



European Society of Veterinary Pathology

**24th Meeting
Edinburgh, Scotland**



Table of Contents

	<i>Page</i>
Welcome from the President.....	3
Welcome from the Chair of the Local Organising Committee	4
Sponsors.....	6
Programme.....	7 - 13
Plenary Speakers	14 - 48
Oral Presentations	49 - 119
Poster Presentations	120 - 229
Social Programme.....	230 - 231
General Information.....	232
Maps.....	233 - 234

Welcome from the President

Dear Participants of the 24th meeting of the European Society of Veterinary Pathology. The ESVP meets in Edinburgh for the second time, having had a previous fall meeting in 1995 in this lively city full of culture and activities. The ESVP and the local organisers have warm memories of that previous meeting. We are all looking forward to refreshing these memories and to gaining new contacts and knowledge. The ESVP thanks the local organisers for all the efforts they undertook in preparing and running the meeting. I wish all participants a rewarding and memorable congress in Edinburgh.

Professor Manfred Reinacher

President, ESVP

ESVP 2006 Organising Committee

Department of Veterinary Pathology, The University of Edinburgh

Dr Roderick Else	Chairman
Ms Arlene Milne	Conference Secretariat
Ms Alys Bradley ¹	
Dr David Buxton ²	
Mr Brian Kelly	
Dr Elspeth Milne	
Dr Susan Rhind	
Dr Sionagh Smith	
Dr Jill Thomson ³	

¹Charles River Laboratories

²Moredun Research Institute

³Scottish Agricultural College

ESVP 2006 Scientific Committee

Prof Cinzia Benazzi	University of Bologna
Dr David Buxton	Moredun Research Institute
Dr Christopher J Clarke	GlaxoSmithKline R&D
Dr Roderick Else	University of Edinburgh
Prof Manfred Reinacher	University of Giessen

Welcome from the Chair of the Local Organising Committee

On behalf of the Organising and Scientific Committees and the European Society of Veterinary Pathology, it gives me great pleasure to welcome you to Edinburgh for the 24th Meeting of the European Society of Veterinary Pathology.

It is eleven years since the first of what has become the Annual Meeting of the Society was held in Edinburgh and since then there have been many developments and changes in veterinary pathology in Europe and worldwide, notably the creation of the European College of Veterinary Pathology.

As always the meeting provides an ideal venue for the older and younger generations of veterinary pathologists from all over the Europe and even further afield to meet, exchange information and learn from each other as well as improve fellowship and friendship. We particularly welcome colleagues from Eastern Europe and further afield and hope that everyone will gain from contributing to and attending the Meeting.

In planning the scientific programme this year we have “gone back to basics” and organised themed sessions starting with a plenary talk followed by contributed papers on the same or related topic. Because poster presentations form a major source of contributions we have provided a specific session so that delegates and poster presenters can discuss findings. We hope that this will stimulate constructive and friendly discussion amongst colleagues. Such sessions do depend on active participants so please come along and participate!

There are also some additional sessions taking place: The British Society of Toxicological Pathologists have kindly organised a day session on spontaneous pathology of non-rodent species. The International Society for Veterinary Dermatopathology is holding a day workshop on 2 September.

As in previous years the Annual Symposium of the CL Davis Foundation for Veterinary Pathology precedes the ESVP Meeting.

The European College and European Society Annual General Meetings will take place after the scientific sessions on Thursday, 31 August and Friday, 1 September, respectively. There will also be an inaugural meeting of the British Society of Veterinary Pathology on Thursday 31 August.

But there is also time for relaxation and play! Edinburgh is a beautiful and vibrant city with much entertainment on offer, mostly within a short distance of the city centre and conference venue. The meeting location is within easy walking distance of Pollock Halls accommodation and other hotels and the city centre, but for those who wish, there are excellent bus services and taxis available. An added bonus for this conference is that the world famous International Festival of the Arts will still be running during the conference week. We are therefore confident that you will find plenty to entertain and delight you. There is no proscribed accompanying

persons programme - most people will find abundant attractions available through the tourist board and city guides that are available from the registration desk.

We therefore hope that you will enjoy Edinburgh and find the 24th Meeting of the ESVP stimulating and rewarding from both a scientific aspect and a personal one be it meeting old friends and colleagues, or making new ones.

Ceud Mile Failte! (A hundred thousand welcomes!)

Roderick (Rod) Else

Chair, Organising Committee

Sponsors

The Organising Committee of the 24th Congress of the European Society for Veterinary Pathology are grateful to the following sponsors who have generously contributed to this year's meeting:

Armed Forces Institute of Pathology

AstraZeneca

British Society of Toxicological Pathology

British Society of Veterinary Pathologists

Cellpath

Dako UK Ltd

Elsevier

GlaxoSmithKline

IDEXX

Nationwide Laboratories

Olympus

SlidePath

Surgipath

Thermo Electron

Vector Labs

Wednesday 30 August*Welcome Reception and Early Registration***Thursday 31 August***0830: Registration**0900: Opening*

<i>Session 1</i>	<i>0915 - 1030</i>	A	B
		<p>Cardio/Pulm (1) CHAIRS: Brian Sheahan, Susan Rhind</p> <hr/> <p>Plenary lecture: David Collie UK "THE LUNG: A REALISTIC TARGET FOR GENE THERAPY?"</p> <hr/> <p>Mark Dagleish UK "VARIATIONS IN THE SEVERITY OF PULMONARY LESIONS INDUCED BY UK OR USA CALF ISOLATES OF PASTEURELLA MULTOCIDA A3 IN A CALF MODEL OF PNEUMONIC PASTERELLOSIS."</p> <hr/> <p>Joe Cassidy Ireland "ADJUVANT MODULATION OF THE PROTECTIVE RESPONSE OF A SUBUNIT VACCINE AGAINST MYCOBACTERIUM TUBERCULOSIS INFECTION IN A MOUSE MODEL"</p> <hr/>	<p>Alimentary/Liver Disease (1) CHAIRS: Jill Thomson, Geoff Pearson</p> <hr/> <p>Plenary lecture: Valentin Perez Perez Spain "INTESTINAL MYCOBACTERIOSIS IN RUMINANTS. PARATUBERCULOSIS: PATHOLOGY AND PATHOGENESIS"</p> <hr/> <p>Maria Muñoz Spain "EXPERIMENTAL INFECTION OF VACCINATED AND UNVACCINATED CALVES WITH MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS"</p> <hr/> <p>Sandra Jolly Belgium "DENSITY AND HETEROGENEITY OF BOVINE MAST CELLS IN INTESTINAL TRACT: DISTRIBUTION OF TRYPTASE, CHYMASE, HISTAMINE AND SEROTONIN IN DUODENUM."</p> <hr/>

Coffee/tea 1030 - 1100

<i>Session 2</i>	<i>1100 - 1230</i>	<p>Cardio/Pulm (2) CHAIRS: Brian Sheahan, Susan Rhind</p> <hr/> <p>Friederike Range Germany "CALCIUM-ACTIVATED CHLORIDE CHANNELS IN EQUINE RECURRENT AIRWAY OBSTRUCTION (RAO): WHAT IS THE ROLE OF ECLCA1?"</p> <hr/> <p>Ebba Nilsson Sweden "SUDDEN DEATH IN RACING HORSES. PATHOLOGICAL AND BACTERIOLOGICAL FINDINGS."</p> <hr/> <p>Orlando Paciello Italy "EFFECTS OF ENVIRONMENTAL AIR POLLUTION IN CANINE LUNGS AND BRONCHIAL LYMPH NODES AND THE ASSOCIATED EXTRACELLULAR MATRIX REMODELING"</p> <hr/>	<p>Alimentary/Liver Disease (2) CHAIRS: Jill Thomson, Geoff Pearson</p> <hr/> <p>Alexandra Schock UK "COCCIDIOSIS IN BRITISH ALPACA (VICUGNA PACOS)"</p> <hr/> <p>Richard Ducatelle Belgium "LESIONS ASSOCIATED WITH CLOSTRIDIUM PERFRINGENS INFECTION IN A CHICKEN INTESTINAL LOOP MODEL"</p> <hr/> <p>Anja Kipar UK "CONTRIBUTION OF THE LIVER TO CLINICAL AND PATHOLOGICAL CHANGES IN FELINE INFECTIOUS PERITONITIS"</p> <hr/>
------------------	--------------------	--	---

		A	B
<i>Session 2 continued</i>	<i>1100 - 1230</i>	Cardio/Pulm (2) continued	Alimentary/Liver Disease continued
		Heike Aupperle Germany "ALTERATIONS OF THE EXTRACELLULAR MATRIX IN CHRONIC DEGENERATIVE MITRAL VALVE DISEASE (ENDOCARDIOSIS) IN DOGS"	Valerie Vandenberghe Belgium "CHRONIC INTESTINAL PSEUDO-OBSTRUCTION IN A BERNESE MOUNTAIN DOG"
		Ken Smith UK "PULMONARY LESIONS IN FOXHOUSES INFECTED WITH EQUINE INFLUENZA H3N8"	Denise Thaller Austria "LONGTIME PATHOLOGICAL SURVEY OF A COLONY OF CAPTIVE HELD LESSER HEDGEHOG TENRECS (ECHINOPS TELFAIRI)"
		Heike Aupperle Germany "CARDIAC FUNCTION AND EXTRACELLULAR MATRIX METABOLISM IN RABBITS WITH DOXORUBICIN CARDIOMY- OPATHY AFTER AUTOLOGOUS MESENCHYMAL BONE-MARROW DERIVED STEM CELL INJECTION"	Trpe Ristoski Macedonia "IMMUNOHISTOCHEMICAL DETECTION AND DISTRIBUTION OF THE VIRAL GP55 ANTIGEN IN PIGS NATURALLY INFECTED WITH CLASSICAL SWINE FEVER VIRUS"
<i>Lunch 1230 - 1400</i>			
<i>Session 3</i>	<i>1400 - 1530</i>	Neoplasia (1) CHAIRS: Marion Hewicker- Trautwein, Donald Kelly	Wildlife Pathology CHAIRS: Antti Sukura, Carl Hård
		Plenary lecture: David Argyle UK "CANCER: LIVING LIFE ON THE EDGE OF GENOMIC STABILITY"	Plenary lecture: Nadia Robert Switzerland "NEW WORLD CAMELIDS: BASIC ANATOMY AND PATHOLOGY"
		Marcelo De las Heras Spain "IMMUNOLOGICAL DIVERSITY BETWEEN CLASSICAL AND ATYPICAL OVINE PULMONARY ADENOCARCINOMA."	Mark Stidworthy UK "THE NATURAL HISTORY OF AN OUTBREAK OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS (LCMV) IN A CAPTIVE COLONY OF GEOFFROY'S MARMOSETS (CAL- LITHRIX GEOFFROYI) IN THE UNITED KINGDOM."
		Marcelo De las Heras Spain "BETARETROVIRUSES-RELATED ANTIGENS IN HUMAN LUNG ADENOCARCINOMAS."	Valéria Café Marçal Switzerland "CHEETAH MYELOPATHY - PATHO- LOGICAL FINDINGS AND IMMUNO- HISTOCHEMICAL INVESTIGATION"
		Alessandro Poli Italy "CYCLOOXYGENASE-2 EXPRES- SION IN CANINE AND FELINE SQUAMOUS CELL CARCINOMAS AND ITS CORRELATION TO VEGF EXPRESSION"	Stephanie Rossteuscher Switzerland "SCUTICOCILIOSIS IN SEA DRAGONS IN BASEL ZOO, SWITZERLAND"

Coffee/Tea 1530 - 1600 hours

<i>Session 4</i>	<i>1600 - 1730</i>	A	B
		<p>Neoplasia (2) CHAIRS: Ronny Lindberg, Peter Brown</p> <hr/> <p>Francesca Millanta Italy "ROLE OF CYCLOOXYGENASE-2, EP2 RECEPTOR, AND MICRO-SOMAL PGE SYNTHASE-1 EXPRESSION IN FELINE INVASIVE MAMMARY CARCINOMAS"</p> <hr/> <p>Nikos Papaioannou Greece "TELOMERASE ACTIVITY AND IMMUNOHISTOCHEMICAL EXPRESSION OF PCNA, P53 AND KI67 IN TESTICULAR TUMOURS OF DOGS"</p> <hr/> <p>Adrian Philbey UK "PATHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISATION OF CANINE VISCERAL HAEMANGIOSARCOMAS"</p> <hr/> <p>Laura Peña Spain "DIFFERENT SITES ARE INVOLVED IN THE METASTATIC INFLAMMATORY MAMMARY CARCINOMA RESPECT TO OTHER METASTATIC NON-INFLAMMATORY MAMMARY TUMORS."</p> <hr/> <p>Eugenia Gabriela Tirifon Romania "EXPERIMENTAL PROCEDURE FOR RS-1 ALVEOLAR-TYPE HEPATOMA"</p>	<p>Varietal Pathology (1) CHAIRS: Christopher Clarke, Sionagh Smith</p> <hr/> <p>James Baily UK "THE PATHOLOGY OF CHRONIC EROSIIVE DERMATOPATHY IN MURRAY COD, MACCULLOCHELLA PEELII PEELII (MITCHELL)."</p> <hr/> <p>Jozef Szarek Poland "PATHOMORPHOLOGICAL PATTERN OF CARP LIVER CAUSED BY THE HERBICIDE AVANS 330 SL IN FISH CLINICALLY HEALTHY AND WITH ICHTHYOPHTHIRIASIS"</p> <hr/> <p>Michal Reichert Poland "PATHOLOGICAL CHANGES IN THE BRAIN AND EXCRETORY KIDNEY OF RAINBOW TROUT FRY EXPERIMENTALLY INFECTED WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS."</p> <hr/> <p>Ahmad Reza Movassaghi Iran "PATHOLOGIC STUDY OF THE PROTECTIVE EFFECTS OF THIAMINE ON EXPERIMENTAL LEAD POISONING IN RABBIT"</p>
<i>ECVP AGM 1730 hours</i>			

