the dysenteric porcine intestine, and the increased number of SOM-positive neurons can indicate an inhibition of release of somatostatin from intestinal nerve endings which may escalate symptoms of diarrhea (lack of SOM neutralizes inhibitory effects of SOM on enteric functions, especially on the motility and absorption).

INTRAPELVIC RENAL NEPHROBLASTOMA IN A MEERKAT (*SURICATA SURICATTA*)

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Introduction: Nephroblastoma or Wilms' tumour is a primary renal tumour, which occurs in children and has been reported in number of animal species. Intrapelvic renal nephroblastoma is a rare variant of this tumour type in human patients but has not previously been reported in the veterinary literature. This report describes the histological and immunohistochemical features of intrapelvic renal nephroblastoma in a captive meerkat (*Suricata suricatta*).
Materials and Methods: A 7 months old female meerkat from Bristol zoo gardens was found dead, and a post-mortem examination was performed. Appropriate tissue samples were collected, processed routinely for microscopic examination and stained with haematoxylin and eosin. Immunohistochemical labelling were performed using antibodies against cytokeratin, vimentin, desmin, smooth muscle actin (SMA), S-100, neuron-specific enolase (NSE) and glial fibrillary acid protein (GFAP).
Results: Gross examination revealed an enlarged and pale left kidney which when sectioned contained a large white mass. Microscopically, the papillary neoplastic mass markedly expanded the pelvis and was covered by pelvic transitional epithelium. The tumour mass was comprised of three cell types: primitive renal blastema, epithelial cells in glomerular and tubular arrangements and mesenchymal stroma. All the cellular components showed diffuse positive cytoplasmic immunolabelling for vimentin. There were small numbers of desmin-positive cells in the stroma.
Conclusion: The gross, histological and immunohistochemical findings were consistent with a diagnosis of triphasic intrapelvic renal nephroblastoma. To the authors' knowledge this is the first report of spontaneous nephroblastoma involving the renal pelvis in a nonhuman species.

COINCIDENCE OR ASSOCIATION? INVESTIGATION OF AN EQUINE INFLUENZA CASE EXHIBITING ENCEPHALOPATHY

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Introduction: Equine influenza is usually a transient and self-limiting disease. However, during an outbreak of equine influenza in the UK during 2003, there were reports of unusually severe clinical signs among unvaccinated animals including two horses with neurological signs, one of which was euthanized. Neurological involvement during influenza infection of people has been described and is often associated with serious sequelae or death. It remains unclear whether influenza virus may directly damage nervous tissue. Recent results suggest that influenza-associated encephalopathy might be a consequence of high plasma concentrations of cytokines.

Materials and Methods: A novel immunohistochemical test for demonstration of equine influenza antigens in formalin fixed paraffin embedded tissue was developed. Influenza-naïve ponies were experimentally challenged by exposure to a nebulised aerosol of influenza A/eq/Newmarket/5/03 (H3N8) virus. Clinical signs were monitored following challenge. Interferon alpha levels in nasal swabs and serum samples taken after challenge were measured using a bioassay.

Results: An Irish Draught mare presented with clinical signs of nasal discharge and lethargy, progressing to respiratory distress, marked depression and ataxia that necessitated elective euthanasia on the ninth day of disease. A nasal swab collected on the eighth day after the onset of clinical signs was positive for equine influenza antigen by nucleoprotein ELISA. The head and neck were submitted for post mortem examination. Gross post mortem examination revealed excessive mucus coating the nasal passages and nasopharynx, with enlargement of the upper respiratory tract-associated lymph nodes. The brain was markedly congested. Histological examination of the upper respiratory tract confirmed mucosal hyperaemia and congestion, with mild to moderate inflammatory changes. Hyperplastic changes were present in local lymph nodes. Sections of brain and spinal cord demonstrated multifocal marked lymphocyte-rich perivascular cuffing, with local gliosis where inflammatory changes were most severe. PCR and immunohistological testing of the affected tissues for equine herpesviruses-1 and 4, and for West Nile Virus proved negative. Results of the application of the immunohistochemical test to the post mortem tissues will be presented. Experimental challenge infection of ponies with the 2003 strain gave rise to more severe clinical signs than are usually seen following challenge infection. Furthermore, not only were levels of inflammatory cytokines significantly elevated in nasal secretions, cytokines were detected in serum samples, which was not observed following challenge infection with other virus strains.

Conclusion: In view of the lack of evidence for an alternative aetiology, the possibility that infection with the currently circulating highly pathogenic strains of equine influenza could give rise to neurological complications cannot be ruled out.

EFFECT OF HOST GENOTYPE ON LESION PROFILE AND PrP^{sc} ACCUMULATION IN NATURALLY AFFECTED SHEEP AND GOATS IN GREECE

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Introduction: The aim of this study was to determine the lesions and the distribution of PrP^{sc} in various regions of the brain of sheep and goats with natural scrapie carrying different PrP genotypes.

Materials and Methods: Eight sheep (2-4 years old) with ELISA confirmed scrapie infection were selected on the basis of their PrP genotype (codons 136, 154 and 171): ARQ/ARQ (n=4), ARQ/AHQ (n=4). Six goats (2-4 years old) with ELISA confirmed scrapie infection was selected on the basis of their PrP genotype (codons 21, 142, 143, 154 and 240): VV_{21}, II_{142}, HH_{143}, RR_{154}, PP_{240} (n=2), VV_{21}, II_{142}, HH_{143}, RR_{154}, SP_{240} (n=2), VV_{21}, II_{142}, HH_{143}, RR_{154}, SS_{240} (n=2). One ELISA negative sheep carrying the ARQ/ARR genotype and one ELISA negative goat carrying the VV_{21}, II_{142}, HR_{143}, RR_{154}, PP_{240} genotype were selected as negative control animals. For histological examination, one spinal cord segment at the level of vertebra C_2 and eight coronal slices of the brain (cerebrum, brainstem and cerebellum) 3-4 μm thick were selected as follows: medulla at the obex and at caudal cerebellar peduncles including the trapezoidal body; middle of the pons; mesencephalon through the rostral colliculi just posterior to the pineal body; middle transverse section of the cerebellum; diencephalon at the mamillary body and at the hypophyseal infundibulum-optic tract levels; and frontal cortex rostral to corpus callosum. The slices were processed and embedded in paraffin, and sections of 4-6 μm thick were stained with haematoxylin and eosin. Unstained sections from the same samples were stained according to the LSAB method using the L42 monoclonal antibody.

Results: In four scrapie affected sheep (ARQ/ARQ) lesions and deposition of PrP^{sc} were detected in all examined regions. In two of the other four sheep the lesions and deposition of PrP^{sc} were detected only in the obex, pons and diencephalon. In the remaining two sheep the lesions were seen in all regions except in mesencephalon, cerebellum and thalamus. In these sheep PrP^{sc} was detected in the same regions. In two scrapie affected goats (VV_{21}, II_{142}, HH_{143}, RR_{154}, PP_{240}) lesions were seen in all examined regions except the cerebellum, thalamus and frontal cortex. In two scrapie affected goats (VV_{21}, II_{142}, HH_{143}, RR_{154}, SS_{240}) lesions were found only in the obex, pons and thalamus. In the last two scrapie affected goats (VV_{21}, II_{142}, HH_{143}, RR_{154}, SP_{240}) lesions were found in all regions except the spinal cord, diencephalons and frontal cortex. On the contrary PrP^{sc} was detected in all examined regions. Neither lesions nor PrP^{sc} were detected in the controls.

Conclusion: From our results is concluded that there was a different distribution pattern of the brain lesions and PrP^{sc} deposition according to the PrP genotype among sheep and goats.

THE DENDRITIC CELL PATTERN IN CANINE LYMPHOMAS

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Introduction: Dendritic cells (DC) are commonly detected in human lymphomas. These cells may represent the vestiges of dendritic cells network in lymphoid tissue or newly appear as the result of stromal cells differentiation toward DC de novo. It has been postulated that DC may create a microenvironment that promote tumour growth. *In vitro* studies indicate that DC may also protect malignant B cells from apoptosis, including its induction by some anticancer agents. Little is known about DC in canine lymphomas.
Materials and Methods: Forty three lymph nodes obtained from dogs with multicentric lymphoma were included in this study. All specimens were fixed in 10% neutral buffered formalin and paraffin embedded. Lymphoma phenotype was determined by immunohistochemistry using anti-CD3 policlonal antibody and anti-CD79α monoclonal antibody, detecting T and B lymphocytes, respectively. DC were identified by staining with anti-S100 protein polyclonal antibody.
Results: Among 43 lymphomas of various histological subtypes, 14 had T cell phenotype (CD3+CD79α-) and the other 28 were of B cell origin (CD79α+CD3-). S-100 positive cells were identified in all the cases. Both, the number and the distribution pattern of DC varied among cases. On the basis of these differences all cases were classified into 4 patterns: 1 – vaguely follicular organization (DC were arranged in a loose and ill-defined mesh with radiating outline, solitary or confluented); 2 – diffuse organization (single DC were diffusely scattered throughout the neoplastic cells); 3 – mixed organization (characterized by the presence of both: scattered DC and DC arranged in a loose and ill-defined spherical meshwork); 4 – diffuse organization with DC aggregates (DC were scattered but also located in clusters of various size and shape, usually well-defined). Pattern 1 was observed in 13 of 43 lymphomas, all of them had B cell phenotype; pattern 2 was identified in 18 cases, 10 tumours of T cell and 8 of B cell origin. Five lymphomas were assessed as pattern 3, one had T cell and 4 had B cell phenotype. Pattern 4 was found in 6 cases (3 tumours of each phenotype). The number of S-100 positive cells also varied among cases. In some samples positive staining was evident only in single cells, but in the other, number of DC was high and constituted a dense network. The most significant variations occurred in lymphomas with diffused organization of DC (pattern 2).
Conclusion: DC are frequently identified in canine lymphomas, often as a dense network. DC architectural pattern vary between B and T cell lymphomas, however, it was not possible to identify the unique pattern which might have been used as a criterion for discriminating between these phenotypes of lymphoma.