Friday 1 September

Session 5 0900 - 1030	A	B	C
	CNS/muscle (1) CHAIRS: Wolfgang Baumgaertner, Alun Williams	Clinical Pathology CHAIRS: Elspeth Milne, Adrian Philbey	BSTP - Non-rodent laboratory animals pathology
	Plenary lecture: Lorenzo Gonzalez UK IMMUNOHISTOCHEMICAL PHENO- TYPES OF NATURAL AND EXPERIMENTAL SHEEP TSES	Plenary lecture: Carlo Masserdotti Italy ARCHITECTURAL PATTERNS IN CYTOLOGY	SPONTANEOUS PATHOLOGY OF THE DOG
	Rosa Bolea Spain "CORRELATION BETWEEN BAX OVEREXPRESSION AND PRION DEPOSITION IN MEDULLA OBLONGATA FROM NATURAL SCRAPIE WITHOUT EVIDENCE OF APOPTOSIS"	Second Plenary lecture Reinhard Mischke Germany "APPROACH TO BONE MARROW CYTOLOGY IN THE DOG AND CAT"	Cheryl Scudamore (Covance)
	Silvia Siso UK "THE PATTERNS OF ACCUMULA- TION OF PRION PROTEIN DURING PRECLINICAL SHEEP SCRAPIE AND BSE SUGGEST DIFFERENT PATHWAYS OF NEUROINVASION"	Adrian Philbey UK "DIAGNOSIS OF BOID INCLUSION BODY DISEASE BY EXAMINATION OF BLOOD SMEARS AND PULMONARY LAVAGE"	SPONTANEOUS PATHOLOGY OF NON-HUMAN PRIMATES Alys Bradley (Charles River)
Anna Oevermann Switzerland "CNS LESIONS IN SMALL RUMINANTS: A HISTOPATHOLOGI- CAL ANALYSIS OF FALLEN STOCK BRAINS COLLECTED IN A ONE YEAR TSE SURVEY"			

Coffee/tea 1030 - 1100

Session 6 1100 - 1230	A	B	C
	CNS/muscle (2) CHAIRS: Federico Valenza, Alun Williams	Clinical Pathology continued CHAIRS: Elspeth Milne, Adrian Philbey	BSTP
	John Callanan UK "IMMUNOHISTOCHEMICAL CHARACTERISATION OF THE CNS CELL INFILTRATIONS IN GREYHOUND MENINGOENCEPHALITIS"	Second Plenary lecture: Kathleen Freeman UK "THE USE OF EQUINE CYTOLOGY IN PRACTICE: ACUTE AND CHRONIC CONDITIONS"	MICROANATOMY OF POULTRY Ronnie Chamanza (Charles River)
Katharina Kramer Germany "VIRAL SPREAD IN BRAINS OF BORNA DISEASE VIRUS- INFECTED TNF-TRANSGENIC MICE"	Pathology (2) Peter Brown UK "DIGITAL MICROSCOPY EQ IN VETERINARY PATHOLOGY – AN RCVS TRUST FUND PROJECT."	SPONTANEOUS PATHOLOGY OF POULTRY Robert LaRagione (VLA)	

		A	B	C
<i>Session 6 (continued)</i>	<i>1100 - 1230</i>	CNS/muscle (2) continued	Varietal Pathology (2) continued	BSTP
		Doris Porombka Germany “REGIONAL TROPISM OF BORNA DISEASE VIRUS TRANSCRIPTION AND REPLICATION IN DIFFERENT BRAIN AREAS OF EXPERIMENTALLY INFECTED LEWIS RATS”	Udo Hetzel UK “ANAPLASMA PHAGOCYTOPHILUM: SITES OF PRIMARY INFECTION”	continued from previous page
		Catherine Botteron UK “NEUROLOGICAL CHANGES AND BRAIN COPPER ELEVATION IN NORTH RONALDSAY SHEEP: A MODEL FOR WILSON’S DISEASE?”	Franco Mutinelli Italy “STUDY OF THE PATHOGENESIS OF THE HPAI H5N1 EXPERIMENTAL INFECTION IN PECKIN DUCKS, BASED ON IMMUNOHISTOCHEMISTRY AND IN SITU HYBRIDIZATION”	
		Julio Benavides Silvan Spain “CHARACTERIZATION OF THE CENTRAL NERVOUS SYSTEM INFLAMMATORY RESPONSE IN NATURAL CASES OF OVINE VISNA”		
		Hanne Jahns Ireland “NEUROPATHOLOGICAL CHANGES IN APPARENTLY HEALTHY MINK ON ALEUTIAN DISEASE VIRUS-INFECTED IRISH FUR FARMS AND SEQUENCE VARIATIONS IN THE VP2 REGION OF THE VIRUS.”		

Lunch 1230 - 1400**ESVP Committee Meeting 1300 -1400**

<i>Session 7</i>	<i>1400 - 1530</i>	Varietal Pathology (3) CHAIRS: Jakob Walter, Richard Ducatelle	Reproductive Pathology CHAIRS: Andreas Pospischil, Rod Else	BSTP
		James O’Donovan Ireland “THE HIGH INCIDENCE OF BILATERAL THYROID LESIONS IN HYPERTHYROID CATS SUPPORTS BILATERAL THYROIDECTOMY AS THE SURGICAL TREATMENT OF CHOICE IN FELINE HYPERTHYROIDISM.”	Plenary lecture: David Buxton UK “INFECTIONS OF PREGNANCY IN RUMINANTS; A DEVELOPING STORY.” Nicole Borel Switzerland “CHLAMYDIA-RELATED ABORTIONS IN CATTLE FROM GRAUBUNDEN, SWITZERLAND”	PECULIARITIES OF THE MICROANATOMY OF FERRETS, RABBITS, GPS AND HAMSTERS Bruce Williams, AFIP

Saturday 2 September		
<i>Session 9</i>	<i>0900 - 1030</i>	A
		Varietal Pathology (4) CHAIRS: Jozef Szarek, Jill Thomson
		Mercedes Marquez Spain “LAFORA’S BODIES CHARACTERIZATION IN CANINE LAFORA DISEASE: TOPOGRAPHICAL, HISTOPATHOLOGICAL, IMMUNOHISTOCHEMICAL AND ULTRASCTRUCTURAL STUDIES.”
		Alun Williams UK “PRION-INDUCED NEURODEGENERATION - A TALE OF RAFTS AND ANCHORS”
		Lieve Vangeel Belgium “MORPHOLOGICAL CHARACTERISATION OF EOSINOPHILIC MYOSITIS IN BOVINES IN BELGIUM”
		Frédérique Nguyen France “MICROVASCULATURE IN SKELETAL MUSCLES OF GRMD (GOLDEN RETRIEVER MUSCULAR DYSTROPHY) DOGS”
		Henny Martineau UK “BILATERAL JUGULAR PHLEBECTASIA IN A PEDIGREE BELTEX TUP”
<i>Coffee/tea 1030 - 1100</i>		
<i>Session 10</i>	<i>1100 - 1230</i>	<i>MYSTERY SLIDE SESSION</i>
		<i>End of Conference</i>

PLENARY SPEAKERS

THE LUNG: A REALISTIC TARGET FOR GENE THERAPY?

D David S Collie

University of Edinburgh, Royal (Dick) School of Veterinary Studies, Division of Veterinary Clinical Studies, Easter Bush Veterinary Centre, Roslin, Midlothian, EH25 9RG

Respiratory disease now kills more people than coronary heart disease – that is one in four people in the UK. It is the most common long term illness among children, the most common illness responsible for an emergency admission to hospital, and costs the NHS more than any other disease area (Petersen S, Wolstenholme J . The Burden of Lung Disease, British Thoracic Society 2001).

Gene therapy may have a future role to play in alleviating this burden. In addition to highlighting the major aspects relating to delivery and vector design this brief introduction should serve as a reminder of some recognised limitations surrounding lung-directed gene therapy.

The major routes available for targeting the lungs with gene therapy are via the airways and via the bloodstream. Available airway delivery strategies include instillation, injection or even aerosolisation via the bronchoscope, and whole lung aerosol delivery.

The process of clearing the lung of instillates or aerosols will change the composition of the epithelial lining fluid (ELF) containing the gene transfer agent (GTA). Changes with respect to salt concentration, concentration of gene transfer agent, and protein concentration are all aspects which can impact (positively or negatively) on the performance of a particular GTA. Indeed, such changes are likely to influence particle size and hence the mechanism of uptake and/or clearance of a given GTA – whether by epithelial cells or alveolar macrophages. Our current understanding as to the relative influence of these different variables *in vivo* is limited.

Aerosol delivery is inefficient. The amount of aerosol deposited in the lungs from a metered dose inhaler is roughly 15%-20% of that released; the majority is deposited in the mouth and eventually swallowed, providing no or limited therapeutic benefit.

As far as using the bloodstream to deliver genes to the lung there are two basic delivery routes – either to target the venous circulation entering the lung via the pulmonary artery, or to target the arterial supply to the bronchial tree. Whilst the latter requires catheterisation under fluoroscopic guidance, jugular vein or pulmonary artery catheterisation can be used to target the vascular endothelial cells lining the pulmonary circulation. As with predicted effects following airway delivery, when a GTA is injected into the systemic circulation it is

known that the physical properties of the preparation will change very rapidly. For a lipoplex, exposure to serum will cause particles to aggregate and then disintegrate – causing the release of DNA. Such aggregation is in fact the basis for certain non-viral GTAs being successful in targeting the pulmonary endothelium.

In the context of selecting an appropriate vector it should be emphasised that a vector is not always a necessity - it is possible to deliver naked DNA to the airways and mediate gene expression. However, there are problems associated with delivery in that naked DNA is extremely sensitive to shear stress and cannot be aerosolised using conventional jet or ultrasonic nebulisers. And, even if these problems are solved, the DNA still has to somehow spontaneously interact with the cell surface. Unfortunately, the phosphate backbone that traces round the DNA double helix carries a negative charge; the airway cell surface is also negatively charged – so DNA will tend to be repulsed. In addition – if it remains for long enough in the ELF – the nucleases present in that environment will rapidly cleave the DNA into smaller fragments. With these barriers in mind it is surprising that expression occurs to any extent at all following delivery of naked DNA to the lung.

One way to circumvent these barriers is to incorporate the pDNA into liposomes. The core and layers of a liposome can contain the pDNA in solution – thus shielding the negative charge on the DNA from the cell surface and protecting it from nucleases and shear forces present during nebulisation. In addition, by incorporating lipids with positive charges, the chance increases that the lipoplex will non-specifically interact with negatively charged cell surface groups. From such interaction the lipoplexes are susceptible to uptake by endocytosis and encapsulation in cytoplasmic vesicles. As soon as it gets into the cell the lipoplex is liable to be degraded by nucleases following fusion with lysosomes – so it must release early from the endosomal compartment.

Although theoretically liposomes can be as small as 20nm diameter, in practice, because the cationic lipids are unable to alter the conformation of the DNA in any ordered sense, they tend to be substantially larger than this. In contrast, when DNA associates with cationic polymers the DNA collapses, or condenses, into compact particles containing only one or a few molecules of nanoparticle dimensions. Even very large DNA molecules can be condensed in this manner.

Once taken into the cell and released from the endosomal compartment the DNA has to enter the nucleus through the nuclear pores (~50nm diameter). Entry is facilitated by interaction with the nuclear pore complex. This interaction can be mediated through the DNA being associated with a nuclear localization signal – a polypeptide sequence recognised by chaperone proteins linked to the pore complex.

An important influence on transgene expression is promoter attenuation brought about as a consequence of inflammatory cytokines; these cytokines in turn arising as a consequence of the innate immune system recognising particular unmethylated CpG dinucleotide sequences in bacterial DNA. Constructing plasmids with no immunostimulatory CpG motifs has been shown to result in reduced inflammation and sustained transgene expression relative to unmodified plasmids.

The area of non-viral vector design is very active at the present time and such efforts are currently focussed on understanding the link between the structure of complexes and transfection efficiency – for only in understanding that link can directed improvements be brought to bear.

Viruses, which basically consist of genetic material contained within a protein shell, have evolved mechanisms to penetrate cell membranes and use the host cell's machinery and metabolism to replicate. By removing or altering the genes responsible for viral replication and replacing them or adding transgenes it is possible to engineer recombinant viruses that can be used to effect gene transfer and expression.

However, the major limitation of viruses as vectors relates to the induction of immune responses. Even with the genes responsible for replication removed, the viral vector still has to enter the cell and therefore leave the cell to deal with its protein coat. This will be presented to the immune system. Secondly the virus may induce the host cell to make viral proteins which again will potentially stimulate an immune response. Finally, even when the genes responsible for expression of viral proteins are removed it is still possible to get some 'leaky' expression, presumably as a consequence of inadequate separation of 'helper' viruses during production.

Adenoviral vectors can infect non-dividing cells and, because they don't integrate, the virus and transgene are lost over time as those transduced cells are replaced in the normal course of tissue renewal and homeostasis. They are therefore useful in gene therapy strategies where the expression of the transgene is only required for a short time.

Where sustained expression would be an advantage then integrating vectors such as retrovirus derivatives of murine leukemia virus could be considered. One drawback of such integrating viruses is the risk of perturbing the function of the host's genes or activating oncogenes. Another specific drawback of such retroviruses is that they are incapable of infecting non-dividing cells and thus would be inappropriate for terminally differentiated cells such as neurones or alveolar macrophages.

Fortunately, by using lentiviruses, the latter problem is solved, for these

viruses are capable of infecting both dividing and non-dividing cells. As with retroviruses, lentiviruses are integrating viruses.

Adeno-associated viruses (AAV) can't replicate in cells unless the cells are also infected with adenoviruses or herpesviruses. In the absence of such 'helper' viruses a small proportion of virus DNA does integrate into the host cell genome and remains quiescent. This integration is however extremely inefficient. AAV hasn't been associated with any disease in humans and its close relatives don't cause disease in animals. This doesn't mean that they don't elicit an immune response however and pre-existing or induced neutralising antibodies can be a major hurdle to the use of these vectors in humans.

Whilst the perceived goal for lung-directed gene therapy is often seen in the context of inherited disorders such as cystic fibrosis the potential application is realistically far more extensive. Gene therapy, in offering the potential to modulate susceptibility to, or progression of major pulmonary diseases such as asthma, emphysema, COPD and cancer, is likely to assume greater prominence in respiratory medicine in the years ahead.

**INTESTINAL MYCOBACTERIOSIS IN RUMINANTS.
PARATUBERCULOSIS: PATHOLOGY AND PATHOGENESIS**

Valentín Pérez

Anatomía Patológica. Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, 24071 Leon, Spain.