IMMUNOHISTOCHEMICAL STUDY OF PrP^{sc} IN NEURAL, LYMPHOID AND OTHER PERIPHERAL TISSUES OF A CAT WITH FELINE SPONGIFORM ENCEPHALOPATHY

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Introduction: Feline Spongiform Encephalopathy (FSE) is a prion disease affecting domestic cats and other feline species. Lesion profile, transmission experiments in mice and western blotting studies suggest that FSE is caused by the same strain which causes BSE in cattle and nvCJD in humans. In a recent study, PrP^{sc} was detected in lymphoid organs (spleen, Peyer's patches) and kidneys of cats affected with FSE.
Materials and Methods: A domestic shorthair cat presented with progressive hind-limb ataxia and increased aggressiveness was necropsied. A wide spectrum of tissue samples representing the nervous system, the lymphiod system and other peripheral tissues was collected and stained by haematoxylin and eosin (HE). The presence and the distribution of PrP^{sc} were investigated by immunohistochemistry (IHC) using 2 mouse monoclonal andibodies (mAbs: 34C9 and 6H4) and a polyclonal antibody (pAb: C15S).
Results: Histopathological changes consisted of a diffuse vacuolation of the grey matter neuropil, vacuolation of neuronal perikarya and gliosis. A strong PrP^{sc} immunostaining was detected in brain (grey matter neuropil of brain stem, cerebrum and cerebellum), retina (inner and outer plexiform layer, rod and cone layer and ganglion cell layer), optic nerve, pars nervosa of the pituitary gland, trigeminal ganglion and myenteric plexus. In addition, some positive labelling was demonstrated in spleen (lymphoid follicles) and kidney (tubular epithelial cells) by the use of the pAb, but not by the mAbs. Nictitating membrane, nasal mucosa, salivary gland, heart, pancreas, thyroid gland, parathyroid gland, striated muscle and bone marrow were negative.
Conclusion: The PrP^{sc} distribution within the brain is similar to that described in other FSE-affected cats. The pattern of abnormal PrP in the retina corresponds to that found in a captive cheetah with FSE, in sheep with scrapie and in nvCJD in humans. The immunoreactivity in the spleen and kidney is probably the result of an unspecific staining, since it was observed solely using the polyclonal antibody.

EFFECT OF MICROSPHERE SIZE AND TEMPERATURE ON THE UPTAKE BY EXCISED RAT ILEUM MAINTAINED IN A NOVEL SYSTEM OF DIFFUSION CHAMBERS

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Introduction: Use of microspheres in gastrointestinal absorption studies is well established. Their uptake is influenced by particle size and, in the case of small intestines, whether or not Peyer's patches (PP) are present. The aim of present study was to investigate the functionality of diffusion chambers by studying the uptake of 0.5 and 1.0 μm microspheres as a function of time and temperature in excised ileal PP and adjacent non-Payer's patches (NPP) tissue.

Materials and Methods: Freshly excised ileal PP and NPP tissues from adult male Wistar rats were mounted vertically in diffusion chambers. Suspensions of 1.7×10^8 and 3.4×10^7 FITC-labeled carboxylated 0.5 and 1.0 μm latex microspheres/ml in Krebs-Henseleit solution were added to the mucosal compartment. Tissues were incubated at 37°C for 5, 10, 20, 40, 60 min. and at 4°C for 60 min. Tissues were subjected to quantitative and qualitative analysis by fluorescence microscopy. Quantitative data was analyzed by repeated measure ANOVA and the interaction of microspheres size, tissue and time was assessed.

Results: There was no uptake of 0.5 or 1.0 μm microspheres at 4°C. In PP at 37°C, microspheres were absorbed into the follicle-associated epithelium (FAE) surface, within FAE and in lymphoid tissue in a time dependent fashion. In NPP, microspheres were present in the villous epithelium, lamina propria, pericryptal stroma and the cryptal epithelium also in a time dependent manner. Absorption of microspheres was significantly enhanced in ileum containing PP as against ileum without PP (Table 1).

Table 1. Mean microspheres percentage[1,2] association with excised rat ileal PP and NPP at 37°C.

Tissue	Microsphere diameter, μm	Time, min.				
		5	10	20	40	60
PP	0.5	0.08±0.004	0.14±0.007	0.19±0.021	0.25±0.080	0.36±0.010
	1	0.03±0.002	0.05±0.003	0.09±0.004	0.14±0.005	0.18±0.004
NPP	0.5	0.05±0.006	0.09±0.003	0.11±0.004	0.13±0.010	0.14±0.011
	1	0.01±0.001	0.02±0.001	0.03±0.002	0.05±0.003	0.05±0.003

[1]Microsphere size, tissue, time and their interactions were different at $p<0.0001$, n=10 each time point.
[2]Percentage = (number of microspheres in homogenized tissues/number of microspheres at 0 min.) x 100.

Conclusions: Excised rat ileal tissues retained its capacity to absorb microspheres when maintained in diffusion chambers. Particle uptake appears to be a rapid, energy- and time- dependent process. The results indicate that rat ileal tissue can be successfully maintained *in vitro* in diffusion chambers for the purpose of absorption studies.

MYXOMA OF THE SMALL BOWEL IN 14 YEARS OLD GELDING

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Introduction: Reports of equine myxomas are rare. In those very few ones anatomical locations have been such as nasal cavity, respiratory tract and mandible but not intestine. We report a case of equine jejunal myxoma.

Materials and Methods: A 14 years old pony gelding was referred to the University of Helsinki Equine Hospital. The gelding had an 8 h history of mild to moderate abdominal discomfort that has not been responsive to routine medical care. Six hours after presentation an exploratory laparotomy was performed. Intussusceptions approximately 20 cm in length was found in the mid jejunum. It was manually reduced but a firm palpable mass was present in the bowel wall which partially occluded the lumen. Approximately 50 cm of jejunum was resected. Specimen of resected intestine was fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned and stained with haematoxylin and eosin, alcian blue, and PAS. Immunohistochemistry for vimentin, cytokeratin and protein S-100 antigens were performed with commercial detection kit.

Results: On presentation the gelding was bright and alert with no obvious signs of abdominal discomfort. Routine clinical examination showed: heart rate of 40, respiratory rate 60, no gastrointestinal sounds, mucous membranes were pink and moist. Rectal examination was unremarkable. Abdominal ultrasound revealed several loops of dilated small intestine (up to 5 cm) with minimal motility. Peritoneal fluid was within normal limits. Analyze of haematology and electrolytes showed mild azotemia, polycythemia and hypocalcemia. Post operatively the gelding did well and complications were limited to a mild incisional infection and thrombophlebitis of the left jugular vein. The gelding was re-examined one month after discharge and was in good body condition and had no further episodes of colic reported. Echocardiography performed at that time was unremarkable. A 16 cm long piece of resected intestine was received for histopathological examination. At the opposite side of mesentery was located approximately 8.5 cm long nodular peduncultaed tumour which almost totally occluded the jejunal lumen. The mucosal surface was dark and cut surface showed gelatinous appearance of the mass. Microscopically, *tunica mucosa* showed congestion and autolytic changes, *tella submucosa* was filled with alcian blue and PAS positive matrix which also forced between muscle cells in *tunica muscularis*. Scant number of stellate tumour cells was located in the mucoid matrix. The cells did not showed nuclear atypia and no mitotic activity was recorded. The tumour cells were vimentin and protein S-100 positive. The tumour was classified as myxoma.

Discussion: Myxoma is most often reported as a heart tumour of man. Also the few reports of intestinal myxoma are from man and some are associated with cardiac tumour as well. In our case myxoma has caused intestinal intussusceptions which had a sequela of obstruction. The histological appearance of the tumour cells was absolutely benign and bland.

DEGENERATION OF THE VAGUS NERVE DUE TO TUMOUR INVASION – A RETROGRADE LYMPHATIC METASTASING PRIMARY LUNG CARCINOMA?

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Introduction: In a cat with a history of wheezing, coughing, vomiting and endoscopically diagnosed unilateral laryngeal paralysis a pleomorphic pulmonary and mediastinal tumour was found. Our observations demonstrate the correlation between tumour growth and neuronal (vagal) alteration.
Materials and Methods: Necropsy was performed on a 10 years old, male, serologically FIV-positive Maine Coon cat. Among other tissues, lung, precardiac mediastinum, trachea, larynx and accompanying nerves and vessels were fixed in 10% formalin and paraffin embedded. Serial transversal sections of the cervical and thoracic region (larynx, precardiac mediastinum, lungs) were examined. In addition, immunohistology with double labelling of cytokeratin and a glial marker (GFAP) was performed to further characterize the tumour cells and to demonstrate the neuronal lesions.
Results: Necropsy revealed multiple solid, unencapsulated, whitish tumour masses of variable size in the lung. The precardiac mediastinum, including vessels and nerves, was occupied by a macroscopically similar tumour mass. On the serosal surface, prominent lymphatic vessels were obvious. Histologically, the lung tumour was a solid to tubuloacinar, multifocally bilayered, highly invasive carcinoma. The cuboidal to cylindrical tumour cells demonstrated a high percentage of atypia and three to five mitoses per high power field. Mediastinal serial transversal sections revealed focal axonal degeneration of the left vagus nerve due to perineural tumour cell invasion with extensive desmoplasia. Along the neck lymphangiosis carcinomatosa and metastases in regional lymph nodes were detected. Abundant cytokeratin-positive cells close to GFAP-positive remnants of nerve tissue could be demonstrated.
Conclusion: A unilateral vagal degeneration with consecutive hemiplegia laryngis was caused by tumour invasion into the precardiac mediastinum. The “diagnostic dilemma” in this case was to differentiate between the two pathogenetic possibilities: a primary lung carcinoma with evident retrograde lymphatic spread or a tumour deriving from epithelial tissues along neck and head (i.e. salivary glands, dispersed thyreoid tissue, parathyreoidea or other branchiogenic epithelium) with pulmonary metastasis. Because neither morphologically nor immunohistologically any other epithelial tumour could be detected, a bronchial adenocarcinoma with retrograde lymphatic metastasis seems to be possible.

EQUINE ABORTION CAUSED BY ENCEPHALITOZOON CUNICULI

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Introduction: Aim of the study was to describe the results of gross, histological, immunohistochemical, ultrastructural and molecularbiological investigations of a case of equine abortion caused by *Encephalitozoon cuniculi*.
Materials and Methods: Bacteriological, serological, light microscopical, electron microscopical and PCR examination were made for detecting microorganisms and lesions in the fetus and fetal membrane.
Results: Focal lympho-histiocytic hepatitis and multiplex focal lympho-histiocytic villitis accompanied by villus necroses and marked hypertrophy of chorionic epithelial cells in the arcades was observed. Elongated nucleated organisms (1-2 mm) were seen in groups in vacuoles or solitary situated in the cytoplasm of the chorionic epithelial cells. The organisms were in large number and often extra cellular in areas of villitis and villus necroses. They were Gram-positive, stained with haematoxylin and eosin (HE), PAS, and Giemsa, but they were not stained with Gomori methenamine silver staining, and only weakly stained with Warthin-Starry silver staining. Ultrastructurally the organisms were identified as Microsporidia, which were proved to be Encephalitozoon by the molecular means. The organisms reacted strong with *E. cuniculi* specific rabbit sera used for immunohistochemistry. No *E. cuniculi* was detected in the fetal organs. Few Chlamydia was also present in the chorionic epithelial cells, but no histological lesions were obvious in conjunction with its presence.
Conclusions: This is the first reported case of *E. cuniculi* induced equine abortion in Europe. Although *E. cuniculi* induced abortion is rare, Microsporidia should be considered a differential diagnosis for intracellular organisms observed in chorionic epithelial cells of horses.

RHABDOMYOSARCOMA IN A BUDGERIGAR (*MELOPSITTACUS UNDULATES*) (THE FIRST CASE REPORT FROM IRAN)

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Introduction: Rhabdomyoma or rhabdomyosarcoma are names given respectively to benign or malignant neoplasm's arising from striated muscle, skeletal, or cardiac. Although known in many animal species but it is in frequent. Rhabdomyosarcoma has been reported to arise from skeletal muscle of the tongue, pharynx, and panniculus, and from the myocardium and urinary bladder of dog which are locally invasive and tend to metastases early. Primary tumours of striated muscle are rare. Malignant striated muscle tumours are twice as frequent as benign ones, and about half of the striated muscle tumours in domestic animals arise from sites other than skeletal muscles. Rhabdomyosarcoma was reported from budgerigar (Petrak 1969, Raphael and Nguyen 1980) and fowl.

Materials and Methods: A tumour mass was found on the muscle of left thigh of a male budgerigar. According to the owner, a swelling was presented in the left thigh area, and has been enlarging rapidly during a month. Physical examination revealed oval, moderately firm mass, about 2.5 cm in diameter in the region of the left thigh. The bird was weak and died following day. This tumour mass was separated from soft tissue and bone of left thigh. Tumour mass was fixed in a 10% buffered formalin solution. After fixation and tissue processing, 7 μm paraffin sections were stained with haematoxylin and eosin. The special staining was carried out for differentiating from other mesenchymal malignant tumour of connective tissue.

Results: Necropsy revealed a roughly oral man measuring 2.5 x 1.5 x 1 cm in the muscle of left thigh, the external surface of the mass was pale red and grey on cut surface. Tumour was poorly circumscribed and was not encapsulated. There were no metastases. At the cut surface, the evidence of necrosis and invasion of the adjacent tissue were observed. The microscopic appearance was similar to that of a fibrosarcoma. The tumour cells were poorly difference. Large vacuolated cells (spider cells) were present. Mitotic figures were numerous, nuclei varied greatly in size and pleomorphism in tumour cells were seen, cell out-lines were not clear. The mass contained irregular areas of necrosis.

Conclusion: According to the microscopic characteristic of this tumour, neoplasia diagnosed as a pleomorphic type of rhabdomyosarcoma. This finding was confirmed by Van Gieson special staining. In the stain the cytoplasm of tumour cells and skeletal muscle as well as epithelium cells stained yellow and collagen fibres stained red.

OCCURRENCE OF UNUSUAL CONTAGIOUS ECTHYMA (ORF) IN INDOGENOUS GOATS OF IRAN (A CASE REPORT)

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I**ntroduction:** Contagious pustular dermatitis (Orf, Sore mouth) is a common localized infection of young sheep and goats caused by a *Parapoxvirus* with wordwide distribution. Less commonly human beings, cattle wild ungulates, and dogs are infected. Morbidity in lambs is usually great and although mortality is usually low. It can approach 15% in lambs. The lesions usually are seen on lips, nostril and sometimes in mouth and seldom because of development of lesions they can be observed in oesophagous and rumen. This lesions may can be seen in teats and inguinal region, too.
Materials and Methods: A one 4 months old kid with several popular, elevated grayish lesions on lips, around the nostril, fore and hind limbs, inguinal region, flanks and under the abdomen was referred to clinic of faculty of veterinary medicine of Tehran University. After necropsy, several samples from these nodules at different parts of skin, in 4 mm thickness were selected and for histopathological examination fixed in 10% buffered formaldehyd. After fixation and tissue processing 7 µm paraffin sections were stained with haematoxylin and eosin.
Results: The most important clinical signs were anorexia and emaciation. These warty papules subsequently became vesicles and pustules. The popular lesions were seen widely in different parts of the skin. Clinical signs and microscopic lesions made us suspicious to ecthyma. Microscopically the changes consisting of proliferation of the basal layer of epidermis with, spongiosis, hydropic degeneration, vesicle and pustul formation in the upper layers, eosinophilic intracytoplasmic inclusion bodies were seen in these affected cells.
Conclusion: The disease is known in the United States, Europe and Australia. Usually lesions resolve in 2 to 4 weeks, but there are reports of lesions persisting for several months. According to macroscopic and microscopic observations and clinical signs, the disease was diagnosed Contagious Ecthyma certainly, but these lesions exceptionally and widely were observed in whole of the body. At necropsy, abdominal cavity components were tested too, but no lesions were seen.

OCCURRENCE OF CUTANEOUS HAEMANGIOMA IN CHICKEN; THE FIRST REPORT FROM IRAN

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Introduction: Haemangiomas are benign tumour of vascular endothelium. Haemangiomas are characterized by increased numbers of normal or abnormal vessels field with blood. Difficult to distinguish with certainly from malformations or hamartomas. This tumour is common in dog, but rare in other domastic animals. Although this tumour diagnosed in lungs, spleen, kidneys, ovary and gut wall, but they commonly present in skin.
Materials and Methods: This tumour recognized in a broiler chicken approximately
2 months old in one of the slaugtherhouses in Qazvin.
Results: Macroscopically this tumour appears as dark reddish masses with 0.5-1 cm in diameter. These tumour masses are located subcutaneously in thighs. Histopathology revealed that this tumour is a cavernous haemangioma with distended blood vessels with thin walls composed of endothelial cells. Tumour cells are characterized by vesicular nuclei and some of them contain a prominent nucleolus. A few mitotic figures were showed in tumour cells.
Conclusions: Campbell et al. believe that haemangioma particularly occurs in broiler chickens. Sola et al. reported a cutaneous haemangioma in chickens in 1997. Fallavena et al. studied on 800 skin lesions in condemended broiler carcasses. They explained different histologic lesions and also diagnosed haemangioma (0.25%). Gilead et al. isolated retrovirus from haemangiomas in an outbreak of a neoplastic disease in layers in the Jerusalem area.

ENDOCRINE AND METABOLIC CHANGES IN *ANGUILLA ANGUILLA* L. FOLLOWING EXPOSURE TO β-NAPHTHOFLAVONE – A MICROSOMAL ENZYME INDUCER

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Introduction: Previous research work concerning mammals showed that typical microsomal enzyme inducers affected thyroid function. Nevertheless, to our knowledge, there are no studies on fish concerning the effects of this type of chemicals over the hypothalamo-pituitary-thyroid axis. The information of the effects of microsomal enzyme inducers on corticosteroid hormones is also scarce. The purpose of the present study was to investigate the effects of β-naphthoflavone (BNF) on the *Anguilla anguilla* L. (eels) plasma thyroid-stimulating hormone (TSH), free triiodothyronine (T3), free thyroxine (T4), cortisol, as well as glucose and lactate levels.
Materials and Methods: Eels were exposed during 24 and 48 h to 2.7 μM of BNF, a known microsomal enzyme inducer. The determination of cortisol, TSH, T3 and T4 were performed in plasma, using diagnostic ELISA direct immunoenzymatic kits (Diametra, Italy). Plasma glucose was measured according to the method modified from Banauch et al. (1975). Plasma lactate was determined according to the method modified from Noll (1974).
Results: BNF significantly decreased eels plasma T4 levels, whereas TSH, T3 and cortisol plasma remained constant. However, plasma glucose levels were significantly increased, demonstrating that intermediary metabolism has been affected.
Conclusion: Eels revealed a similarity with the known mammal responses in terms of plasma T4. These results demonstrate that typical microsomal enzyme inducers, namely BNF, can also be important endocrine disruptors. BNF affected the intermediary metabolism since plasma glucose levels were significantly increased; however, it seems difficult to establish a correlation between cortisol, thyroid hormones and carbohydrate metabolism. In the future, it would be important to evaluate BNF effect along a wide exposure length and different concentrations in order to better understand its effects in the studied parameters. It would also be of interest to assess thyroid hormones conjugation and deconjugation. The present results confirm the studied parameters as important biomonitoring tools to assess the presence of stressors in aquatic environment. Additionally, they provide significant information concerning fish intermediary metabolism and endocrine responses to complex environmental mixtures.

PHYSIOLOGICAL AND GENOTOXIC RESPONSES OF EUROPEAN EEL (*ANGUILLA ANGUILLA* L.) AFTER SHORT-TERM EXPOSURE TO CHROMIUM OR COPPER – THE INFLUENCE OF PRE-EXPOSURE TO A PAH-LIKE COMPOUND

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Introduction: Heavy metals are widespread contaminants released into aquatic systems from numerous anthropogenic sources, constituting a serious threat to fish populations. In the present work alterations on the endocrine function were evaluated as plasma levels of cortisol, thyroid-stimulating hormone (TSH), free triiodothyronine (T3), and free thyroxine (T4). The intermediary metabolism was assessed as plasma glucose and lactate concentrations. The frequency of erythrocytic nuclear abnormalities (ENA) was determined as a genotoxicity indicator. The same responses were studied in chromium- (Cr-) or copper- (Cu-) exposed fish following a β-naphthoflavone (BNF) pre-treatment, simulating a sequential exposure to a PAH and heavy metals, in order to assess the interaction between these two classes of compounds.
Materials and Methods: *Anguilla anguilla* L. was exposed during 24 h to chromium (Cr – 100 μM and 1 mM) or copper (Cu – 1 μM and 2.5 μM), with or without a 24 h pre-exposure to BNF – 2.7 μM, a PAH-like compound. Plasma cortisol, TSH, free T3 and T4 were performed by ELISA direct immunoenzymatic methods. Plasma glucose was measured according to the method modified from Banauch et al. (1975). Lactate was determined according to the method modified from Noll (1974).
Results: The single exposure to the highest Cu dose induced an increase in plasma cortisol level. Regarding the BNF pre-exposure influence, an antagonistic effect over the Cu-induced cortisol increase seems to occur. Plasma T4 revealed to be a more responsive parameter than TSH and T3. Moreover, the T4 Cu-induced decrease was potentiated by BNF pre-exposure, suggesting a synergistic interaction. Considering Cr, the same mechanism seems to occur for the lowest concentration. ENA induction was only observed for BNF + 2.5 μM Cu exposure probably indicating a synergism of these sequential treatments.
Conclusion: Eels cortisol levels were significantly increased only by the highest Cu concentration. Though, an antagonistic interaction between BNF and this Cu concentration were observed. Thyroid function is affected by both concentrations of the two tested heavy metals. Eels carbohydrate metabolism seems to be affected as the highest copper concentration increased glucose whereas the lowest copper concentration increased plasma lactate. Genotoxicity, expressed as an ENA increase was only observed at the highest Cu concentration in BNF pre-exposed eels. Metabolic alterations caused by the eel's previous exposure to PAH-like compounds may alter some of their physiological responses to Cr and Cu in a synergistic or antagonistic way. Therefore, the interpretation of heavy metal effects in environmental contaminated waters should always take into consideration, the fish metabolic pre-disposition resulting mainly from other contaminants pre-exposure, namely PAHs.