Intestinal mycobacteriosis are considered as one of the major diseases of ruminants. Two are the main causative agents: *Mycobacterium avium* subsp. *avium* (Maa) and, mainly, *Mycobacterium avium* subsp. *paratuberculosis* (Map). Severe infections by Maa appear rarely in natural conditions.

Subclinical infections can occur in the mesenteric lymph nodes, especially in cattle. However, Map infections are very common worldwide in all the ruminant species and are the cause of paratuberculosis or Johne's disease, a chronic infectious disease of both domestic and wild ruminants. Map replicates extremely slowly and many isolates are refractory to culture.

Animals are infected when young but the disease only appears clinically in adult animals, older than one year. This prolonged incubation period together with the difficulty in reproducing the characteristic features of the disease by experimental infection makes difficult the study of host-pathogen interactions.

Pathology of the disease is characterized by the development of a granulomatous inflammatory response in different areas of the intestine and related lymph nodes. It has been characterized by the study of natural and experimental cases. Individual variations in the pathological responses occur and a variety of lesions can be detected among infected animals. They range from the presence of focal granulomas, exclusively located in the gut associated lymphoid tissue or lymph nodes, seen in animals showing no clinical signs, up to diffuse forms affecting wide areas of the intestinal wall that appear in animals showing clinical signs. It has been seen that the development of lesions is closely related to the immune response mounted by the animals against Map and, therefore, an immunopathological spectrum of the disease has been defined. Animals with focal forms are located in the tuberculoid extreme of the spectrum, associated with high cellular immune responses. They appear in the initial phases of the infection or in those animals showing latency or resistance against Map infection that mount an effective response capable of controlling the progression of the disease. These animals shed low numbers of bacteria in their feces and show elevated antigen-specific interferon-gamma (IFN- γ) production. As infection progresses to a more clinical state, bacterial shedding in the feces is increased, IFN- γ production declines and antibody titers increase. These animals show diffuse lesions that correspond to borderline tuberculoid or lepromatous forms of the spectrum where a decrease in cell-mediated responses is observed,

contributing to the progressive nature of paratuberculosis. The relevance that these findings have in the interpretation of immune-based diagnostic tests will be discussed.

Differences in the pathological responses have been observed also among the different ruminant species showing paratuberculosis. They vary according to the location of lesions and the nature of the inflammatory infiltrate. In sheep or goats, focal intestinal lesions, formed by small, well-demarcated granulomas are located exclusively in the ileocaecal and jejunal Peyer's patches and, in animals showing clinical signs, two well-defined pathological forms are described, namely the "paucibacillary" form, in which the inflammatory infiltrate was composed of lymphocytes with some macrophages but few, if any, mycobacteria and the "multibacillary" form in which macrophages filled with numerous mycobacteria were the main inflammatory cells. However, in cattle, focal forms appear more frequently in the lymph nodes, with no involvement of the intestine; in animals with clinical signs and diffuse lesions, intermediate forms, sharing characteristics of both multibacillary and paucibacillary lesions are encountered, besides the two main types described in sheep. The presence of high numbers of multinucleated Langhans giant cells in all the lesion types in cattle, makes this a characteristic feature of bovine paratuberculosis, in contrast to the disease in sheep and goats. For explaining such differences, several hypotheses have been proposed such as the existence of morphological or functional differences between the intestinal compartments according to the ruminant species, variations in the immune response and susceptibility to mycobacterial infections. The causative organism plays also an important role. Map strains can be clearly separated into sheep types and cattle types, on the basis of bacteriological and molecular biology methods and these types are so-called because they are prevalent in those species. Recently, an experimental infection in lambs using both types of strains has shown that lesions induced by bovine strains in lambs are close to the cattle lesional pattern encountered in natural cases, with focal granulomatous lesions located in the lymph nodes and a high proportion of giant cells, whereas ovine strains produce lesions resembling the pattern observed in natural cases in sheep.

At present, the knowledge of the underlying mechanisms of paratuberculosis pathogenesis is still a challenge, from the perspective of both the pathogen and the host. Several virulence factors of Map have been recently identified. Young animals are most susceptible to Map infection which is transmitted via milk and environmental contamination. M-cell rich Peyer's patches are the major portal of entry of Map into the organism. Following expulsion at the basal side of the M-cell, Map is taken up by macrophages where it can persist, surviving its microbicidal mechanisms by altering the normal phagosomal maturation

pathways and preventing apoptosis. Infected macrophages, restricted in their capacity to migrate, accumulate at the site, forming the granulomatous lesion and activating cell mediated immunity. Animals can remain at this stage for years but a limited number of them rapidly progress to the clinical status. It is unclear what mechanism causes the progression from subclinical to clinical stage of the disease. It has been hypothesized to occur due to a switch in immune reactivity from type 1 to type 2 responses where IL-4 and IFN- γ play a crucial role. The decrease in IFN- γ production in the clinical stage is mediated by the increase in production of regulatory cytokines, IL-10 and TGF- β , which inhibit macrophage microbicidal and antigen-presenting ability. A progressive loss of CD4⁺ helper T cells that recognize mycobacterial antigens permits the infection to progress beyond control. The influence of host genetics resistance to paratuberculosis is a field that will need further investigation in the coming years.

CANCER: LIVING LIFE ON THE EDGE OF GENOMIC STABILITY

*Professor David J. Argyle BVMS PhD DECVIM-CA (Oncology) MRCVS
Royal (Dick) School of Veterinary Studies, University of Edinburgh*

Introduction

Cells of multi-cellular organisms form part of a specialized society that cooperate to promote survival of the organism. In this, cell division, proliferation and differentiation are strictly controlled and a balance exists between normal cell birth and the natural cell death rate. Derangement of these normal homeostatic mechanisms can lead to uncontrolled proliferation or loss of appropriate death leading to a normal cell taking on a malignant phenotype. This lecture will review our current understanding of carcinogenesis, and challenge some existing theories based upon new evidence derived from animal models.

Multi-Step Carcinogenesis: Our Current View.

Cancer is the phenotypic end result of a whole series of genetic and non-genetic events that may take place a long period of time to develop. The application of a cancer-producing agent (carcinogen) to tissues does not lead to the immediate production of a cancer cell. Following the initiation step produced by the agent, there follows a period of tumor promotion. This promotion may be caused by the same initiating agent or by other substances such as normal growth promoters or hormones. The initiating step is a rapid step and affects the genetic material of the cell. If the cell does not repair this damage, then promoting factors may progress the cell toward a malignant phenotype. In contrast to initiation, progression may be a very slow process, and may not even manifest in the lifetime of the animal. Each stage of multi-step carcinogenesis reflects genetic changes in the cell with a selection advantage that drives the progression towards a highly malignant cell. The age-dependent incidence of cancer suggests a requirement for between four and seven rate limiting, stochastic events to produce the malignant phenotype. These sequential events in tumor formation are a consequence of changes at the genetic level. Over the past twenty-five years, cancer research has generated a rich and complex body of information revealing that cancer is a disease involving dynamic changes in the genome. Seminal to our understanding of cancer biology has been the discovery of the so called “cancer genes” or Oncogenes and Tumour Suppressor Genes. Mutations that produce oncogenes with dominant gain of function and tumour suppressor genes with recessive loss of function have been identified through their alteration in human and animal cancer cells and by their elicitation of cancer phenotypes in experimental models.

Cancer arises through multiple molecular mechanisms.

The advances in our understanding of normal cell biology and the processes

that lead to malignancy have increased dramatically over the past 30 years. The last decade has shown us that transformation of a normal cell into a malignant cell requires very few molecular, biochemical and cellular changes that can be considered as acquired capabilities. Further, despite the wide diversity of cancer types, these acquired capabilities appear to be common to all types of cancer. An optimistic view of increasing simplicity in cancer biology is further endorsed by the fact that all normal cells, irrespective of origin and phenotype carry similar molecular machineries that regulate cell proliferation, differentiation, ageing and cell death. It has been suggested that the vast array of cancer genotypes is a manifestation of only six alterations in cellular physiology that collectively dictate malignant growth. These acquired characteristics can be summarized under the following headings:

- Self sufficient growth
- Insensitivity to anti-growth signals
- Evasion of programmed cell death (apoptosis)
- Limitless replicative potential
- Sustained Angiogenesis
- Tissue Invasion and Metastasis

Metastasis.

Metastasis is defined the dissemination of neoplastic cells to distant secondary (or higher order) sites, where they proliferate to form a macroscopic mass. Implicit in this process is the presence of a primary tumor. Metastases are not a direct extension of the primary tumor and are not dependent upon the route of spread (i.e hematogenous vs. lymphatic vs. peritoneal seeding). The process of metastasis is believed to occur through the completion of a series of step-wise events . In order for this process to occur a cancer cell must leave the site of the primary tumor, pass through the tumor basement membrane, and then through or between endothelial cells to enter the circulation (extravasation). While in the circulation tumor cells must be able to resist anoikis (programmed cell death associated with loss of cellular contact), evade immune recognition and physical stress, and eventually arrest at distant organs. At that distant site the cell must leave the circulation and survive in the hostile microenvironment of the foreign tissue. This distant site may be the eventual target organ for metastasis or may be a temporary site. In either case the cancer cell is thought to lie dormant for a protracted period of time before moving to its final location. Following dormancy, cells receive signals to proliferate, create new blood vessels (angiogenesis) or co-opt existing blood vessels and then successfully grow into a measurable metastatic lesion. It is likely that further progression is associated with the repetition of this process and the development of metastases from metastases; as such the steps outlined above continue not only after the

detection of the primary tumor but also after the detection metastases. The basic tenants of this model of metastasis have been intact for over 40 years; however, a greater understanding of biological principles associated with the each metastasis process is emerging. The opportunity provided by this emerging understanding is the development of novel strategies for the management of metastases in pet animals. One recent exciting development has been the observation that metastatic growth is preceded by an influx of bone marrow progenitor cells that create a metastatic niche at predetermined sites.

The Pathways to Cancer.

It is important to stress that the pathways for cells becoming malignant are highly variable. Mutations in certain oncogenes can occur early in the progression of some tumors, and late in others. As a consequence, the acquisition of the essential cancer characteristics may appear at different times in the progression of different cancers. Furthermore, in certain tumors, a specific genetic event may, on its own, contribute only partially to the acquisition of a single capability, whilst in others, it may contribute to the simultaneous acquisition of multiple capabilities. However, irrespective of the path taken, the hallmark capabilities of cancer will remain common for multiple cancer types and will help clarify mechanisms, prognosis and the development of new treatments

Cancer Stem Cells.

It was first extensively documented for leukemia and multiple myeloma that only a small subset of cancer cells is capable of extensive proliferation. For example, when mouse myeloma cells were obtained from mouse ascites, separated from normal haematopoietic cells and put in clonal in vitro colony-forming assays, only 1 in 10,000 to 1 in 100 cancer cells were able to form colonies. Even when leukaemic cells were transplanted in vivo, only 1–4% of cells could form spleen colonies. Because the differences in clonogenicity among the leukemia cells mirrored the differences in clonogenicity among normal haematopoietic cells, the clonogenic leukaemic cells were described as leukaemic stem cells. It has also been shown for solid cancers that the cells are phenotypically heterogeneous and that only a small proportion of cells are clonogenic in culture and in vivo. For example, only 1 in 1,000 to 1 in 5,000 lung cancer, ovarian cancer or neuroblastoma cells were found to form colonies in soft agar. Just as in the context of leukaemic stem cells, these observations led to the hypothesis that only a few cancer cells are actually tumorigenic and that these tumorigenic cells could be considered as cancer stem cells.

If the growth of solid cancers is driven by cancer stem cells, it would have profound implications for cancer therapy. At present, all of the phenotypically diverse cancer cells are treated as though they have unlimited proliferative potential and can acquire the ability to metastasize. For many years, however,

it has been recognized that small numbers of disseminated cancer cells can be detected at sites distant from primary tumors in patients that never manifest metastatic disease. One possibility is that immune surveillance is highly effective at killing disseminated cancer cells before they can form a detectable tumor. Another possibility is that most cancer cells lack the ability to form a new tumor such that only the dissemination of rare cancer stem cells can lead to metastatic disease. If so, the goal of therapy must be to identify and kill this cancer stem cell population. If solid cancer stem cells can be identified prospectively and isolated, then we should be able to identify more efficiently new diagnostic markers and therapeutic targets expressed by the stem cells. If tumour growth and metastasis are driven by a small population of cancer stem cells, this might explain the failure to develop therapies that are consistently able to eradicate solid tumours . Although currently available drugs can shrink metastatic tumors, these effects are usually transient and often do not appreciably extend the life of patients. One reason for the failure of these treatments is the acquisition of drug resistance by the cancer cells as they evolve; another possibility is that existing therapies fail to kill cancer stem cells effectively.

Existing therapies have been developed largely against the bulk population of tumour cells because they are often identified by their ability to shrink tumours. Because most cells with a cancer have limited proliferative potential, an ability to shrink a tumour mainly reflects an ability to kill these cells. It seems that normal stem cells from various tissues tend to be more resistant to chemotherapeutics than mature cell types from the same tissues. The reasons for this are not clear, but may relate to high levels of expression of anti-apoptotic proteins or ABC transporters such as the multi-drug resistance gene. If the same were true of cancer stem cells, then one would predict that these cells would be more resistant to chemotherapeutics than tumor cells with limited proliferative potential. Even therapies that cause complete regression of tumors might spare enough cancer stem cells to allow re-growth of the tumors. Therapies that are more specifically directed against cancer stem cells might result in much more durable responses and even cures of metastatic tumors. The concept of cancer stem cells is an exciting one and prospective studies need to be employed in veterinary cancer patients to identify these populations.

NEW WORLD CAMELIDS: BASIC ANATOMY AND PATHOLOGY

Nadia Robert, Dr.med-vet., Dipl. ACVP

Centre for Fish and Wildlife Health, Institute of Animal Pathology, Vetsuisse Faculty, University of Berne, 3012 Bern, Switzerland

Domesticated New World camelids (NWCs), including llamas and alpacas, have gained much popularity among private holders during the last 10 years, mostly as companion animal or for trekking or breeding purposes. Accordingly, knowledge concerning diseases in these species has increased and showed that the spectrum of diseases differs significantly from those in domestic ruminants. Even though camelids are not taxonomically classified as ruminants, they are functional ruminants. They are classified in the order Artiodactyla, suborder Tylopoda.