INTERNAL HYDROCEPHALUS AND GLIOMATOSIS CEREBRI IN AN ENGLISH BULLDOG

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Introduction: Gliomatosis cerebri is well-recognised in human medicine and is characterized by widespread infiltration of the neuropil by neoplastic glial cells, generally with preservation of the normal brain architecture; it is classified by the World Health Organisation (WHO) as a neuroepithelial neoplasm of unknown origin. This report describes a case of bilateral internal hydrocephalus associated with gliomatosis cerebri in 6 years old, male English bulldog, which presented with signs of ataxia, syncope and collapse.

Materials and Methods: Full post mortem and histological examinations were performed. Tissue for histological examination was fixed in 10% buffered formalin, routinely embedded in paraffin and sections were taken for microscopic examination (haematoxylin and eosin stain). Immunohistology was performed for the demonstration of astrocytes (glial fibrillary acidic protein), microglial cells (MHC class II), neurofilament and cytokeratin.

Results: Gross findings were limited to a moderate, bilateral, internal hydrocephalus. Histological examination identified a neoplastic infiltrate, morphologically compatible with gliomatosis cerebri (neoplastic cells negative for neurofilament, cytokeratin, GFAP and MHC II), encompassing the third ventricle and extending to the lateral ventricles, with only focal infiltration of the adjacent parenchyma. It was, in areas, associated with moderate hyperplasia of the overlying ependyma. The third ventricle was narrowed due to protrusion of the neoplastic infiltrate. The infiltrate itself was enveloped by astrocytes and associated with focal microgliosis.

Conclusion: Gliomatosis cerebri is rare in dogs. In most cases, there is massive infiltration of the brain parenchyma at the time of diagnosis (Porter et al. 2003). In the present case, however, the neoplastic infiltrate was not extensive. Nonetheless the animal presented with neurological signs, which may have been due to internal hydrocephalus. The location and growth pattern of the neoplastic infiltrate suggests an association with the hydrocephalus, however, as the animal was brachycephalic, we cannot entirely exclude the possibility that the hydrocephalus was a separate, concurrent entity. Immunohistological findings on the neoplastic infiltrate confirm those of a recent study on canine gliomatosis cerebri (Porter et al. 2003), suggesting that these neoplasms are not of glial origin.

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ECCRINE CARCINOMA OF THE FOOTPAD IN A DOG

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Introduction: Eccrine sweat glands are normally located in the footpads of dogs and eccrine carcinoma is extremely rare, as too few cases have been well documented in veterinary literature.
Material and Methods: A 9 years old, male bobtail dog was referred for the evaluation of right front leg lameness of 2 months duration, due to swelling and ulcerated mass of the 5th digit. During clinical examination, besides the firm, painfull cutaneous mass and the radiographic evidence of 3rd phalangeal bone lysis, no other abnormalities were detected. Incisional biopsy was performed and the neoplastic tissue was fixed in 10% formalin and processed routinely. Sections were cut at 4 μm and stained with haematoxylin and eosin for histopathological evaluation. An immunohistochemical study was performed for cytokeratin (ck 8, 18, 19), epithelial membrane antigen (EMA), S-100, carcinoembryonic antigen (CEA), desmin and smooth muscle actin (SMA). As therapy, 5th digit amputation was performed.
Results: The histological examination showed neoplastic epithelial pleomorphic basaloid cells arranged in irregular cords, tubules or/and acini, in one or multiple layers. The cells had amphophilic or eosinophilic cytoplasm, large hyperchromatic nuclei, prominent nucleoli and increased mitoses. In some areas a reactive abundant stroma was seen. Invasive growth patterns were observed in the deep dermis and also the phalangeal bone. Neoplastic cells in tissue sections strongly expressed cytokeratins, weakly positive was in some areas S-100, whereas EMA, CEA, SMA and desmin were negative. Cytokeratins 8 and 18 are normally co-expressed and found in ductal and glandular epithelia, which helps to distinguish between eccrine and squamous cell carcinoma, suggesting sweat secretory differentiation.
Conclusions: Eccrine carcinoma has to be differentiated also from other rare adenocarcinomas which metastasize to the skin of digits and immunohistochemistry is useful. Although high aggressiveness of the tumour with rapid metastasis to lymph nodes and subcutaneous tissues of the affected limb is reported in the literature, no evidence of recurrence or metastasis has been noticed to date (9 months post-operatively) in our case.

CYTOLOGY, HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY OF METASTATIC LIPID-RICH MAMMARY CARCINOMA IN A DOG

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Introduction: According to latest international histological classification of domestic animal tumours, canine mammary lipid-rich carcinoma is a special type, extremely rare and reported also as a type of canine inflammatory mammary carcinoma. Lipid-rich carcinoma is an aggressive tumour with a very poor prognosis.
Material and Methods: An 11 years old Doberman spayed bitch was presented because of a cutaneous plaque of 12 cm in diameter in the left axillary region, an enlargement of the prescapular lymph node and marked oedema in the affected extremity. Seven months ago, tumours from 3 mammary glands were surgically removed (right regional mastectomy of glands 4 and 5, left aggressive lumpectomy between 1st and 2nd thoracic gland) and sent to a pathologist who made a diagnosis of carcinoma. Moreover, no erythema, oedema, firmness or warmth of the mammary glands was noticed. At presence, FNA of affected lymph node and skin punch biopsy from the mass were performed. Cytology smears were stained with Diff-Quik. The tissue sample was fixed in 10% neutral buffered formalin and processed for routine histologic and special immunohistochemical evaluation.
Results: Cytology indicated neoplasmatic infiltration of the lymph node and skin by round to polygonal pleomorphic cells, round to oval nuclei with large variation in nuclear size and density and prominent nucleoli. The neoplastic cells, individual or in small clusters, resembled mainly epithelial and less mesenchymal origin (spindle cells) and moreover, most of them had abundant foamy cytoplasm and some had large vacuoles displacing nucleus at the edge (signet-ring cells). Mitotic figures were seen. Diagnosis of malignancy was made and differential diagnosis of pleomorphic liposarcoma or lipid-rich carcinoma was set. Histopathology of haematoxylin and eosin sections revealed dermal infiltration of neoplastic cells with marked atypia, arranged in a solid-alveolar pattern, having an abundant clear or light eosinophylic foamy cytoplasm. Neoplastic emboli were seen in dilated dermal lymphatic vessels. Some neoplastic nests had mild infiltration of PMNs. The diagnosis of lipid-rich mammary carcinoma was made. The tumour was negative for PAS-diastase, indicating that clear cytoplasm was not due to glycogen storage. Moreover, the neoplastic epithelial cells immunohistochemically were positive for cytokeratin (8, 18, 19) and oestrogen receptor was expressed by most tumour cell nuclei, whereas tumour cells were negative for vimentin and progesterone receptors.
Conclusions: Lipid-rich carcinoma is not commonly reported in the veterinary literature and moreover, precise characteristics in cytological specimens are not well defined and immunohistochemical data concerning hormone receptors are controversial. Probably many cases seen in routine histologic sections are underestimated, while unfixed specimens are required in order to detect lipid with special stains.

CUTANEOUS HISTIOCYTOMA WITH LEISHMANIA-LADEN NEOPLASTIC CELLS IN A DOG

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Introduction: Canine cutaneous histiocytoma is a common, benign cutaneous (epidermal Langerhans cell) neoplasm of the dog, representing approximately 5.5% of all skin tumours. Leismaniasis (endemic in Greece) is a severe, often fatal, systemic disease, which is rarely presented with an atypical nodular form. Recently reported, *Leishmania* positive dogs had concurrent neoplastic disease and *Leishmania amastigotes* was easily cultured from diverse neoplastic tissue. The parasitization of TVT cells by *Leishmania spp*. has also been documented, suggesting the histiocytic origin of the neoplasm.
Materials and Methods: A 12 years old, male, boxer dog was examined by the referring veterinarian for a 2.5 cm in diameter cutaneous ulcerated nodule of 2 months duration, located in the left thigh region. No other systemic clinical sings were noticed. Initially a FNA was performed and furthermore the tumoural nodule was surgically resected, fixed in 10% formalin and processed routinely, 4 μm sections were obtained and stained with haematoxylin and eosin (HE) and Giemsa. Immunohistochemistry was applied using monoclonal antibodies for CD1a. Leishmaniasis was diagnoses by IFA and bone marrow PCR techniques.
Results: Cytological examination of FNA samples from the nodule, revealed population of characteristic cells with a round to oval nucleus and light blue cytoplasm lacking vacuoles and granules, having frequent mitoses and moreover a less number of "butt cells", as well as few PMNs, plasma cells and lymphocytes were seen. Presence of *Leishmania amastigotes* within neoplastic histiocytic-like cells was observed. On the basis of light microscopic characteristics on HE stained sections, cutaneous histiocytoma was diagnosed. The tumour was characterized by a non-encapsulated, poorly demarcated, diffuse proliferation of fairly monomorphic roundish cells, with an eccentric, oval to cleaved nucleus, with inconspicuous and small nucleoli, and an abundant, pale blue, non-vacuolated cytoplasm. Numerous mitotic figures and binucleated cells were present. Some areas of secondary infiltration with few PMNs, plasma cells and lympocytes were seen. Immunohistochemistry of tumour sections showed numerous macrophages staining positive for *Leishmania infantum* and CD1a positivity of the neoplastic cells.
Conclusions: We describe an unusual case of cutaneous histiocytoma with leishmania-laden neoplastic cells in a dog. We suggest that leishmaniosis was pre-existent (with no clinical signs) to the cutaneous histiocytic tumour induction. The neoplastic nodule must be distinguished from the rarely seen cutaneous nodular form of leishmaniasis, which is characterized by dense accumulation in superficial and deep dermis of macrophages with no signs of neoplasia.