Camelids have a complex, three compartmented stomach. Digestion is similar but not analogous to ruminant digestion, and is much more efficient in extracting protein and energy from poor quality forages. Compartments one and two (called C1 and C2) are anaerobic fermentation chambers and the ingesta is homogenous and relatively dry. Both compartments are lined with non-keratinized stratified squamous epithelium, except for the ventral portions, which contain a large number of recessed glandular pouches. The glandular sacs of C1 and C2, as well as 4/5th the elongated third compartment (C3) are lined with a cardiac glandular mucosa. Proper gastric and pyloric glands are confined to the terminal fifth of C3. The intestinal tract is relatively long and almost equally divided between small and large intestine; the large intestine forms a spiral colon with 5.5 centripetal coils and 4.5 centrifugal coils. The narrow diameter Ampulla duodeni and the colon are primary sites of impaction in lamoids.

The liver is entirely located on the right side and there is no gall bladder. The caudal border of the liver is strikingly fimbriated. The kidneys are non-lobulated, shaped like those of a sheep.

Further important anatomical and physiological differences between camelids and true ruminants include: elliptical small red blood cells; foot with toenail and softpad; horizontal position of the second and third phalanges; induced ovulation, no estrous cycle; copulation in prone position with prolonged copulation; diffuse placenta; epidermal membranes surrounding the fetus; elongated soft palate (obligate nasal breather); suburethral diverticulum in females.

During the past five years, about 130 llamas and alpacas have been necropsied in our institution. Among the neonatal crias (< 1 mo of age), the main causes of mortality correlate with the literature and include low body weight and “poor doers”, lack of milk intake and failure of passive immunoglobulin transfer, *post*

partum infectious diseases (Rotavirus, *Streptococcus* sp., *Enterobacter* sp.) and malformations. The exact cause of the “poor doers” is mostly not known, but prematurity and “intrauterine growth retardation” (IURG), as defined by Adams, have to be considered. Based on our experience, dicrocoeliosis in the cow should be considered as possible cause of abortion and IUGR, secondary to suboptimal uterine environment.

In juveniles and adults, the most important problems are related to parasite infestation, including liver flukes, gastro-intestinal nematodes and coccidia. Other significant causes of mortality are mostly comparable with the standard literature on new world camelids and include dental problems, infectious diseases (esp. *Listeria monocytogenes* and *Mycobacterium microti*), tumors (lymphosarcoma, C1-squamous cell carcinoma, hemangiosarcoma), obstructive urolithiasis in males, and intoxications (moldy hay, toxic mushrooms, oak bud).

In contrast to the common liver fluke *Fasciola hepatica*, which is found throughout tropical and temperate regions in the world and has commonly been described in NWC, there are only very rare reports concerning infestation with the lancet fluke *Dicrocoelium dendriticum*. It is however a common problem in NWC in Central Europe. Based on our necropsy material, the lancet fluke has a prevalence of about 40%, and is diagnosed as cause of mortality in > 25% of NWC older than 6 mo. This parasite lives in the bile duct and gall bladder of mammal species, mainly ruminants, and its biological cycle requires two intermediate hosts (a terrestrial snail and an ant). In the end host, the young flukes migrate directly up the biliary duct system of the liver, without penetration of the gut wall, liver capsule, and liver parenchyma in contrast to fascioliasis. Diagnosis and treatment still remains a difficult issue. Clinical diagnosis of dicrocoeliosis is often challenging as symptoms are unspecific and usually associated with very rapid decline in general condition or sudden death. When reported, clinical signs last only hours to few days duration and include decreased appetite, recumbency, dyspnoe, cardiac arrhythmia and/or abortion. Blood analysis may indicate increased liver values, hypoproteinemia and/or anemia, however these changes are often not evident and therefore of little diagnostic value. Parasite eggs are passed irregularly in batches into the feces and therefore clinical diagnosis of dicrocoeliosis requires repeated coprological examinations. At necropsy the animals are usually in a good body condition. Severe pulmonary edema and sero-fibrinous exudations in the body cavities (thorax, pericardium and/or abdomen) are consistent striking changes. The liver is severely enlarged (up to 4 times the normal weight), with increased consistency and a mottled appearance emphasizing the lobular pattern, and variable numbers of parasites gush out of the biliary ducts on the cut surface. Histologically the major changes

consist of portal bridging fibrosis associated with bile duct proliferation and variable inflammatory infiltrates. Occasionally focal abscesses or granulomas can form around degenerated parasites or eggs. In the lungs, beside severe congestion and alveolar and interstitial edema, a vasculopathy is commonly observed in the middle and small arteries, characterized by endothelial and medial thickening, and presence of edema and/or fibrin within or surrounding the vessel wall, accompanied by a variable number of inflammatory cells. Severe exudations in the body cavities are not typical findings in domestic ruminants with microcoeliosis, but these exudations in the body cavities and lungs found in NWC correlate with the acute symptoms and sudden death. It is however unclear how the vascular changes and permeability problems arise and how they relate to the liver problems. However, pulmonary hypertension is a well-documented sequel to hepatic disease in human patients. The underlying pathophysiology is unknown but it is thought to involve the buildup of circulating vasoactive substances not filtered by the diseased liver.

Over the last five years Mycobacteriosis caused by *Mycobacterium microti* was diagnosed in our institute in 5 llamas and 1 alpaca from three different owners. Clinical signs lasted from several weeks to several months and were unspecific, including appetite- and weight loss, recumbency, increased respiratory and cardiac frequency, cardiac arrhythmia, and / or abortion. *Antemortem* intradermal tuberculin testing was performed on three llamas several months prior to death and revealed negative. At the time of death or euthanasia the body condition varied from good to cachectic and necropsy revealed in all cases caseous nodules (1 to 10 cm in diameter) in various organs, including lungs, liver, spleen, mediastinal and mesenteric lymph nodes, and serosal surfaces. Histologically, the granulomas were composed of large numbers of closely packed, epithelioid macrophages and multinucleated giant cells, admixed with various numbers of lymphocytes, plasma cells, and neutrophils. Ziehl-Neelsen and Fite-Faraco staining may revealed abundant or very rare acid-fast bacilli (AFB) within the macrophages. The AFB were identified by spoligotyping as *Mycobacterium microti*, vole type. *M. microti* belongs to the *M. tuberculosis* complex whose members share an identical 16S rRNA gene and show >90% relatedness (85 to 89% relatedness for *M. microti*) at the DNA level. The natural hosts and reservoirs of *M. microti* are small rodents such as field voles (*Microtus agrestis*), wood mice (*Apodemus sylvaticus*) and shrews (*Sorex araneus*). Sporadic cases of *M. microti* infections were previously reported in Europe in cats, pigs, a ferret, a cow, a badger, and a captive vicuña. *M. microti* has to be considered as a potential zoonotic agent, since it has been detected in pulmonary tuberculosis in humans in several European countries, affecting immunocompromised as well as immunocompetent patients. As direct access and close contact to llamas

and alpacas often occur in zoos and under private ownership, especially with children, recognition of potential zoonoses represents a critical issue.

Although skin diseases are not common in our necropsy material, these disorders represent a major part of the clinical problems. The most common problems are hyperkeratosis and alopecia associated with *Sarcoptes*, *Chorioptes* or *Psoroptes* infestation, lice, mallophaga and idiopathic hyperkeratosis (including zinc-responsive dermatosis and nasal/perioral or generalized mange).

REFERENCES

- Adams, R., and F. Garry. 1994. Llama Neonatology. In: The Veterinary Clinics of North America – Food Animal Medicine - Update on Llama Medicine. E. Hunt and L.W. Johnson (eds). W.B. Saunders Company, Philadelphia. Pp. 209-227.
- Budhiraja, R. and P.M. Hassoun. 2003. Portopulmonary hypertension: a tale of two circulations. *Chest* 123:562-576.
- Fowler, M.E. 1998. Medicine and Surgery of South American Camelids. Iowa State University Press, Ames.
- Fowler, M.E. 2003. Camelidae. In: Zoo and Wild Animal Medicine. M.E. Fowler and R.E. Miller (eds). Saunders Elsevier Science. Pp. 612-624.
- Gunsser, I., T. Hänichen, and J. Maierl. 1999. Leberegelbefall bei Neuweltkameliden: Parasitologie, Pathologie, Klinik und Therapie. *Tierärztl. Prax.* 27(G):187-192.
- Oevermann, A., G.E. Pfyffer, P. Zanolari, M. Meylan, and N. Robert. 2004. Generalized Tuberculosis in Llamas (*Lama glama*) due to *Mycobacterium microti*. *J. Clin. Micr.* 42(4):1819-1821.
- Puente, G.L. 1997. Acute and subacute fascioliasis of alpacas (*Lama pacos*) and treatment with triclabendazole. *Trop. Anim. Health Prod.* 29:31-32.
- Rickard, L.G. 1994. Parasites. In: The Veterinary Clinics of North America – Food Animal Medicine - Update on Llama Medicine. E. Hunt and L.W. Johnson (eds). W.B. Saunders Company, Philadelphia. Pp. 239-247.
- Rosychuk R.A.V. 1989. Llama Dermatology. *Vet Clin North Am Food Anim Pract.* 5:203-215.
- Rosychuk R.A.V. 1994. Llama Dermatology. *Vet Clin North Am Food Anim Pract.* 5:228-239.
- Smith, J.A. 1989. Non-infectious Diseases. In: The Veterinary Clinics of North America – Food Animal Medicine. L.W. Johnson. W.B. Saunders Company, Philadelphia. Pp. 101-143.
- Wenker, Ch., J.-M. Hatt, H. Hertzberger, P. Ossent, T. Hänichen., A. Brack, and E. Isenbügel. 1998. Dikrozöliose bei Neuweltkameliden. *Tierärztl. Prax.* 26(G):355-361.

IMMUNOHISTOCHEMICAL PHENOTYPES OF NATURAL AND EXPERIMENTAL SHEEP TSES

Lorenzo González, Sílvia Sisó, Stuart Martin and Martin Jeffrey

Veterinary Laboratories Agency (VLA-Lasswade). Pentlands Science Park, Bush Loan, Penicuik, Midlothian EH26 0PZ

With the advent of molecular techniques, immunohistochemistry (IHC) appears to have been relegated to a second plane, as it is generally regarded labour intensive and of subjective interpretation. In the field of the transmissible spongiform encephalopathies (TSEs), other techniques such as immunochemical “rapid” tests and Western immunoblotting are preferred for surveillance purposes, with IHC being employed for diagnostic confirmation of suspected cases. The use of IHC, however, extends to studies of the pathogenesis of TSEs, particularly in relation with the routes of entry to and dissemination within the brain of the infectious agent or rather its marker, the disease-associated form of the prion protein (PrP^d). While several groups are advocating the use of Western immunoblotting for the definition of molecular phenotypes to characterize TSE agents and strain, this is another area where IHC has a relevant role. In summary, there are several means by which we can investigate sheep scrapie strains diversity:

1. The Western immunoblot profile of PrP^d is very useful for discrimination between BSE, classical scrapie and atypical scrapie (Stack *et al.*, 2002, Benestad *et al.*, 2003). These methods, however do not distinguish between naturally occurring or experimental scrapie strains.
2. Different scrapie strains have been identified following transmission of sheep scrapie isolates to mice. These strains are characterised by their relative incubation periods in different mouse strains and by their spongiform lesion profile (Bruce *et al.*, 1991). However, the process by which these strains have emerged over time has involved multiple passages at limiting dilution, often involving transmission between different rodent species. The relationship between these rodent-adapted scrapie strains and the original sheep isolates is therefore unclear.
3. The profile of spongiform change in sheep brains has been assessed for its contribution to scrapie strain characterization (Ligios *et al.*, 2002). However, individual variability appears to be too high to allow consistent strain definition (Begara-McGorum *et al.*, 2002) and suggest that there are factors other than strains and PrP genotype which influence the patterns and severity of spongiform change in sheep.
4. We have designed and employed two different approaches for the characterization of IHC phenotypes in the brains of TSE-affected sheep: the

“Epitope mapping” and the “PrPd profiling” methods.

Epitope mapping (Jeffrey *et al.*, 2001, 2003, 2006; Martin *et al.*, 2005)

This approach is based in the availability or not of different epitopes of the prion protein depending on its intra- or extra-cellular location. We have observed that this availability and, consequently, the detection or otherwise of the prion protein with a panel of antibodies, is dependant on the infecting strain. This notion has been successfully applied to the discrimination between classical scrapie, BSE and CH1641, an experimental scrapie strain that, on Western immunoblot grounds is indistinguishable from BSE.

Reactivity to	Monoclonal antibody and epitope recognized				
	BG4 46-54	P4 93-99	IDPrP4 101-106	L42 144-166	R145 221-226
Extra-cellular PrP^d					
Scrapie	+	+	+	+	+
BSE	+	+	+	+	+
CH1641	-	-	-	-	-
Intra-neuronal PrP^d					
Scrapie	-	+	+	+	+
BSE	-	-	+	+	+
CH1641	-	-	-	+	+

We have observed that extra-cellular PrPd reacts with antibodies raised against epitopes situated all across the protein, from the N to the C terminus, and this is interpreted as this extra-cellular or cell membrane-associated PrPd being full length. That is however not the case in CH1641 infection, where PrPd is restricted to intra-cellular accumulation. In contrast, extreme downstream antibodies like BG4 do not recognize intra-neuronal PrPd suggesting that intracellular protein is truncated. This truncation occurs at different levels depending on the infecting TSE agent: further from the N-terminus in BSE than in scrapie and even more towards the C terminus in CH1641 infection. Nevertheless, differences in truncation sites between sheep scrapie strains, both natural and SSBP/1, have not yet been demonstrated with the available antibodies.

Because the host (sheep) and the cell type (neurons) are the same in all cases, the diversity of truncation points can only be attributed to differences in the conformation of the prion protein generated in those different infections. These distinct conformations would constitute the basis of strains. However, in the case of sheep BSE, the point in the amino acid sequence at which truncation occurs also depends on the cell where PrPd locates. Thus, PrPd in glial cells is cleaved further upstream than in neurones and truncation occurs even further upstream in tingible body macrophages in lymphoid tissues. This is possibly best explained by differences in the enzymatic activity in those different cell populations, and the fact that it does not occur in scrapie cases, where truncation

appears to occur at the same point regardless of the cell, would also be related to differences in conformation of the prion protein from different TSE agents.

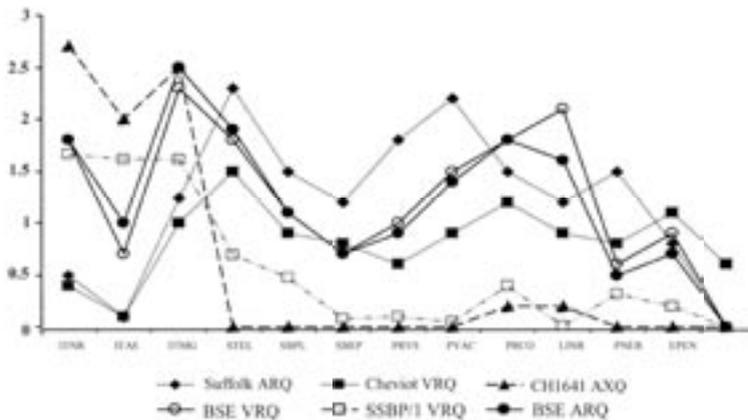
PrPd profiling (González *et al.*, 2002, 2003, 2005)

Looking at the way PrPd accumulates in the brain of TSE-affected sheep, it becomes clear that there are different morphological, topographical and cell-associated types of PrPd. Thus, PrPd deposits can be related to neurons, glial cells, ependymal cells, endothelia,... and those accumulations can be intra- or extra-cellular and adopt a variety of morphologies. In the majority of clinically affected sheep, at least after natural infection, PrPd deposits are widely distributed throughout the brain, therefore providing little help towards the characterization of pathological phenotypes. On the other hand, we have been studying the morphology and cell-association of PrPd deposits in the brains of sheep of different breeds and PrP genotypes, which developed clinical disease after infection with different natural and experimental TSE sources, including BSE.

The PrPd profiling consists in the identification and subjective scoring of different PrPd types in a systematic selection of brain areas (cerebral cortex, striatum, thalamus/hypothalamus, midbrain, cerebellum and obex). The PrPd types that we currently recognize are the intra-neuronal (ITNR), intra-astrocytic (ITAS), intra-microglial (ITMG), stellate (STEL), sub-pial (SBPL), sub-ependymal (SBEP), peri-vascular (PRVS), peri-vacuolar (PVAC), particulate-coalescing (PRCO), linear (LINR), peri-neuronal (PNER), ependymal (EPEN) and vascular plaques (VASC). These types are scored from 0 (absent) to 3 (prominent) in all those different brain areas and the averages for each animal are later obtained and represented as in the graph. This approach enables us to provide further evidence of how unique and stable sheep BSE is, so that it produces the same profile regardless of route and dose of infection, PrP genotype and breed of the host, etc. Equally, it has also showed that CH1641 and SSBP/1 produce consistent pathological phenotypes in the brain, which are little or not influenced by host factors, and that are different from BSE and natural scrapie. A comparison of PrPd profiles between different natural and experimental sheep TSEs is provided on the next page.

We have hypothesized that these distinct PrPd profiles are the result of different TSE agents and strains having tropism for different cell types within the brain and/or resulting in different PrP processing.

In summary, the detailed examination of PrPd accumulation in the brains of TSE affected sheep and the use of different antibodies are valid tools for the characterization of pathological phenotypes, the analysis of which can help the definition of TSE agents and strains and the effect that other factors, host or



environment, may have in those phenotypes.

References

- Begara-McGorum, I., González, L., Simmons, M., Hunter, N., Houston, F. and Jeffrey, M. (2002). Vacuolar lesion profile in sheep scrapie: factors influencing its variation and relationship to disease-specific PrP accumulation. *Journal of Comparative Pathology* **127**, 59-68.
- Benestad, S.L., Sarradin, P., Thu, B., Schonheit, J., Tranulis, M.A. and Bratberg, B. (2003). Cases of scrapie with unusual features in Norway and designation of a new type, Nor 98. *Veterinary Record* **153**, 202-208.
- Bruce, M.E., McConnell, I., Fraser, H. and Dickinson, A.G. (1991). The disease characteristics of different strains of scrapie in Sinc congenic mouse lines: implications for the nature of the agent and host control of pathogenesis. *Journal of General Virology*, **72**, 595-603.
- González, L., Martin, S., Begara-McGorum, I., Hunter, N., Houston, F., Simmons, M. and Jeffrey, M. (2002). Effects of agent strain and host genotype on PrP accumulation in the brain of sheep naturally and experimentally affected with scrapie. *Journal of Comparative Pathology* **126**, 17-29.
- González, L., Martin, S. and Jeffrey, M. (2003). Distinct profiles of PrPd immunoreactivity in the brain of scrapie- and BSE-infected sheep: implications on differential cell targeting and PrP processing. *Journal of General Virology* **84**, 1339-1350.
- González, L., Martin, S., Houston, F.E., Hunter, N., Reid, H.W., Bellworthy, S.J. and Jeffrey, M. (2005). Phenotype of disease-associated PrP accumulation in the brain of bovine spongiform encephalopathy experimentally infected sheep. *Journal of General Virology* **86**, 827-838.

Jeffrey, M., Martin, S., González, L., Ryder, S.J., Bellworthy, S.J. and Jackman, R. (2001). Differential diagnosis of infections with the bovine spongiform encephalopathy (BSE) and scrapie agents in sheep. *Journal of Comparative Pathology* **125**, 271-284.

Jeffrey, M., Martin, S. and González, L. (2003). Cell-associated variants of disease-specific prion protein immunolabelling are found in different sources of sheep transmissible spongiform encephalopathy. *Journal of General Virology* **84**, 1033-1046.

Jeffrey, M., González, L., Chong, A., Foster, J., Goldmann, W., Hunter, N. and Martin, S. (2006). Ovine Infection with the Agents of Scrapie (CH1641 Isolate) and Bovine Spongiform Encephalopathy: Immunochemical Similarities can be Resolved by Immunohistochemistry. *Journal of Comparative Pathology*, **134**, 17-29.

Ligios, C., Jeffrey, M., Ryder, S.J., Bellworthy, S.J. and Simmons, M.M. (2002). Differentiation of scrapie phenotypes by lesion profiling. *Journal of Comparative Pathology* **127**, 45-57.

Martin, S., González, L., Chong, A., Houston, F., Hunter, N. and Jeffrey, M. (2005). Immunohistochemical characteristics of disease-associated PrP are not altered by host genotype or route of inoculation following infection of sheep with bovine spongiform encephalopathy. *Journal of General Virology* **86**, 839-848.

Stack, M., Chaplin, M. & Clark, J. (2002). Differentiation of prion protein glycoforms from naturally occurring sheep scrapie, sheep-passaged scrapie strains (CH1641 and SSBP1), bovine spongiform encephalopathy (BSE) cases and Romney and Cheviot breed sheep experimentally inoculated with BSE using two monoclonal antibodies. *Acta Neuropathologica* **104**, 279-286.

ARCHITECTURAL PATTERNS IN CYTOLOGY

Carlo Masserdotti, DVM, Dipl ECVCP, Brescia

Clinica Veterinaria S.antonio, Via E.montale N°2 Salo' (Bs) Italy

INTRODUCTION.

In general, cytologic arrangements can be considered as reciprocal dispositions of exfoliated or aspirated cells that retain the full or partial architectural features of parent tissues. A cardinal usefulness in the identification of arrangements is the ability to translate the cytologic pattern into histologic tissue pattern of diagnostic value.

Despite the common opinions about the difficulty in interpretation of the architectural features in a cytological sample, cellular architectures are maintained in many specimens. This can best be achieved if careful attention is given to tissue collection and in smear preparation, in order to obtain cellular samples wherein reciprocal relationships among cells are respected. High cellularity represents the best background to look for remnants of cellular architecture. The possibility to obtain samples with high cellularity is related to the sample, smearing and fixation methods, and obviously, to the skill of the cytopathologist; recent improvements in veterinary imaging, the development of new sampling methods, like ultrasound-guided fine needle aspiration, tomographic-guided fine needle aspiration and recent improvements in tissue collection, like FNCS (fine needle capillary sampling) or the tissue crush specimen have permitted the easy access to internal organs and have improved the possibility of obtaining high-cellularity samples that might retain more architectural features. The use of more delicate and less traumatic techniques for smear preparation has also contributed to the possibility of obtaining more integral cellular aggregations. The aim of this lecture is to describe the principal patterns of cellular arrangement and the current nomenclature in order to provide supplementary information for cytological diagnosis.

ARCHITECTURAL PATTERNS.

The most common models of cellular arrangements can be described as follows:

Pavement arrangement. The cells are disposed as pieces of a mosaic in a monolayer, also called "cobblestone arrangement".

Honeycomb arrangement. This is similar to a pavement arrangement, although cells are not flat, but rather are cuboidal or columnar.

Acinar arrangement. This is characteristic of glandular tissues, where the basic unit is represented by cells arranged around an empty central area, sometimes depleted of secretory material.

Palisade arrangement. In this type of arrangement, the cellular bodies frequently show a columnar shape and are arranged in regular rows that resemble a palisade; nuclei of columnar epithelial cells are basally oriented.

Papillary arrangement. A papilla is commonly composed of a stromal or vascular axis and a mono or multilayered epithelial layer, where the outer cells may show a palisade arrangement. Frequently the axis is not evident or may be lacking entirely, and the papillary arrangement, defined by some authors as “morulae” or more simply “three-dimensional clusters”, is represented by clusters of cells where the cytoplasmic surface underlines a smooth profile.

Trabecular arrangement. Cells exfoliate in large, elongated three-dimensional or two-dimensional clusters that exhibit multiple ramification.

Storiform arrangement. This is a typical mesenchymal arrangement: the spindle cells are arranged in wave-like bundles, sometimes associated with stromal strands, where they intersect or intertwine at various angles.

Perivascular arrangement. Cells are disposed around single or multiple vascular structures that are recognizable as endothelial elongated elements, sometimes containing a few erythrocytes.

CONCLUSIONS

Cellular arrangement may represent a model for improvement of cytologic diagnosis, because together with classic criteria, it provides a useful tool for diagnosis of many diseases, mostly neoplastic. The review of cytologic architecture establishes a useful relationship with the histologic pictures of a normal or pathologic tissue.

The present abstract is the summary of a paper, that will be published in the coming issues of *Veterinary Clinical Pathology*.

Reprinted with permission, American Society for Veterinary Clinical Pathology.

INFECTIONS OF PREGNANCY IN RUMINANTS; A DEVELOPING STORY.

David Buxton

Moredun Research Institute, Edinburgh, Scotland.

Several infectious agents, including protozoa (eg *Toxoplasma gondii*, *Neospora caninum*), bacteria (eg *Chlamydophila abortus*, *Bacillus licheniformis*, *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* sp. *Brucella abortus*, *B. melitensis* and *Coxiella burnetii*) and viruses (eg bovine virus diarrhoea virus, bovine herpesvirus 1; border disease virus) have the potential to cause abortion in farm ruminants (Brownlie and England, 1999; Buxton and Henderson, 1999; Dubey *et al.*, 2006). Together this group of pathogens is of considerable significance worldwide through the economic losses caused, the welfare of livestock and the potential zoonotic risk that several of them carry. This brief review will explore some of the mechanisms that may act alone or in combination to endanger the fetus.

Pregnancy in mammals represents a very special biological compromise made by the mother in order that she can harbour and nurture the developing fetus which is, to all intents and purposes, a foreign body (Entrican and Wheelhouse, 2006). As part of this subtle balance, maternal immunity at the materno-fetal interface is modulated so that mechanisms that activate inflammatory cells are suppressed. Thus there is minimal maternal expression of cytokines such as interleukin 2 (IL-2), tumour necrosis factor alpha (TNF- α) and interferon gamma (IFN- γ) (Entrican and Wheelhouse, 2006). However in helping to orchestrate a successful pregnancy these mechanisms represent a chink in the animals' immune "armour" making the placenta and fetus peculiarly susceptible to certain pathogens.

Abortifacient pathogens almost always infect the placenta and fetus via the maternal blood as part of a primary infection or during recrudescence of a persistent infection in the dam. In the former the mother attempts to control infection by innate and adaptive immune mechanisms but the pathogen only needs to be released briefly into the circulation to gain access to the placenta and fetus. In a typical case of ovine toxoplasma abortion a naïve pregnant ewe ingests *T. gondii* sporulated oocysts, they excyst in the gut lumen to invade the intestinal wall and multiply in the draining mesenteric lymph nodes before being released into the blood to be disseminated to the placenta and other tissues (Buxton, 1998).

While infection is readily controlled in the tissues with the formation of bradyzoites in tissue cysts and the onset of a persistent but normally benign infection, in pregnancy *Toxoplasma* also invades the placenta where control is

more complicated. Here tachyzoites initially multiply in the maternal placental caruncular septum before invading the fetal placental villi. The multifocal necrosis initiated in this way gives rise to the typical “white spot” placenta seen at the time of abortion. However the timing of this primary infection also influences the outcome (Buxton and Finlayson, 1986). A maternal parasitaemia in mid-pregnancy would be expected to induce typical lesions of multifocal placental necrosis with invasion of the fetus and the development of characteristic focal inflammation in the brain and some other tissues. At this stage the fetal immune system may be sufficiently developed to suppress and control the parasite so that infection is not always fatal and fetal development continues. However the placental lesions are not totally controlled and their progressive expansion may eventually limit the availability of oxygen at a late stage in gestation, when fetal demand is at its maximum, triggering secondary anoxic brain damage (Buxton *et al.*, 1982).

In an infection initiated earlier in gestation, before the fetal immune system is sufficiently mature, *T. gondii* is able to multiply unhindered causing rapid and extensive tissue damage, death of the fetus and its subsequent reabsorption. Ewes affected in this way may give the outward appearance of being barren. Placental lesions initiated in the latter stages of pregnancy do not have time to develop to the extent that they endanger the fetal supply of oxygen. While the parasite will be transmitted across the placenta it is more readily controlled by the now better developed fetal immune system, so that the lamb is born healthy, infected and immune.