HAEMANGIOSARCOMA OF THE CORNEA IN A DOG

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Introduction: Haemangiosarcoma in the dog occurs more commonly in internal sites (spleen, liver, lungs, heart) and skin, presented as multicentric disease or primary tumour with metastasis. Rarely the tumour affects the eyes and is confined to the conjunctiva, orbit, choroid, iris, cornea, sclera and the 3rd eyelid of the dog. Although, it is stated in some textbooks that vascular endothelial neoplasms even of the avascular cornea are seen most commonly in dogs, reviewing the international literature we could not find journal articles presenting canine primary corneal haemangiosarcoma clinical cases.
Material and Methods: A 14 years old, female dog was referred for the evaluation of a corneal mass. Initially, the owner had noticed a small reddish lesion highly vascularized, involving the cornea of the left eye, which grew significantly within a month. On clinical examination, the animal was in a good health condition, besides of the tumour and signs of kerato-conjunctival inflammation. The tumour, about 1.2 cm^2 size, clearly defined and localized exclusively on the corneal tissue, was surgically removed after superficial keratectomy using an operating microscope, fixed in 10% formalin and processed routinely. Sections were cut at 4 μm and stained with haematoxylin and eosin for histopathological evaluation and moreover immunohistochemistry was performed using the following antibodies: vimentin, CD31, CD34 (class II) and von Willebrand's factor.
Results: The histological examination showed a subepithelial, non-encapsulated but well demarcated, mesenchymal neoplasm composed of vascular channels lined by pleomorphic endothelial cells. The tumour had areas of blood-filled vessels with well differentiated endothelium, as well as solid areas with high cellularity consisting of proliferating atypical cells, a marked mitotic index and small capillaries with irregular shapes. Immunohistochemistry confirmed the initial histopathological diagnosis of haemangiosarcoma. The neoplastic cells were negative for cytokeratine and positive for vimentin. The antibodies anti-factor VIII-related antigen and anti-CD34 reacted immunohistochemically moderately or intensely with the neoplastic endothelial cells, whereas CD31 and vimentin labelled very strongly the neoplastic cells.
Conclusions: In our opinion, primary corneal haemangiosarcoma is a rare tumour in dogs. Solar radiation has been suggested in the pathogenesis of haemangiosarcomas of the eyelid in animals. Corneal haemangiosarcoma has been experimentally induced by ultraviolet radiation. Concerning the biological behaviour of the tumour, although haemangiosarcomas show malignancy, no evidence of recurrence or metastasis has been noticed to date (7 months post-operatively) in our case.

SAFETY STUDIES FOR THE ANTIBIOTIC UNIFENICOL 10% IN BROILERS

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Introduction: Unifenicol 10% is an antibiotic (florfenicol a.i.) produced by United Animal Health Ltd. Budapest, Hungary, with a known intense antibacterian activity, recommanded in broilers against *Chlamydia spp*., *Escherichia coli*, *Pasteurella spp*. and *Mycoplasma*. The aim of the study was to assess the consequences of the therapeutic dose (TD), 1 ml Unifenicol 10%/l drinking water, double TD (TDx2), three and five times TD (TDx3, TDx5) upon some bioproductive and health parameters in broilers.

Materials and Methods: The study was carried out along five days (recommanded therapy period) on 5 groups (4 experimental – E and one control – C), 10 individuals each – Cobb 500 hybrid broilers, one week old, as follows: E1: TD; E2: TDx2; E3: TDx3; E4: TDx5; C=no treatment. Water and fodder consumption, daily weight gain were measured. Erythrocytes (E), leukocytes (L) count, hemoglobin (Hb), hematocrit (Ht) values, leukograme (LK) were determined by authomatic MS – 9 VEET analyzer and total plasmatic proteine (P), albumine (A), globuline (G), uric acid (Ua), creatinine (C); GOT, GPT, ALP enzymes by VET SCREEN analyzer. Local and general tolerance were evaluated. After euthanasia, followed post-mortem assessment and microscopic examination (haematoxylin and eosin stain).

Results: The investigations pointed out a good local and general tolerance in E1 and E2 groups but diarrhoea in E3 and E4 groups. Comparative to C group was registered: a limited decrease of appetite for fodders (more evident in E3: -22.82%) and water (more evident in E3: -8.02% and E4: -28.86%) and of daily weight gain (aprox. -23.16% in E2, E3, and E4); no significant ($p>0.05$) and in physiological range dynamics of E, L, Ht, Hb, low amplitude fluctuation of plasmatic P, A, G, not related to dose and in physiological limits; creatinine increase ($p>0.05$) only in E3 (+28.57%) and E4 (+42.85%) groups but in physiological limits; decrease of Ua, significant for $p=0.05$ in E1 (-69%), E2 (-34.37%), E3 (-50%) groups but not in E4 (-25.62%), the values being lower than the psyhological limits; progressive, limited, not significant ($p>0.05$), related to dose increase of GOT (E1: +6.55%, E2: +9.28%, E4: +14.2%) and pronunced and significant for $p=0.05$ in E4 group of GPT (+118.18%); decrease of ALP concentration, significant ($p<0.05$) in E1 (-27.02%), E2 (-31.96%), not significant ($p>0.05$) in E3 (-12.33%) and a not significant increase in E4 (+1.19%). The assessment of post-mortem state and microscopic examination showed: slight liver enlargement, diffuse hepatic degeneration, liver nuclear heterochromatinization, vacuolar degeneration, slight degeneration sings in nephrons and renale corpuscules, slight spleen enlargement, distension and gazeous, respectively brownish foamous content in cecum in individuals from E3 and E4 groups.

Conclusion: Unifenicol 10%, in the framework of the studied parameters, has no registrable adverse effects in broilers at doses until three times higher than the TD.

A MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF RABBIT ILEUM AFTER *ESCHERICHIA COLI* INFECTION

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Introduction: Enteropathogenic *Escherichia coli* (EPEC) is an attaching and effacing (A/E) pathogen. EPEC strains belonging to serotype O103:K-H2 strains and to rhamnose-negative biotypes are responsible for severe diarrheas in after-weaning rabbits. The beneficial effects of vitamin E dietary supplementation on the immune response of animals against a variety of infectious agents have been reported. However, the reports about vitamin E influence on the resistance of animals to disease are discrepant. We sought to study the pathology induced by the E22 EPEC strain in experimentally infected rabbits and test whether dietary supplementation with vitamin E had any influence at the first stages of disease progression.

Materials and Methods: Ninety 30 days old New Zealand weaned rabbits were used for experimental infection. Rabbits were divided into four groups and treated as follows: group I – 30 rabbits infected with 2 x 10^6 cfu of EPEC strain E22; group II – 30 rabbits infected with 2 x 10^6 cfu of EPEC strain E22 and daily administered 60 mg/kg b.w. of vitamin E *p.o*., throughout the experiment starting 10 days prior to infection; group III – 15 rabbits were inoculated with 10^9 cfu of the apathogenic strain BM21; group IV – 15 rabbits were inoculated with 10^9 cfu of the apathogenic strain BM21, and daily administered 60 mg/kg b.w. of vitamin E *p.o*., throughout the experiment starting 10 days prior to infection. Tissue from the distal ileum was removed, while the animal was under anaesthesia, and processed by routine methods for the observation by light microscopy. The EPEC strain E22 was detected by immunohistochemical technique.

Results: The ileal lesions between group I and group II had qualitative similarities and were: 1) *lamina propria* and submucosal oedema; 2) polymorphonuclear cells and lymphocytes infiltrations; 3) diminution of villus length; 4) villous fusion; 5) colonization by EPEC; 6) degeneration and exfoliation of epithelial cells. The above lesions between groups I and II showed to differ at the time of appearance and the intensity. For this reason, we used morphometric and statistical analysis of the data for more reliable evaluation of the lesions. No histological alterations were observed in control rabbits (group III and group IV).

Conclusion: Total mucosal thickness, villous height, villous width, crypt depth, crypt width, villous height/crypt depth ratio, lymphocytes and polymorphonuclear cell infiltrations at the submucosa and mucosa showed significant differences between groups I and II, indicating that vitamin E should activate the immune system and provide some protection to intestinal mucosa.

THE ROLE OF HEAT SHOCK PROTEIN 70 IN HELICOBACTER-ASSOCIATED GASTRITIS

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Introduction: The aim of the study was to clarify the pathogenesis of gastritis associated with *Helicobacter heilmannii* in cheetahs. It has been suggested that an autoimmune mechanism related to heat shock protein 70 (HSP70) plays a role in chronic active hepatitis associated with *H. hepaticus* infection in certain strains of mice. We carried out the following experiment using a murine helicobacter infection model.
Materials and Methods: Gastric tissue samples from mice infected with *H. heilmannii* originally obtained from cheetahs were homogenized and orally inoculated into Crj:CD-1(ICR), A/J, AKR/N, C3H, C57BL, DBA, CBA and BALB/C strains of mice aged 4-6 weeks. Stomach samples from mice in the study sacrificed at 4, 8, 12 and 52 weeks were used for diachronic pathological examination. The distribution of HSP70 in the stomach was observed using immunostaining. Untreated mice for each mouse strain were used as controls and the gastric lesions of infected mice compared with those of untreated mice.
Results: *H. heilmannii* infection was observed throughout the entire stomach of infected mice of all strains. Although the severity of lesions differed depending on the mouse strain, gastritis was present in all strains, but was restricted to the fundus area. The severity of gastric lesions progressed diachronically and the number of lymph follicles observed increased with time in all strains except the DBA mouse, in which inflammation was consistently mild. Parietal cells, macrophages and the bodies of infecting Helicobacter were strongly positive for HSP70 in all mouse strains.
Conclusion: HSP is believed to play a role in the adhesion of bacillus to stomach epithelium. An immune-system mediated mechanism in which the antibody which helicobacter HSP induced attacks the HSP of gastric epithelium, is also believed. Results of the present study suggested that HSP70 may play a role in the inducement of *H. heilmannii*-related gastritis. However, many factors including urease, vacuolation toxin and various cytokines are believed to be involved in the pathogenesis of *H. pylori*, and future more detailed examination is required concerning *H. heilmannii*-related gastritis.

TRACHEAL CARCINOSARCOMA IN A BELGIAN BLUE HEIFER

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Introduction: Neoplasms of the trachea are very rare. Any tissue in or adjacent to the wall of the trachea can give rise to a tumour, so a variety of epithelial and mesenchymal tumours has been found in the trachea. Mixed tumours (salivary gland type) of the trachea occur occasionally in humans and most of these are benign. Only two malignant mixed tumours of the trachea have been described in humans. We describe a primary malignant mixed tumour arising in the tracheal wall in a heifer, which to our knowledge is the first mixed tumour of the trachea ever reported in any domestic animal.