A similar set of circumstances appears to influence the outcome of infection in cattle by the closely related protozoan, *Neospora caninum*. However, while in ovine toxoplasmosis the major danger to pregnancy is a primary infection in the ewe being transmitted to the placenta and fetus, in bovine neosporosis both this risk (exogenous transplacental transmission) and recrudescence of an established persistent neospora infection (endogenous transplacental transmission) occurs (Dubey *et al.*, 2006). Evolutionary pressures appear to have favoured this recrudescence of infection being triggered later in gestation, so that *N. caninum* is passed on to the next generation without causing disease. However undefined circumstances, but possibly sometimes involving some form of management stress, may precipitate a maternal parasitaemia earlier in gestation. In this case fetal immunity is insufficiently developed to suppress the invading parasite, with consequent acute placental and fetal necrosis and abortion (Dubey *et al.*, 2006). In fetuses that survive longer, lesions of focal necrosis and associated microgliosis are seen in the brain and focal necrosis and inflammation may also be present in the heart and liver (Dubey *et al.*, 2006), similar to those of ovine toxoplasmosis.

Other factors may influence the outcome of bovine neosporosis. For example it has been proposed that placental damage may also cause the release of maternal prostaglandins that in turn cause luteolysis and abortion (Dubey *et al.*, 2006). Also it has been hypothesised that in some circumstances the maternal placental inflammation evoked by *N. caninum* causes the local release of maternal pro-inflammatory cytokines that may well trigger an immune mediated failure of pregnancy (Innes *et al.*, 2005). This latter suggestion is supported by evidence that in experimental bovine neosporosis the maternal inflammatory response in the placentome is predominantly composed of T-lymphocytes, with evidence of local production of interferon gamma, suggesting a Th-1 type immune response, potentially incompatible with pregnancy (Maley *et al.*, in press). While these proposed mechanisms may all be linked, one or more of them may be of particular importance in a given instance and all will be influenced by the stage of gestation.

The stage of gestation is also a key factor in the pathogenesis of ovine chlamydial abortion caused by *C. abortus*, an obligate intracellular bacterium. While in ovine toxoplasmosis the clinical outcome is influenced by the time of onset of infection, in ovine chlamydial abortion a reasonably consistent clinical syndrome is seen, with abortion usually confined to the last two weeks of gestation with no evidence of earlier fetal death or mummification. Examination of the pathogenesis of experimental infections in sheep has shown that *C. abortus* does not induce lesions in the placenta until after 90 days gestation (Buxton *et al.*, 1990), despite the fact that the ewe might have become infected before this, including the initiation of a latent infection prior to pregnancy. The earliest lesions are seen in the placenta after 100 days gestation when the organism is seen multiplying in fetal placental trophoblast cells in the hilus of the placentome. Why this rather precise timing occurs is not completely understood but once started progressively more trophoblast cells become infected, triggering their destruction and further dissemination of the bacterium. The underlying basement membrane is disrupted and there is an associated intense suppurative fetal inflammatory response (Buxton *et al.*, 2002; Sammin *et al.*, in press). The mesenchyme becomes oedematous, arteritis and arteriolitis develop, and some blood vessels become thrombotic. While the fetal inflammation includes CD4+, CD8+ and $\gamma\delta$ T cells, macrophages appear to be the most numerous, many of which can express TNF- α (Buxton *et al.*, 2002). It is likely that the latter cells are of considerable significance in the pathogenesis of abortion. The factors that define this remarkably precise onset of lesion development in ovine chlamydial abortion are only partially understood but they will include a site of latency (possibly the ewe's immune system) where *C. abortus* persists, conditions that encourage a maternal recrudescence of this latent infection and

subsequent chlamydaemia, a route of entry to the placenta and the provision of an environment in which *C. abortus* can parasitise and multiply in the placental trophoblast cells.

The dangers of a pathogen circulating in the dam's bloodstream during pregnancy and the central role of the host immune response are exemplified by the pestiviruses. In ruminants transplacental transmission of Border disease virus (BDV) in sheep or bovine virus diarrhoea virus (BVDV) in cattle, occurs in pregnant dams that develop a viraemia while suffering a primary infection. When this occurs either virus can infect the placenta and cross to the fetus where the outcome will be influenced by the stage of pregnancy. Early on, before immunocompetence, BDV and BVDV may infect the fetus without always causing fetal death or developmental abnormalities (Nettleton *et al.*, 1998; Brownlie and England, 1999). In these circumstances when the fetus subsequently becomes immunocompetent it recognises the virus as "self" rather than as "foreign", and does not mount an immune response to the virus, allowing the establishment of a persistent infection. In Border disease and bovine virus diarrhoea offspring may be born live, seronegative for the respective virus but actively shedding virus, so that in-contact naïve animals become infected. Similarly persistently infected females that survive to maturity and pregnancy will pass virus on to their offspring to cause fetal pathology.

Thus pregnancy offers a special set of conditions dictated by the mother's need to produce genetically distinct offspring to a stage where they are sufficiently mature to survive "outside". These special circumstances have also allowed certain protozoa, bacteria and viruses to evolve to take advantage of the situation, not always with a fatal outcome for the fetus, thus helping to ensure their own survival.

References

Brownlie, J. and England, G.C.W. (1999). Viral infections with pathological consequences for reproduction in veterinary species. In *Viral Infections in Obstetrics and Gynaecology*, Eds D.J. Jeffries and C.N. Hudson, Arnold, London.

Buxton, D., Gilmour, J.S., Angus, K.W., Blewett, D.A. and Miller, J.K. (1982). Perinatal changes in lambs infected with *Toxoplasma gondii*. *Research in Veterinary Science* 32, 170-176.

Buxton, D. and Finlayson, J. (1986). Experimental infection of pregnant sheep with *Toxoplasma gondii*: pathological and immunological observations on the placenta and foetus. *Journal of Comparative Pathology*, 96, 319-333.

Buxton, D., Anderson, I.E., Longbottom, D., Livingstone, M., Wattedgedera, S. and Entrican, G. (2002). Ovine chlamydial abortion: characterization of the inflammatory immune response in placental tissues. *Journal of Comparative Pathology* 127, 133-141.

- Buxton, D., Barlow, R.M., Finlayson, J., Anderson, I.E. and Mackellar, A. (1990). Observations on the pathogenesis of *Chlamydia psittaci* infection on pregnant sheep. *Journal of Comparative Pathology*, 102, 221-237.
- Buxton, D. (1998). Protozoan infections (*Toxoplasma gondii*, *Neospora caninum* and *Sarcocystis* spp.) in sheep and goats: recent advances. *Veterinary Research* 29, 289-310.
- Buxton, D. and Henderson, D.C. (1999). Infectious abortion in sheep. *In Practice* 21 (7), 360-368.
- Dubey, J.P., Buxton, D. and Wouda, W. (2006). The pathogenesis of bovine neosporosis. *Journal of Comparative Pathology*. 134, 267-289.
- Entrican, G. and Wheelhouse, N.M. (2006). Immunity in the female sheep reproductive tract *Veterinary Research* 37, 295-309.
- Innes, A.E., Wright, S., Bartley, P., Maley, S., Macalldowie, C. Esteban-Redondo, I. and Buxton, D. (2005). The host-parasite relationship in bovine neosporosis, *Veterinary Immunology and Immunopathology* 108, 29-36.
- Maley, S.W., Buxton, D., Macalldowie, C.N., Anderson, I.E., Wright, S.E., Bartley, P.M., Esteban-Redondo, I., Hamilton, C.M., Storset, A.K. and Innes, E.A. (2007). Characterization of the immune response generated in the placenta of cattle experimentally infected with *Neospora caninum* in early gestation. *Journal of Comparative Pathology*. In press.
- Nettleton, P.F., Gilray, J.A., Russo, P. and Dlissi, E. (1998). Border disease of sheep and goats. *Veterinary Research* 29, 327-340.
- Sammin, D.J., Markey, B.K., Quinn, P.J., McElroy, M.C. and Bassett, H.F. (2006). Comparison of foetal and maternal inflammatory responses in the ovine placenta following experimental infection with *Chlamydia abortus*. *Journal of Comparative Pathology*, (in press).

THE USE OF EQUINE CYTOLOGY IN PRACTICE: ACUTE AND CHRONIC CONDITIONS

Kathleen P. Freeman, BS, DVM, MS, PhD, DipECVCP, MRCVS

Idexx Laboratories, Wetherby, West Yorkshire

Introduction

Cytology has become a common technique in equine practice. Situations in which cytology is most likely to provide valuable information are presentations for acute and chronic conditions. Equine clinicians appreciate pathologists who know about conditions specific to the horse and are willing to work with them in determining the aetiologic diagnosis, differential diagnoses and/or pathologic-anatomic diagnoses for various clinical presentations. This presentation will summarize various clinical presentations and types of cytologic specimens that may be of value for investigation. Examples of the types of cytologic interpretations that may be helpful will be included. It may be difficult to win the approval of equine practitioners who often have high expectations regarding pathologist performance, but once a good working relationship is established, the equine practitioner may be a regular contributor to the cytologic caseload in the laboratory.

Types of Cytologic Specimens for Investigation of Various Clinical Presentations

Acute colic, lameness or joint swelling, neurologic signs, respiratory distress or dyspnoea or post-foaling bleeding or post-breeding/post-foaling colic are examples of acute clinical presentations in which cytologic evaluation may provide valuable information. Chronic or recurrent colic prolonged or recurrent lameness or joint swelling, chronic or recurrent respiratory disease, infertility or poor performance, weight loss, or persistent swellings, masses or enlargements are examples of chronic or recurrent conditions in which cytologic evaluation may provide valuable information. The presentations and types of cytologic specimens which may be of benefit for their investigation, and examples of useful cytologic information that may be obtained are summarized in the table on the next page.

Advantages of Cytology

Cytologic evaluation is often less invasive than biopsy and less expensive than other techniques, such as advanced imaging. In some circumstances, cytology may provide information not available by other means, allow identification of patterns corresponding to clinical conditions, or provide a rapid diagnosis of sepsis that requires immediate treatment in order to provide for the best welfare, prognosis and future performance of equine athletes. In some cases it may identify abnormalities that indicate a need for additional investigation or

Clinical Presentation	Cytologic Specimen(s) for Investigation	Useful Cytologic Information
<i>Acute Conditions</i>		
Acute colic	Abdominal fluid	Vascular compromise/possible need for surgical intervention
Acute lameness or joint swelling	Synovial fluid	Active inflammation or its absence <i>Reactive features</i>
Acute neurologic signs	Cerebrospinal fluid	Inflammatory/ noninflammatory or other categories of
Acute respiratory signs or dyspnoea	Bronchoalveolar lavage/Tracheal washing Guttural pouch washing/brushing	Presence or absence of increased mucus and inflammation/ detection of allergy/ obstruction or other patterns corresponding to clinical syndromes or conditions
Acute reproductive bleeding post-foaling or post-foaling or post-breeding colic	Uterine swab/washing/lavage	Presence or absence of haemorrhage or inflammation/ stage of reproductive activity
	Abdominal fluid	Vascular compromise/ inflammation/ spermatozoa that may indicate accidental uptake during breeding
<i>Chronic Conditions</i>		
Chronic or recurrent colic	Abdominal fluid	Presence or absence of inflammation or vascular compromise/possible parasitism/ possible draining abscess or other conditions
Chronic lameness or joint swelling	Synovial fluid	Presence or absence of neutrophilic inflammation/ sepsis/ reactive features
Chronic or recurrent neurologic signs	Cerebrospinal fluid	Presence or absence of inflammation/haemorrhage or other conditions
Chronic or recurrent respiratory disease	Bronchoalveolar lavage/Tracheal washing	Presence or absence of increased mucus and inflammation/ detection of allergy/ obstruction or other patterns corresponding to clinical syndromes or conditions
Chronic infertility	Uterine washing/ swab/lavage	Presence or absence of inflammation/ infection/ stage of reproductive activity/response to treatment
Poor performance	Bronchoalveolar lavage/Tracheal washing Abdominal fluid Synovial fluid	Confirmation of system or likely site(s) responsible for poor performance/ demarcation of pathology involving a particular site
Persistent swellings, masses or enlargements	Fine needle aspiration	Detection and identification of the source, aetiology (if possible), presence or absence of inflammation or malignancy, or other conditions
Weight loss	Abdominal fluid Synovial fluid Endoscopic gastrointestinal brushing/washing Fine needle aspirates Percutaneous aspirates	Confirmation of system or likely site(s) resulting in weight loss/ determination of pathology involving a particular site

medical or surgical intervention. It is often complementary to other avenues of investigation, including biopsy with histologic evaluation.

Disadvantages of Cytology

There are a limited number of ways that cells can respond to a variety of insults. However, sometimes knowledge of specific conditions or disease, their aetiopathogenesis and progression may enable an interpretation of a high probability of lesser probability of a particular disease or condition.

Sometimes the features may be nonspecific, but supportive of a particular clinical suspicion or helpful in confirming that a particular system, location, or joint is likely to be the site responsible for the clinical signs observed. In those cases in which only a pathologic-anatomic diagnosis is possible, this may provide a reference point for further investigations, monitoring or serial evaluations to determine if features are persistent, progressive or changing with time or treatment.

Knowledge of the particular system, organ or site of cytologic origin is needed in order to determine the limitations of the cytologic specimen. In most organs and systems cytology is poorly sensitive for the detection of fibrosis, which may herald an end-stage or irreversible phase of disease progression.

Role of the Pathologist

It is the duty of the clinical pathologist interpreting cytologic specimens from the horse to understand equine anatomy, the methods of collection, and the features that are required to determine specimen adequacy and likely representation of the site of collection. Knowledge of disease processes and those that can be detected cytologically is important in providing the most specific information possible, but also being able to advise regarding the limitations of the technique with regard to diagnosis of certain conditions, as well as conditions that could present with a similar appearance. Ongoing evaluation of the literature, as well as attention to cytologic/histology correlation and clinical followup is vital to remaining current with regard to cytologic interpretation. Knowledge of changing terminology and paradigms for understanding disease processes and current treatment justifications is important in providing information relative to these facets of equine medicine and practice. There is no excuse for claiming that equine practitioners fail to submit 'good specimens'. Education regarding specimen collection, handling and submission is of paramount importance in fostering a good pathologist-clinician relationship. Methods for collection of 'good specimens' of all types are readily available in the veterinary literature.

Conclusions

Equine cytology interpretation is a unique niche of cytopathologic practice. It may provide a rewarding opportunity for a good working relationship between the pathologist and the practitioner. It may help contribute to provision of the best possible care for equine patients and contribute to equine health and welfare.