Materials and Methods: A two years old Belgian Blue heifer was presented with a respiratory rate of 36 per minute, open mouth breathing and an inspiratory and expiratory stridor. At necropsy there was a tracheal mass of 17 cm in length by 10 cm in diameter attached to the ventral side of the tracheal rings, with severe compression of the tracheal lumen. Multiple samples for microscopic examination were taken. All sections were stained with haematoxylin and eosin, periodic acid Schiff and alcian blue at pH 2.5. Immunohistochemistry for cytokeratin, vimentin, smooth muscle actin, S-100 protein and Ki67 was performed.

Results: On histology the tumour was well demarcated, encapsulated and composed of epithelial elements dispersed throughout a matrix showing varying degrees of myxo-chondroid and chondroid stroma. The epithelial component was consistent with an adenoid cystic carcinoma. The stromal component was consistent with a chondrosarcoma.

Immunohistochemically the epithelial component was positive for cytokeratin, moderately positive for smooth muscle actin and weakly positive for vimentin and S-100 protein. The mesenchymal cells were positive for vimentin and smooth muscle actin.

Conclusion: The diagnosis of mixed tumour is dependent on the identification of a prominent myoepithelial cell component against a myxoid or chondroid background. In this case there was immunoreactivity for cytokeratin, vimentin and smooth muscle actin, a phenotype that is regarded as distinctive for cells exhibiting myoepithelial differentiation. Additionally the background stroma in this case was myxo-chondroid, thus fulfilling the criteria for the diagnosis of a mixed tumour.

The pathogenesis of mixed tumours of the salivary gland type in the trachea remains a matter of controversy. Although the lesions are regarded as originating from the myoepithelium of tubulo-acinar seromucous tracheal glands, their occurrence in peripheral locations unrelated to a gland would argue for an origin from a primitive stem cell bearing the capability to differentiate towards ductular structures, myoepithelium and chondromyxoid matrix. In this case the bulk of the tumour was located peripherally with only moderate involvement of the submucosa which might argue for a possible primitive stem cell origin.

DIFFERENCES IN THE PATHOLOGY OBSERVED AFTER THE EXPERIMENTAL INFECTION OF LAMBS WITH DIFFERENT STRAINS OF *MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS*

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Introduction: Different strains of *Mycobacterium avium subsp. paratuberculosis* (Map) have been recognized according to the animal species, microbiological properties or, recently, genetic heterogeneity. However, the pathogenicity of the strains and their ability to infect other animal species different from the original, are not well documented. In this study, a histopathological method has been used to assess the pathology induced by different Map strains in an experimental infection in lambs.

Materials and Methods: A total of thirty 1 month old lambs, divided in 6 groups, were orally challenged with the following Map strains: two bovine strains, with different genetic patterns, A and E (groups 1 and 2); an ovine strain, directly purified from the intestinal mucosa of a clinical case (group 3); a bovine strain, directly purified from the intestinal mucosa of a clinical case (group 4) and the same strain grown in Herrold media (group 5). A 6th group was kept as uninfected control. Humoral and cellular peripheral immune responses as well as the presence of Map DNA in blood were evaluated along 5 months post infection. At this time, lambs were humanely killed for pathological examination, focused on the histological evaluation of intestinal lymphoid tissue. The presence of Map in tissues was assessed by Ziehl-Neelsen, immunohistochemistry and PCR from frozen and paraffin embedded samples.

Results: All the strains were able to infect the lambs. Humoral and cellular immune responses were observed in all the groups, with different intensities. Map DNA was found in blood samples from lambs belonging to the five infected groups only at the begining of the experiment. In all the bovine-infected lambs, lesions had a focal or multifocal character. They appeared mostly in the ileocaecal and jejunal lymph nodes and were formed by well-defined granulomata with caseous necrosis, with or without mineralization, neutrophils and a high proportion of giant cells. Severity of the lesion varied among groups. In group 3 (ovine strain), lesions were diffuse and appeared in the intestine and lymph nodes, characterized by a granulomatous infiltrate, without necrosis and scarce giant cells. Map or its DNA were detected in tissues from all the groups. Only in diffuse lesions were in high amounts. PCR was more sensitive when performed from paraffin sections than frozen tissue.

Conclusion: These results suggest that Map strains have an effect on the pathological features of the infection. Bovine strains, in lambs, induced focal or multifocal lesions, mainly limited to the lymph nodes, whereas ovine strains caused more diffuse and severe granulomatous changes.

HISTOPATHOLOGIC CLASSIFICATION OF 171 CASES OF CANINE AND FELINE NON-HODGKIN LYMPHOMA ACCORDING TO THE WHO CLASSIFICATION

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Introduction: A retrospective study on 123 canine and 48 feline lymphomas was carried out in order to confirm the suitability of the classification system for animal lymphomas, proposed in 2002 by the WHO, which is derived from the REAL classification for human Non-Hodgkin lymphoma.
Materials and Methods: All cases were classified according to the WHO classification, the Kiel classification and the National Cancer Institute Working Formulation. Microscopic examination was performed after standard staining (haematoxylin and eosin) and immunohistochemical labelling for B (CD79) or T (CD3) cell phenotypes.
Results: A high prevalence of B cell lymphomas in dogs (97/123; 79.9%) and of T cell lymphomas in cats (31/48; 64.6%) was observed. Particularly, in dogs the most frequent B cell lymphomas were B large cell lymphomas (B-LCL: 56/97; 57.7%) and plasmacytic tumours (PCT: 20/97; 20.6%). Among T cell lymphomas the T lymphoblastic type was the most frequent in dogs (T-LBL: 8/26; 30.8%) as intestinal (ITCL: 16/31; 51.6%) and peripheral (PTCL: 11/31; 35.5%) in cats; B-LCL (8/17; 47.1%) was the commonest feline B cell lymphoma.
Discussion: The main difficulties using the WHO classification have been the presence, among B-LCL, apart from the diffuse large B cell lymphoma (DLBCL) type and the large cell immunoblastic lymphoma (LCIBL) type often present in mixed type, of 13 cases (12 dogs and 1 cat) with a plasmacytoid differentiation which is not considered as a distinct subtype. In addition, two cases of PCT in dogs expressed both CD79 and CD3 positivities. Furthermore, we met a certain difficulty in differentiating B-LBL (3 dogs) from Burkitt-type lymphoma. ITCL (5 dogs and 16 cats) exhibited a huge morphologic variability. Finally, there were 4 cases of multicentric mature small T cell lymphomas and a thymic T cell lymphoma in dogs, which are not codified in the REAL classification.
Conclusion: The WHO classification has been adapted from human to veterinary medicine, but since in some cases no category fits to animal lymphomas and some categories seem to be not represented in veterinary medicine, further studies in cooperation between clinicians and pathologists should be performed to improve its effectiveness; a further separation into specific classifications for canine and feline lymphoma seems to be required.

THE BRAIN PrP^{res} DISTRIBUTION CURVE (BPDC) OBTAINED WITH WESTERN BLOTTING AND A LUMINESCENCE IMMUNOASSAY: A USEFUL TOOL FOR THE STUDY OF BOVINE SPONGIFORM ENCEPHALOPATHY PATHOGENESIS

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Introduction: The Priocat Laboratory, as the animal transmissible spongiform encephalopathies (TSE) reference diagnostic laboratory in Catalonia, gains access to the whole brain of Bovine spongiform encephalopathy (BSE) affected cows. The availability of such interesting material has raised our interest in basic pathogenesis research. Our first approach has been to assess the grade of affection of each case by looking at the histopathology. As all of the cases diagnosed in our laboratory had been detected through the active surveillance program (i.e. none had any report of neurological signs) it was not surprising that a minimal amount of lesion could only be detected, mainly confined to the brain stem. Our next approach was to determine the distribution and spread of the resistant prion protein (PrP^{res}) which is considered to be the only reliable marker of prion diseases.

Materials and Methods: PrP^{res} distribution within the brain was studies by means of immunohistochemistry (IHC), Prionics-Check Western blotting (WB) and Prionics-Check Luminescence immunoassay (LIA).

Results: IHC to detect PrP^{res} is the "Golden Standard" technique for this purpose and gives accurate information on the anatomical as well as the cellular distribution of the mentioned protein. It is however a technique which requires a considerable amount of time not only to perform it, but also to read and interpret it. Furthermore the interpretation always relies on the expertise, and to some extent the subjectivity, of the pathologist. So the need for a more rapid and objective method to asses the whole brain of those cases was clear. By means of the WB and LIA tests a brain PrP^{res} distribution curve (BPDC) has been designed.

Conclusion: Notwithstanding the particularities of each test, both methods yield an accurate and comparable profile of the distribution of the PrP^{res} deposition in the brain, thus providing a quick and effective method to determine the level of affection of a particular case. We also propose the BPDC as a tool to differentiate those classical cases of BSE from the atypical phenotypes recently described.

IMMUNOHISTOLOGICAL DEMONSTRATION OF FELINE INFECTIOUS PERITONITIS VIRUS STRUCTURAL PROTEINS AND THEIR CELLULAR DISTRIBUTION IN FELINE INFECTIOUS PERITONITIS

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Introduction: Immunohistological detection of coronavirus antigen within the lesions is a sensitive method to confirm Feline Infectious Peritonitis infection. By this method it is possible to demonstrate coronavirus antigen within the lesions typical for Feline Infectious Peritonitis (FIP). The current study was undertaken to investigate cellular distribution of the three structural proteins membrane glycoprotein (M), nucleocapsid glycoprotein (N) and spike glycoprotein (S) using monoclonal antibodies from different private and commercial sources (Custom Monoclonals, Hohdatsu, IDEXX).
Materials and Methods: FIP virus structural proteins were demonstrated with monoclonal antibodies after formalin fixation in paraffin embedded tissue using various enzyme-based or buffer-based antigen retrieval protocols and indirect immunoperoxidase as well as streptavidin methods.
Results: The nucleocapsid protein was demonstrated in the cytoplasm of infected macrophages, the membrane protein and the spike protein were mostly demonstrated as granular precipitate near the nucleus, probably the Golgi apparatus.
Conclusions: These findings assist the thesis that the N protein is only produced in the cytosol and mature M and S glycoproteins assemble in the Golgi apparatus.

THE METABOLIC POTENTIAL OF THE LIVER IS AFFECTED IN RATS INTOXICATED SIMULTANEOUSLY WITH DIMETHOATE AND PYRANTEL

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Introduction: Dimethoate (phosphoorganic insecticide) and pyrantel are two different compounds commonly used in agriculture and veterinary medicine, respectively. Some data suggest that may act synergistically and be toxic both in animals and humans. The aim of presented study was to analyze the effect of the above compounds on the selected metabolic activities of hepatocytes and P450 dependent biotransforming potential of the liver.

Materials and Methods: The investigations were carried out on Wistar rats (170-190 g b.w.). One group of animals received dimethoate in drinking water at the dose $^1/_{25}$ DL_{50}/day for 28 consecutive days and the other group received dimethoate (as above) and two doses of pyrantel ($^1/_2$ DL_{50}) at 14 and 28 day of experiment. At selected periods 3, 6, 12h and 2, 7 and 14 days after the delivery of the last dose of pyrantel the rats were sacrificed by decapitation. In the sections of the liver the activities of succinic dehydrogenase (SDH) and glucose-6-phosphatase (G6P-ase) were evaluated by the means of appropriate histochemical methods. In the microsomal fraction of the liver the content of cytochrome P450 and cytochrome b5 as well as activities of their corresponding NADPH and NADH reductases were also measured.

Results: Histochemical analysis of the liver sections has shown that both studied compounds diminished the activities of SDH, being the functional marker of internal mitochondrial membrane, as well as G6P-ase which marks the activity of endoplasmic reticulum. The characteristic zonal distribution of the above enzymatic activities was also affected. Total content of cytochrome P450 increased significantly 2 days after the last dose of pyrantel and remained at increased level till the end of experiment. The effect of both pyrantel and dimethoate on the content/activities of the main donors of electrons in the catalytic cycle of cytochrome P450 (cytochrome b5, NADPH and NADH reductases) was minimal and statistically not significant. There was no synergistic effect of pyrantel and dimethoate on any of the components of the cytochrome P450 system.

Conclusion: These investigations have shown that in the conditions of performed experiment both dimethoate and pyrantel affect the main metabolic activities of hepatocytes involved in energy generation and cytochrome P450 dependent biotransformation of xenobiotics.

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DIAGNOSIS OF DOGS PANCREATITIS

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Introduction: Pancreatitis is a life-threating disease to people as well as to animals. It is possible to detect reasons of this fact in scarce knowledge about its etiology and pathogenesis, but first of all, in diagnostic difficulties. The aim of the research was to elaborate principles of dog's pancreatitis diagnostication *intra vitam*, especially pancreatitis chronica, on the base of clinical symptoms, conventional and newly introduced laboratory tests. Results and diagnosis were confirmed by morphological examinations of the pancreas.
Materials and Methods: Dogs with high risk of pancreatitis have been included in the research. The examination embraced 62 dogs of different breeds, between 5 months and 14 years of age. The accepted occurrence of clinical symptoms, which could suggest pancreatitis became the fundamental criterion of division of animals into two groups. All patients underwent physical examinations. Thirty fife dogs without clinical symptoms were qualified for I group. However, 27 dogs entered II group with clinical symptoms, which could suggest pancreatitis. Analytical research of all patients was carried out, designation of complete blood count and serum analysis with pancreas profile, kidney profile and liver profile, urinalysis and urine concentration of creatinine. Simultaneously it tested urine amylase level and attempts of test urine lipase level were taken. Blood for research was collected after 10-12 hour period of non-eating, and samples of urine were taken during the morning miction. Analyzer ABC Vet (ABX Diagnostics Poland) was used for complete blood count. However, analyzer Pointe 800 (Pointe Scientific Poland) and its reagents were used for the serum chemistry. Diagnosis was made on the basis of all results of the carried research with a special emphasis on the increased serum activity of enzymes of pancreas and/or in urine, with the change of minimum two parameters. Results were verified in histological examination of the pancreas clipping post mortem.
Results: Among 62 investigated dogs 14 animals were stated to have pancreatitis, which amounted to 22.58%. On the basis of the accepted diagnostic criteria in I group *pancreatitis chronica* was identified in 4 dogs (11.43% of the researched animals) and in II group *pancreatitis chronica* was identified in 10 cases (37.04%). Ranges of correct values of activities of pancreas enzymes in serum and urine were roughly elaborated. The elaboration covered also the conditions of materials storage (serum and urine) cooled (2-8°C) and frozen (for designations -20°C), and reference values for the proportion of amylase and creatinine in urine.
Conclusion: The research has shown that the increased activity of amylase and lipase in serum and/or in urine can be accepted as the basic criterion in identifying pancreatitis, especially the chronic one among dogs. Usefulness of the presented clinical research influences the fact that they are continued.

RHODOCOCCUS EQUI INFECTION IN FOALS CLINICAL AND PATHOMORPHOLOGICAL CHANGES

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Introduction: *Rhodococcus equi* is an opportunistic intracellular pathogen of horses. It is an important cause of pneumonia in 1-6 months old foals. About half pneumonic foals have also ulcerative colitis or the others intestinal lesions.

Materials and Methods: Seventeen foals from four study farms at which *R. equi* infection is endemic, both sexes, 2-4 months old, different breeds (xo – 3, xx – 8, oo – 1, crossbreed – 5), all were treated with antibiotics. Clinical signs and dates were collected by local veterinarians. Clinical signs suggested pneumonia (in 17 cases): dyspnoea, fever (39.5-41.0°C). One horse had diarrhoea.

Post-mortem examination, histopathological and bacteriological diagnosis were conducted, using routine methods. Histopathological paraffin slides were stained haematoxylin and eosin.

Results: All foals had pneumonia (in 15 cases there were solitary or numerous abscesses in lungs parenchyma, the diameters of abscesses ranged from 0.1 to 3.0 cm, the largest diameter measured 10 x 25 cm). Histopathologically the lesions in lungs were predominantly pyogranulomatous. In 1 foal there giant cells were seen. Neutrophils were numerous; lymphocytes and plasma cells were present in moderate numbers. In 6 cases there were inflammatory cell infiltrates in interstitial tissue of lungs. Moreover 1 foal had pleuritis, 2 – hydropericardium, 1 – pericarditis. One of foals had colitis and other intestinal lesions. In the colon were also pyogranulomatous changes. There was also clinically diagnosed cornear opacity (2 cases) and oedema of joints (2 cases).

Bacteriological examination: *Rhodococcus equi* was isolated from 9 cases (app.53%).

Conclusions: *Rhodococcus equi* infection in horses has different morphological and clinical forms.

AN INTERLABORATORY COMPARISON OF IMMUNOHISTOCHEMISTRY AND PCR METHODS FOR DETECTION OF *NEOSPORA CANINUM* IN BOVINE FOETAL TISSUES

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Introduction: *Neospora caninum* is an important cause of abortion in cattle all over the world. Histological examination of foetal tissues can be of great value as a diagnostic procedure, because the lesions caused by *N. caninum* are often distinctive, particularly the brain lesions. However, a definitive diagnosis depends on the identification of *N. caninum* in foetal tissues. Seven European laboratories participated in a multi-centre evaluation of immunohistochemistry (IHC) and PCR methods for detection of *Neospora caninum* in bovine foetuses.
Materials and Methods: A coded panel of tissue sections from 36 infected and non-infected foetuses was used to evaluate the IHC detection of parasites. A coded panel consisting of 44 homogenized foetal brain samples from natural bovine abortion cases and 32 spiked samples was used to evaluate the PCR methods. Inclusion of a duplicate dilution series of spiked samples was used to evaluate detection limits and repeatability.
Results: IHC methods had a relatively low sensitivity, but a high specificity. There was considerable variation in IHC results between participating laboratories, which may be partly explained by examination practices that depended on the experience of the operator. In addition, the use of different antibody reagents, different antibody dilutions, and different enzymatic treatments of tissues may have contributed to the observed variation.
PCR methods generally had a higher sensitivity than IHC methods and also a high specificity. The agreement between the majority scores of IHC and PCR methods was low. False positive PCR results indicated contamination problems in some instances. Agreement between the PCR results between the various laboratories was better, compared with the IHC results. There appeared to be no clear relationship between the PCR format (i.e. single or nested) and diagnostic sensitivity. Consequently, an improvement of diagnostic performance of PCR may possibly be achieved by optimizing DNA extraction methods.
Discussion: PCR has a higher sensitivity as a confirmation method for the diagnosis of foetal neosporosis compared with immunohistochemistry. However, it should be performed in combination with a histological examination to confirm that detected *N. caninum* DNA is associated with tissue damage and foetal death as the parasite may be present in foetuses that die from other causes.

RESISTANCE TO ANTIBIOTICS GRAM-NEGATIVE RODS ISOLATED FROM FISH AND FROM WATER DURING INTENSIVE FATTENING

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Introduction: Growing intensification of fish production and an increase in the number of diseases caused by bacteria in fish is accompanied by the problem of increasing resistance of bacteria to antibiotics, both in pathogens and in saprophytes. The issue is particularly important because of the transfer of the genes of resistance, both among pathogens and from saprophytes to pathogens.
Materials and Method: The material used in the study were samples of water, alimentary tract contents and skin mucus of fish *(Silurus glanis* L.), which were taken in the Fish Breeding Centre in Ostrołęka. The resistance of bacteria *Aeromonas hydrophila*, *Pseudomonas fluorescens* and *Enterobacteriaceae* to flumequine (UB, 30 μg), oxytetracycline (OT, 30 μg), novobiocin (NV, 5 μg), trimethoprim (W, 5 μg), enrofloxacin (ENR, 5 μg), canamycin (K, 30 μg), oxolinic acid (OA, 30 μg), and neomycin (N, 30 μg) was examined.
Results: Novobiocin was the antibiotic the resistance to which was found to occur the most frequently. The resistance to trimethoprim was also frequent. Canamycin and enrofloxacin were the antibiotics to which the absence of sensitivity was found to exist the least frequently. No difference was found in the resistance to the medicine among the strains from various environments.

		UB	OT	NV	W	ENR	K	OA	N	Number of isolates
Aeromonas hydrophila	water	1	3	22	8	1	1	2	4	22
	al. tract	3	5	11	5	1	0	4	4	11
	mucus	5	2	9	5	0	3	4	0	10
Pseudomonas fluorescens	water	2	3	27	26	1	3	16	4	29
	al. tract	0	2	3	3	0	0	1	0	3
	mucus	2	1	6	6	2	1	6	1	6
Enterobacteria-ceae	water	3	0	17	10	2	2	2	2	18
	al. tract	1	3	4	4	0	0	0	1	7
	mucus	3	2	5	5	1	1	5	2	8

al. tract – alimentary tract

Discussion: The study revealed that the resistance to novobiocin and to trimethoprim existed in more isolates than to other antibiotics (85.9%-95.7% and 57.12%-70.08% of isolates, respectively). A high frequency of resistance to antibiotics in the bacteria isolated from fish during intensive fattening indicates a role which may be played by fish breeding farms as reservoirs of genes of resistance, which may be transferred to other animals and to humans. Like in the studies conducted by other authors the bacterial strains were the most sensitive to canamycin and enrofloxacin.