APPROACH TO BONE MARROW CYTOLOGY IN THE DOG AND CAT

R. Mischke¹ and A. W. Philbey²

¹*Small Animal Clinic, Hannover School of Veterinary Medicine, Bischofsholer Damm 15, D-30173 Hannover, Germany;*

²*Institute of Comparative Medicine, University of Glasgow Veterinary School, Bearsden Road, Glasgow, G61 1QH, Scotland, UK*

Bone marrow cytology is an important additional diagnostic procedure in small animal medicine. The main advantages in comparison to core biopsy are an easier technique, an earlier availability of specimens for interpretation and a better possibility to assess the morphology of individual cells. The most significant disadvantage is the restricted ability to assess bone marrow architecture, which is essential for the diagnosis of selected diseases (esp. myelofibrosis) and cellularity, as well as a possibly limited sensitivity to detect local alterations, such as granulomas and bone marrow metastases. Table 1 gives an overview of possible indications to perform bone marrow cytology. On the assumption that an adequate technique is used, severe complications, including bone marrow infections, injuries of the surrounding tissue and haemorrhages, are rare. There are no absolute contraindications against the aspiration of bone marrow.

Table 1. Indications to perform bone marrow cytology

- **Clarification of the causes of a (protracted) cytopenia (esp. bi- or pancytopenia)**
 - Non-regenerative anaemia
 - Neutropenia
 - Thrombocytopenia
- **Diagnosis of bone marrow neoplasia (Acute leukaemia, chronic myeloproliferative disease, multiple myeloma)**
 - Cytopenia
 - Persistent cytosis
 - Abnormal cells in blood film (blasts, pathological left shift)
 - Recurrent or persistent fever
 - Hypercalcaemia
 - Monoclonal gammopathy
- **Staging, detection of metastases**
 - Malignant lymphoma
 - Micro/distant metastases of non-haematopoietic tumours (esp. carcinoma)

- **Detection of infectious agents**
 - *Leishmania donovani*
 - *Ehrlichia canis*
 - Feline leukaemia virus (immunofluorescence)
 - *Histoplasma capsulatum*
 - *Toxoplasma gondii*
- **Special stains**
 - Cytochemical procedures
 - Immunophenotyping
 - Iron stains

Collection of bone marrow and preparation of cytological specimens

Bone marrow aspiration in non-anaesthetised dogs and cats is performed using spinal needles primarily from the iliac crest and in some cases from the trochanter major femoris in the area of the fossa trochanterica. To avoid dilution with marrow blood, the aspirated marrow should be limited to a volume of 0.5 ml and immediately transferred into an EDTA containing tube and afterwards into a watch glass or Petri dish. By using a spatula, the fragments can be separated from the marrow blood and transferred to slides. Specimens are prepared using the pull apart technique and stained with routine haematological stains, such as May-Grünwald-Giemsa.

Examination schedule

The cytological examination of bone marrow specimens begins with macroscopic examination. 100x magnification is used to assess cellularity and the number of megakaryocytic cells, as well as to detect localised alterations (e.g. local accumulations of plasma cells in cases of multiple myeloma or metastatic infiltrates of non-haematopoietic neoplasia). This magnification is also used to find suitable areas for detailed examination using 200x, 400x and finally 1000x magnification. The interpretation of bone marrow specimens requires information regarding case history, clinical examination and a complete blood count.

Macroscopic examination

The quality of a cytological bone marrow specimen, i.e. the presence of an adequate number of fragments, can often be evaluated by macroscopic examination. This is indicated by a prominent blue central fragment-rich zone containing high numbers of nucleated cells. In most cases, representative marrow requires the presence of fragments. Exceptions are bone marrow hypoplasia, myelofibrosis and often acute lymphoblastic leukaemias, where even with repeated punctures no or only a small number of fragments can be collected.

Examination at low power view

Cellularity

The cellularity (hypo-, normo-, hypercellular) is estimated from the relationship between the cellular and fatty areas of the bone marrow fragments. The physiological percentage of cellular areas (normocellular bone marrow) decreases with age and is approx. 50% in adult dogs and cats.

Megakaryopoiesis

Megakaryopoiesis is examined at 100x magnification. Normally, every low power field contains a small number of platelet precursors and most of these cells (approx. 80%) are megakaryocytes. As platelet precursors are not homogeneously distributed through the smears, several locations should be examined, including different specimens if available. In the case of thrombocytopenia, the number of platelet precursors gives valuable clues regarding their aetiology (production deficit, increased demand by loss, consumption, destruction or sequestration). A very high number of megakaryocytes (>20 per low power view), associated with a very low number of blood platelets, indicates an immune-mediated thrombocytopenia. A high percentage of immature platelet precursors indicates an early stage of reactive proliferation or, eventually, maturation arrest of megakaryopoiesis.

Examination at higher magnification

For a more detailed examination of the cells using higher power dry lenses (200x or 400x magnification) and finally the oil immersion lens (1000x), it is best to choose areas close to fragments. In these areas, nucleated cells are often present in high number and distributed as a monolayer, with only minimal dilution by marrow blood. For routine diagnostic purposes, a differential count based on 500 or 1000 nucleated cells is dispensable. It is essential to estimate the ratio between the number of granulocytic and erythroid cells, as well as the degree and quality (presence of maturation abnormalities) of differentiation of both cell series. In addition, the percentage of other cells should be determined, especially lymphocytes, plasma cells, macrophages, mast cells and abnormal cells. Macrophages should be examined for blood parasites, haemosiderin deposits and haemophagocytosis.

Myeloid/erythroid ratio

The ratio between myeloid (granulocytic) cells and nucleated precursors of erythropoiesis (M:E ratio) shows a wide variation in healthy animals (dog: 0.5:1 to 3:1). Erythroid cells have nuclei with a coarser chromatin structure and the cytoplasmic colour varies from dark blue to orange/pink (red blood cell colour, dependent on staining). Granulopoietic cells have a more delicate nuclear chromatin structure and granules of different colour in a greyish cytoplasm. An increased M:E- ratio can be caused by increased granulopoiesis or decreased erythropoiesis (hypoplasia or aplasia of erythropoiesis) and vice versa. Interpretation is based on the cellularity of the bone marrow, the

reticulocyte count (a measure of red blood cell production by the bone marrow) and, less reliably, on the granulocyte number, which is significantly influenced by consumption.

Maturation index

The degree of maturation (maturation index), which is especially important for granulopoiesis, is defined as the ratio between the immature cells (granulopoiesis: myeloblast to myelocyte; erythropoiesis: proerythroblast to basophilic normoblast) and the mature cells (granulopoiesis: metamyelocyte to segmented; erythropoiesis: polychromatic and oxyphilic normoblasts). The maturation index reflects the interaction between bone marrow production and the demand for cells. Normally, the percentage of more mature cells exceeds the percentage of the respective precursor cells, i.e. significantly more mature than immature cells are present. Immature cells of one or more cell lineages may dominate in cases of myeloproliferative diseases (e.g. chronic myeloid leukaemia), in the early phase of activation of granulopoiesis or erythropoiesis or if demand exceeds production (esp. granulopoiesis: depletion of storage pool, associated with neutropenia).

Other cells

In comparison with erythroid and myeloid cells, lymphoid cells are less frequent in the bone marrow of healthy dogs and cats (<20% of nucleated cells, mainly small lymphocytes). Normally the percentage of plasma cells is <5%, whereas >20% plasma cells are diagnostic for multiple myeloma. Macrophages occur in the bone marrow of healthy dogs and cats only in low number (<2%). A significant increase in macrophages is associated with specific infectious diseases (leishmaniosis, ehrlichiosis), which may sometimes be diagnosed by direct detection of parasites. Haemophagocytosis is normally low in the marrow of healthy dogs and cats. Distinct haemophagocytic activity indicates ineffective erythropoiesis and mainly occurs with immune-mediated haemolytic anaemia, but may also occur in association with malignant histiocytosis, erythroleukaemia and haemophagocytic syndrome. Histiocytic cells with distinct criteria of malignancy are diagnostic for malignant histiocytosis. Lymphoblasts can infiltrate the bone marrow in the final stages of multicentric lymphoma/lymphosarcoma, where the percentage of blasts rarely exceeds 30%. In most cases of acute leukaemia, the majority of nucleated cells at the time of clinical presentation are blasts and replace physiological haematopoiesis. Cytological bone marrow specimens can be used to perform cytochemical and immunocytochemical staining to identify the cellular origin of the blasts. In individual cases of metastatic, non-haematopoietic neoplasia, esp. carcinoma, micro-metastases can be detected in cytological bone marrow specimens; this is a feasible method for the detection of distant metastases.

SESSION 1A
CARDIO PULMONARY (1)

VARIATIONS IN THE SEVERITY OF PULMONARY LESIONS INDUCED BY UK OR USA CALF ISOLATES OF PASTEURELLA MULTOCIDA A3 IN A CALF MODEL OF PNEUMONIC PASTERELLOSIS.

Mark Dagleish, Jeanie Finlayson, Colin Bayne, Gordon Moon, Jim Williams and J. Christopher Hodgson

Moredun Research Institute, UK

Pasteurella multocida A3 is a significant cause bovine pneumonia worldwide resulting in significant mortality and loss of production. Despite this, little information is available on any variations in bacterial virulence between isolates from different geographical regions. We have compared the time of onset, development of clinical signs, lung and associated lymph node pathology and bacteriological responses and characteristics during experimental disease induced by intratracheal inoculation of either a USA or UK isolate of *P. multocida* A:3. Nineteen calves aged 8 weeks were allocated randomly: Group 1 (n = 3) sham-dosed negative control; Group 2 (n = 8) given 7.1×10^8 cfu UK isolate; Group 3 (n = 8) given 5.8×10^8 cfu USA isolate. Using a bronchoscope, calves were dosed on day 0 with 300ml PBS or PBS + bacteria and lavaged immediately before and on days 1 and 4 post-challenge.

Calves given the UK isolate had reduced appetites, pyrexia (40.0-41.0°C), periods of dullness and some developed a mild nasal discharge and obvious respiratory problems over the course of the experiment (10 days). No clinical abnormalities were detected in the calves inoculated with the USA isolate. Cattle infected with the UK strain of *P. multocida* A3 had significantly ($p=0.01$) more gross lesion of pneumonia, as denoted by mean area of consolidated lung tissue, compared to cattle infected with the USA strain. In addition to this, 6 of the 8 cattle infected with the UK strain had pleurisy indicated by thickening of, and fibrinous adhesions between, the visceral and parietal pleura. The cattle infected with the USA strain had no pleurisy. Histological findings also showed the UK infected calves to have significantly more severe lung ($p=0.006$) and lymph node ($p=0.002$) lesions than those infected with the USA strain. Mean bacterial counts in lung tissue and lavage fluids were not significantly different.

In conclusion it may be necessary to select carefully any candidate isolate with respect to potential vaccine development.

ADJUVANT MODULATION OF THE PROTECTIVE RESPONSE OF A SUBUNIT VACCINE AGAINST MYCOBACTERIUM TUBERCULOSIS INFECTION IN A MOUSE MODEL

Joe Cassidy, Else Marie Agger, Joseph Brady, Karen Smith-Korsholm, Carina Vingsbo-Lundberg, Peter Andersen

University College Dublin, Ireland

Globally, tuberculosis (tb) is a major infectious disease of humans. Subunit vaccines may provide an alternative to the widely used BCG vaccine, whose protective efficacy is variable. However, use of subunit vaccines will require co-administration of adjuvants to appropriately prime the immune system and in particular to promote the Th1-biased response crucial to protecting against an intracellular organism such as *Mycobacterium tuberculosis* (*M.tuberculosis*). The presented study utilises Th1- (DDA/MPL) and Th2- (Aluminium hydroxide) inducing adjuvants in combination with the candidate subunit vaccine Ag85B-ESAT-6 and assesses the pathological, bacteriological and immunological responses to aerosol infection with *M. tuberculosis* in a well established mouse model. Following aerosol infection, mice previously given the subunit vaccine in combination with a Th1-inducing adjuvant (group 1 mice) form pulmonary granulomas more rapidly and have lower pulmonary mycobacterial loads than animals given the subunit vaccine in combination with a Th2-inducing adjuvant (group 2 mice) or mice given the vaccine without adjuvant (group 3). Morphimetry at day 14 post-infection (p.i.) indicates that pulmonary granulomas in group 1 mice are more numerous and cover a larger total surface area than those in group 2 or group 3 animals. Day 14 p.i. granulomas in group 1 mice contain larger numbers of iNOS-activated macrophages and fewer acid-fast bacteria and neutrophils than those in the lungs of the other groups. Furthermore, the iNOS positive macrophages in the group 1 mice exhibit a clustering pattern. At day 28 p.i., although granulomas are similar in number and size, with a similar iNOS-positive macrophage clustering pattern in all three groups, those in group 1 animals contain fewer acid-fast bacteria and more dense infiltrates of lymphocytes. In summary, this study indicates that granuloma kinetics and the control of infection are critically influenced by the adjuvant component of tb subunit vaccines.

SESSION 1B
**ALIMENTARY/
LIVER DISEASE (1)**

EXPERIMENTAL INFECTION OF VACCINATED AND UNVACCINATED CALVES WITH MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS

Maria Muñoz, Andrea Verna, Carlos García-Pariente, Oscar Moreno, M. Carmen Ferreras, J. Francisco García-Marín, Valentín Pérez
Universidad de León, Spain

Variations in the lesions associated with *Mycobacterium avium* subsp. paratuberculosis (Map) infection have been observed. Recently, a classification has been proposed in cattle, showing differences in the location and type of lesions with those seen in small ruminants. Vaccination is one of the most efficient methods used in paratuberculosis control. Its effects could be related to a limitation in the progression of lesions towards severe forms. The objectives of this work were to study lesions caused by Map in calves experimentally infected, making a comparison with those observed in natural cases, and the assessment of the effects of vaccination on the lesional development. Ten 3-month-old calves were immunized with a killed vaccine against bovine paratuberculosis and, two months later, 8 vaccinated (VI) and 6 unvaccinated (NVI) calves were challenged with Map. The remaining 4 animals were kept as controls. Three and two animals from VI and NVI groups were killed at 180 days post-vaccination (dpv) and the remaining 13 calves at 330 dpv. Humoral and cellular peripheral immune responses were assessed at different periods along the study and pathological and bacteriological studies were conducted in samples from the intestine and lymph nodes. Intensity of the lesion was assessed by counting the number of granulomas/section in each sample examined. Humoral responses appeared in vaccinated groups at 90 dpv, whereas cellular responses were detected at 30 dpv, reaching the highest values at 120 dpv. At 330 dpv, IFN- γ values were still high. Focal lesions were seen mainly in the mesenteric lymph nodes, as in natural cases, and they showed giant cells in different proportion. Diffuse and severe lesions were seen in NVI animals and in one calf from VI group. Significant differences in the total number of granulomas and in the amount of lesions located in the mucosa were observed between NVI and VI calves. Vaccination in cattle does not prevent infection but reduces significantly the intensity of lesions.