MICROBIAL STUDY OF POST-COOLING WATER AND FISH DURING INTENSIVE REARING

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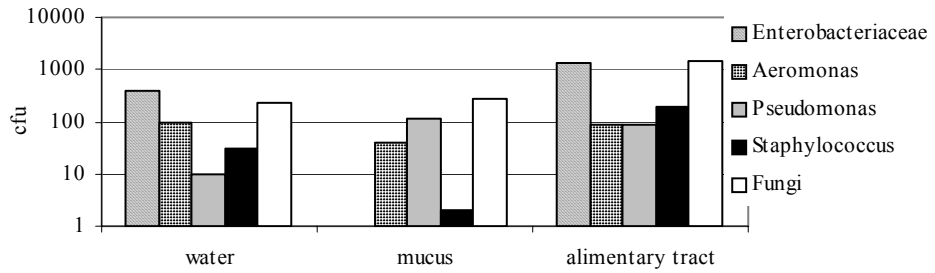
Introduction: Recent years have seen quick development of pisciculture associated to intensive fish rearing. Fish are exposed to various microorganisms, which are either saprophytic microflora of aquatic environment or get there from soil, waste or from air. The aim of the study was to determine potential pathogens present in water, in skin mucus and in the alimentary tract contents of Wels catfish (*Silurus glanis* L.), during intensive fish bin rearing in post-cooling waters.
Materials and Methods: Samples of water and fish were taken in three periods of 2003 – in spring, in summer and in autumn. Bacteria from the family *Enterobacteriaceae,* and the genera *Aeromonas*, *Pseudomonas, Staphylococcus* and fungi were determined in all the samples of water and skin mucus, as well as in the fish alimentary tract contents.
Results: The highest average numbers of microorganisms from the whole study period were found in the alimentary tract contents for the bacteria *Enterobacteriaceae* (1300 cfu/g) and for fungi (1420 cfu/g). These microorganisms also dominated in water – 380 cfu/cm^3 and 230 cfu/cm^3, respectively. These bacteria were not found in the mucus and fungi were found in the amount of 270 cfu/cm^2. The highest average number of bacteria *Aeromonas* was found in water (40 cfu/cm^3), *Pseudomonas* in mucus (110 cfu/cm^2), and *Staphylococcus* in the fish alimentary tract contents (190 cfu/g), which is shown in the figure.



Discussion: Contamination of the aquatic environment is reflected in the microbiological condition of fish. The results of own research have shown that bacteria *Enterobacteriaceae* and fungi dominated in the post-cooling water and in the fish alimentary tract contents. Both these groups indicate organic contamination of the allochtonic type. Bacteria from genera *Aeromonas, Pseudomonas* and *Staphylococcus* were also found. These results confirm and supplement the research conducted by other authors who dealt with these issues.

DIFFERENT ROLES OF PHENOLIC COMPOUNDS ON THE SURFACE AND IN THE INTERIOR OF PLANTS

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The location of phenolic compounds points to the role they play in the plant producing them. Compounds on the surface produce, by evaporation, an atmosphere surrounding the shoots and roots, as well as seeds, which plays an important role in communication between the plant and its environment. Coevolution led to the formation of new compounds involved in two processes – attraction or repulsion. Interior compounds can be released after animals have chewed on and damaged them, making further consumption unattractive. Such compounds released from vacuoles could form a healing film preventing microbes from penetrating the wounded area. Secondary metabolites eaten by animals producing milk for human consumption influence the quality and taste of the milk. Herbs that animals choose to eat naturally, growing in their pastures, and those added as feed, influence concentrations of conjugated linoleic acids, which are known anticancer agents for humans. Increasing the wellness of animals can lead to greater benefits for humans.

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LIST OF EUROPEAN SOCIETY OF VETERINARY CLINICAL PATHOLOGY ABSTRACTS

ETIOPATHOGENIC STUDY IN NATURAL INFECTION PIGS OF PORCINE CIRCOVIRUS

Andrada Marisa[1], Quesada Oscar[1], Venteo Ángel[2], Rodríguez J María[2], Sosa Agueda[1], Sierra Eva[1], Fernández Antonio[1]

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

Andrada Marisa[1], Quesada Oscar[1], Venteo Angel[2], Polo Esther[2], Sanz Antonio[2], García Nerea[3], López Gema[3], Domínguez Lucas[3], Rodríguez J María[2], Fernández Antonio[1]

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EFFECTS OF PRE-ANALYTICAL HANDLING ON SELECTED CANINE HAEMATOLOGICAL PARAMETERS EVALUATED BY THE ABBOTT CELL-DYN 3500® ANALYZER

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DEVELOPMENT OF AN IMMUNOTURBIDIMETRIC ASSAY FOR MEASUREMENT OF FELINE α_1-ACID GLYCOPROTEIN

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PREVALENCE OF THROMBOCYTOPENIA AND OF MACROTHROMBOCYTOSIS IN CAVALIER KING CHARLES SPANIEL

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EXPERIMENTAL CONTAGIOUS AGALACTIA CAUSED BY *MYCOPLASMA AGALACTIAE* IN GOATS: CHRONOLOGICAL STUDY OF CLINICAL AND LESIONAL FINDINGS

Castro Ayoze[1], Fernández Antonio[1], Rodríguez Francisco[1], De la Fe Christian[2], Lorenzo Hector[1], Poveda Bismarck[2], Herráez Pedro[1]
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CYTOMETRIC PATTERNS OF BLOOD FROM DOGS WITH NEOPLASTIC AND NON-NEOPLASTIC DISEASES USING CD18/CD45 DOUBLE LABELLING

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THE STRUCTURAL HETEROGENEITY OF FELINE α-1-ACID GLYCOPROTEIN

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RETROSPECTIVE STUDY OF DIC CASES IN DOGS (2003-2004)

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MORPHOGENETIC ROLE OF THE MYOEPITHELIUM IN APOCRINE AND MODIFIED APOCRINE GLAND TUMOURS OF THE DOG

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NOVEL "GAS EMBOLIC SYNDROME" IN BEAKED WHALES RESEMBLING DECOMPRESSION SICKNESS (DCS)

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LIVER FREE RADICAL, LIPID AND GLYCOGEN CONTENT IN DAIRY COWS AROUND CALVING

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USEFULNESS OF 2,3 DIPHOSPHOGLYCERATE4 (2,3DPG) MEASUREMENT IN THE DIAGNOSIS OF HYPOXIS CONDITIONS IN RACING HORSES

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

Godinho Ana, Monti G, Arbelo Manolo, Mendez Mariña, Sierra Eva, Castro P, Andrada Marisa, José Jaber, Francisco Rodríguez, Fernández Antonio
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FELINE VACCINE-ASSOCIATED SARCOMAS: CYTOLOGICAL RESULTS AND HISTOLOGICAL CORRELATIONS

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ELIMINATION OF *EHRLICHIA CANIS*-DNA FROM THE BLOOD AND SPLEEN OF DOGS WITHIN 16 DAYS OF DOXYCYCLINE TREATMENT

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IMMUNOHISTOCHEMICAL STUDY OF DIGESTIVE LESIONS IN THREE SPECIES OF CETACEANS FOUND STRANDED IN THE CANARY ISLANDS

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CANINE DEMODICOSIS: A PATHOLOGICAL STUDY

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MAST CELL TUMOURS (MCT)

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IMMUNOCYTOCHEMICAL DETECTION OF CYTOKINES IN PNEUMONIC LESIONS OF PIGS NATURALLY INFECTED WITH *MYCOPLASMA HYOPNEUMONIAE*

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INFLUENCE OF DIFFERENT PREANALYTICAL FACTORS ON AN ELISA ASSAY FOR CANINE CRP DETERMINATION

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OIL-RED-O STAIN IN CYTOLOGIC SAMPLES OF FELINE HEPATIC LIPIDOSIS

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CLINICAL AND PATHOLOGICAL FINDINGS IN A DOG WITH DUODENAL ADENOCARCINOMA

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CLINICAL AND PATHOLOGICAL FINDINGS IN SOME CANINE JOINT DISEASES

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LUNG FAT EMBOLISM IN CETACEANS STRANDED IN CANARY ISLANDS

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MEASUREMENT OF OXIDATIVE STRESS AND ANTIOXIDANT ACTIVITY

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COMPARATIVE METHODOLOGY FOR THE QUANTIFICATION OF MEGAKARYOCYTOPOIESIS IN THE BONE MARROW OF HEALHTY BEAGLE DOGS

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VALIDATION OF A NEW METHOD FOR TROPONIN I DETECTION IN HAN WISTAR RATS

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CAN MEASUREMENT OF PROTHROMBIN TIME, PT, DETECT FACTOR VII DEFICIENCY IN DOG?

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PRELIMINARY RESULTS ABOUT STOMATIN EXPRESSION IN ERYTHROCYTES OF MIDDLE SCHNAUZERS WITH HEREDITARY STOMATOCYTOSIS

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C-REACTIVE PROTEIN LEVELS IN CANINE EFFUSIONS: A PRELIMINARY STUDY

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DETECTION OF PORCINE CIRCOVIRUS 2 INFECTION BY IMMUNOHISTOCHEMISTRY AND POLYMERASE CHAIN REACTION-ELISA

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CORRESPONDENCE BETWEEN IMMUNOHISTOCHEMICAL AND ELECTRON MICROSCOPICAL FEATURES OF MERKEL CELLS IN DOGS

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MEASUREMREMENT OF CANINE EXOGENOUS CREATININE CLEARANCE DIRECTLY FROM WHOLE BLOOD SAMPLES: PRELIMINARY RESULTS

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METHOD CHANGES IN ENZYMOLOGY – SWITCHING FROM DGKC TO IFCC METHODS IN A VETERINARY SETTING

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ISO 9000:2000 – CERTIFICATION: EXPERIENCES OF A UNIVERSITY LABORATORY

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

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PLATELETS PARAMETERS OBTAINED WITH THE ADVIA 120 IN DOGS WITH RENAL FAILURE

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ACCURACY OF CSF CYTOLOGY FOR THE DIAGNOSIS OF TUMOURS IN THE CNS OF DOGS AND CATS

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PROTEIN ELECTROPHORESIS AND ALBUMIN QUOTA IN CEREBROSPINAL FLUID OF DOGS WITH NEUROLOGIC DISEASES: 105 CASES

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