DENSITY AND HETEROGENEITY OF BOVINE MAST CELLS IN INTESTINAL TRACT: DISTRIBUTION OF TRYPTASE, CHYMASE, HISTAMINE AND SEROTONIN IN DUODENUM.

Sandra Jolly, Frédéric Farnir, Daniel Desmecht

University of Liege, Belgium

Mast cells (MCs) contain preformed proinflammatory substances including proteases, amines and proteoglycans. The nature of these substances is far from being constant, which led to the concept of “MC heterogeneity”, according to which the distribution of MC subpopulations varies between species, organs and/or tissue compartments. Improved immuno- and enzymo-histochemical methods (IHC & EHC) were developed for distinguishing the different mediators in bovine mast cells. The distribution and relative abundance of distinct subpopulations of metachromatic cells were investigated in duodenum.

Acidic toluidin blue stain in Carnoy fixed tissue was taken as the reference method to identify bovine MCs. Presence and distribution of tryptase (EHC), chymase (EHC), histamine (IHC) and serotonin (IHC) were investigated. Anova-3 analysis was performed (mediator / animal/ compartment effects).

The concentration of total MCs varied among animals ($P < 0.01$) and among compartments ($P < 0.001$). MCs were more numerous in lamina propria but no intraepithelial MC was seen. MCs were generally distributed around nervous and vascular structures and near glands.

Tryptase is the most common mediator (73%). The difference in the proportion of tryptase-positive MCs were not significant in intestinal compartments except for mucosa, where both intensity staining and proportion of positive-cells (38.3 +/- 7.1%) were lower.

The percentage of histamine-positive mast cells was very low in mucosal and submucosal compartments, being highest in the adventice (66.5 +/- 14.1 %). Chymase activity was particularly high around Brunner glands (55.7 +/- 9.5%).

The inner and outer layers of the tunica muscularis were closely comparable, MCs densities and mediators expressions were similar except for chymase detection (a higher percentage of chymase mast cells in outer layer). Serotonin positive MCs is anecdotal, only scarce positive cells were detected in mucosa.

The heterogeneity of total mast cell density among compartments and the striking variations of mediators expression suggest that factors generated in different micro-environments may influence mast cell recruitment, proliferation and differentiation. These differences should be taken into account when investigating the role played by MCs in physiological and pathological processes.

SESSION 2A
CARDIO PULMONARY (2)

CALCIUM-ACTIVATED CHLORIDE CHANNELS IN EQUINE RECURRENT AIRWAY OBSTRUCTION (RAO): WHAT IS THE ROLE OF ECLCA1?

Friederike Range, Ina Leverkoehne, Lars Mundhenk, Wally B Thoreson, Achim D Gruber

Freie Universität Berlin, Germany

The term recurrent airway obstruction (RAO; chronic obstructive bronchiolitis) in the horse includes a variety of distinct entities with common clinical and histologic lesions. One functionally relevant change is hyper- and metaplasia of mucin producing goblet cells in bronchi and bronchioli of affected horses. The resulting overproduction and aberrant composition of mucus are among the key mechanisms that lead to obstruction of the smaller airways.

Members of the CLCA gene family (chloride channels, calcium-activated) have recently been shown to be critically involved in epithelial disorders with aberrant mucus production including asthma and cystic fibrosis. Specifically, the human hCLCA1 induces goblet cell metaplasia and overproduction of mucins and is a prime candidate as genetic modulator in childhood asthma.

We have identified, cloned and characterized the first equine homologue of the CLCA gene family, eCLCA1. Sequence analyses identified eCLCA1 as the direct equine orthologue of the human hCLCA1. Heterologous expression of eCLCA1 in cultured HEK293 cells induced a novel, calcium-activated chloride conductance. Immunohistochemical analyses using antibodies raised against eCLCA1 identified expression in various mucin producing cells in the respiratory, alimentary and reproductive tracts as well as in the kidney and skin. The eCLCA1 expression pattern is similar but not identical to that of the human hCLCA1. Importantly, real-time RT-PCR, Northern and Western blot analyses in 3 horses as well as immunohistochemistry in 9 horses with RAO disclosed a strong upregulation of eCLCA1 in the airways, implying a significant role in the pathogenesis of mucus overproduction. Of interest, we have identified three single nucleotide polymorphisms (SNP) as allelic variations in the equine population with as yet unknown significance. It is tempting to speculate that the identification of eCLCA1 may help to identify new therapeutic targets in equine RAO.

SUDDEN DEATH IN RACING HORSES. PATHOLOGICAL AND BACTERIOLOGICAL FINDINGS.

Ebba Nilsson¹, Viveca Båverud², Carl Hård af Segerstad¹

¹Department of Pathology and ²Department of Bacteriology, National Veterinary Institute, UPPSALA, Sweden.

Every year a few racehorses die during races in Sweden. In 2005 eight horses died during a race on or near the finish line. The horses were submitted for post mortem examination and samples for bacterial culturing were taken from 6 of them. Six of the horses were trotters and 2 were thoroughbreds. The major macroscopic finding in all horses was acute bleedings in the lungs. Histological findings were detected in 3 horses, one had a mild acute degeneration in arterioles with a few leukocytes, one had a chronic, active pharyngitis and aerocystitis and the third had an acute myocarditis. The other 5 horses showed no histological changes. Samples from lungs, lymphnodes and guttural pouches were submitted for routine bacterial culturing and in five of the horses Streptococci were found in at least 2 organs, *S. zooepidemicus* in 4 cases and *S. equisimilis* in one.

None of the horses showed signs of infection before the race.

There are several well documented reports of Toxic Shock-like Syndrome associated with group C streptococci in man. The occurrence of *Streptococcus zooepidemicus* in healthy horses has been reported to be approximately 30%. In that context, in spite of the limited amount of cases, the findings described here are intriguing and inspire to further investigations.

Session 2A**EFFECTS OF ENVIRONMENTAL AIR POLLUTION IN CANINE LUNGS AND BRONCHIAL LYMPH NODES AND THE ASSOCIATED EXTRACELLULAR MATRIX REMODELING**

Orlando Paciello¹, Valeria Russo¹, Manuela Martano¹, Luigi Navas², Serenella Papparella¹.

¹Department of Pathology and Animal Health; ²Department of Veterinary Clinical Science; University of Naples Federico II, Naples – Italy.

Environmental air pollutants including ozone, nitrogen dioxide and respirable particles less than 10 µm in diameter (PM10) contribute to the occurrence of human respiratory diseases including transient changes in lung function, increased respiratory infections, deposition and degradation of extracellular matrix (ECM) leading to pulmonary structural remodelling.

We examined the pathologic changes of lungs and bronchial lymph nodes of 15 dogs from Naples, and the localization of Matrix Metalloproteinase-1,-2 and Tissue Inhibitor of Metalloproteinase by: 1) light -and electron-microscopic (EM)

immunocytochemistry; 2) immunoblots; 3) X-ray microanalysis and scanning electron microscopy. The most common microscopic change was lung and lymph node fibrosis with clusters of macrophages containing intracytoplasmic birefringent particles. The composition of environmental particles was: 1) in lungs Na (3%), S (2%), P (16%) and traces of K, Ca, Al, Si, Cl, Ti, Fe. 2) in the lymph-nodes Si (4%), Al (2%), K (2,6%), Fe (1,5%), Na (2%), S (1,8%), P (11%), and only few particles of Ca, Cl, Ti. MMP-2, MT-MMP1 and TIMP were detected within alveolar and bronchial epithelial cells. Myofibroblasts within fibrotic areas prominently expressed MMPs. Western blotting of lung tissue and lymph nodes homogenates revealed specific bands within the expected molecular weight. The pulmonary fibrosis and the extracellular matrix remodeling associated with these minerals, also detected within lymph nodes, may be triggered by the inflammatory response and mediated by MMPs. The evaluation of the composition and origin of environmental minerals within domestic animals may provide some useful comparative information regarding health hazards and pathogenesis of pulmonary fibrosis in humans.

Supported by grants (to OP) from the Regione Campania L.R. N.5

Session 2A

ALTERATIONS OF THE EXTRACELLULAR MATRIX IN CHRONIC DEGENERATIVE MITRAL VALVE DISEASE (ENDOCARDIOSIS) IN DOGS

Heike Aupperle, Imke März, Alexander Kappe, Heinz-Adolf Schoon*

Institut für Veterinär-Pathologie, Universität Leipzig, An den Tierkliniken 33, 04103 Leipzig, Germany

**Klinik für Kleintiere, Universität Leipzig, An den Tierkliniken 23, 04103 Leipzig, Germany*

Objective: Chronic degenerative valve disease (syn. endocardiosis) is a common disease in dogs. The pathogenesis of this myxomatous degeneration is still unclear. The aim of this study is to characterize the composition and distribution of the extracellular matrix (ECM) of normal versus degenerated mitral valves in dogs.

Methods: 44 dogs of different breeds (dachshound n=11, Yorkshire terrier n=7, spaniel n=5, Chihuahua n=4, poodle n=4, others n=15) and age (1-16 years) were investigated grossly and histologically (H.-E., Picrosirius Red stain). Mitral valves were classified as normal (n=7), or mild (n=8), moderate (n=13), or severe (n=16) endocardiosis. The ECM components collagen I, III, IV, VI, laminin, fibronectin, heparansulfate were detected immunohistochemically.

Results: In normal mitral valves laminin, heparansulfate, fibronectin, collagen

IV and VI are detected as a thin subendothelial layer. The spongiosa contains mainly mucopolysaccharides, collagen I and VI, and small amounts of fibronectin and collagen III. The fibrosa is composed of collagen I, III, VI.

Early stages of endocardiosis are characterized by focal mildly thickened subendothelial layers of laminin and collagen IV at the atrial side. Fibronectin and collagen III are mildly increased in foci of the spongiosa.

Nodular alterations in severe endocardiosis contain markedly increased amounts of fibronectin, collagen III and VI. Furthermore, laminin and collagen IV can be detected in these regions. Subendothelial layers of laminin and collagen IV are multifocally thickened and infiltrate the altered valvular stroma.

Conclusion: The immunohistochemical results show that the composition and arrangement of different ECM proteins are changed during development of endocardiosis. Further investigations of the function, metabolism and differentiation of interstitial valve cells are necessary for a better understanding of the regulation of ECM metabolism in this disease.

Session 2A

PULMONARY LESIONS IN FOXHOUNDS INFECTED WITH EQUINE INFLUENZA H3N8.

Ken Smith, Tony Blunden, Greg Dowd, Shona Macrae, Jodi Miller and Janet Daly.*

*Animal Health Trust and *Royal Veterinary College*

Influenza A viruses are divided into subtypes according to the serological reactivity of the surface glycoproteins haemagglutinin (H) and neuraminidase (N). To date, 16 H and 9 N subtypes have been isolated in various combinations from aquatic birds. Only certain subtype combinations are found in the 3 mammalian species that are commonly infected with influenza A viruses (man, pigs and horses). In April 2004, the University of Florida issued a press release describing the first evidence of equine influenza virus jumping the species barrier. According to this press release, 8 racing greyhounds died from haemorrhagic pneumonia during an outbreak of respiratory disease in Florida in January 2004. This information led us to re-examine an outbreak of severe respiratory disease in a pack of English foxhounds that occurred in the UK in September 2002. The outbreak was marked by a sudden onset of coughing in a pack of 80 hounds, a minority of which became markedly lethargic or moribund prior to death or euthanasia. Two hounds were subject to detailed post mortem examination. Gross changes were limited to the respiratory tract and characterised by bilateral pneumonia. Histological examination of sections of pneumonic lung revealed necrotising bronchitis, bronchiolitis and alveolitis with occlusion of small airways and alveoli by exudate and bacteria. Immunohistochemistry using a

polyclonal antiserum specific for equine influenza H3N8 revealed abundant viral antigen in degenerate epithelial cells and macrophages. Secondary infection by bacteria having the morphology of streptococci was noted. In vitro modelling using tracheal explants from dogs confirmed receptors capable of binding equine influenza at all levels of the tracheobronchial tree and confirmed viral replication in organ culture.

Session 2A

CARDIAC FUNCTION AND EXTRACELLULAR MATRIX METABOLISM IN RABBITS WITH DOXORUBICIN CARDIOMYOPATHY AFTER AUTOLOGOUS MESENCHYMAL BONE-MARROW DERIVED STEM CELL INJECTION

Heike Aupperle, Jens Garbade, Andreas Schubert, Stephan Dhein*, Heinz-Adolf Schoon, Friedrich-Wilhelm Mohr**

Institut für Veterinär-Pathologie, Universität Leipzig, An den Tierkliniken 33, 04103 Leipzig, Germany

**Herzzentrum Leipzig, Universität Leipzig, Strümpell Str. 39, 04289 Leipzig, Germany*

Objective: Cell therapy is becoming increasingly important as a potential new therapy for patients with advanced heart failure. The aim of this study was to investigate the effects of bone-marrow derived mesenchymal stem cell (MSC) transplantation into chronic doxorubicin induced cardiomyopathy on the composition and metabolism of extracellular matrix in a rabbit model.

Methods: 36 rabbits were divided into four groups. Group 1: healthy controls (n=6), groups 2-4: six weeks doxorubicin treatment (3 mg/kg). Group 2 received no further treatment (n=6). In group 3 (n=9) culture medium and in group 4 (n=15) autologous MSCs ($1.5\text{-}2.0 \times 10^6/\text{ml}$) were injected in the left ventricular wall (LV). Animals were investigated clinically by echocardiography. Samples of the hearts were stained with H.-E. and picosirius red. The expression of matrix metalloproteinases MMP-1, -2, -3, and -9, and their tissue inhibitors TIMP-2 and -3 were detected immunohistochemically. mRNA levels were determined in frozen specimens by real-time PCR.

Results: Echocardiography showed a better cardiac function in stem cell than in the medium treated animals. Cardiomyocytes expressed MMP-1, -2, -9, TIMP-2 and -3. Fibrocytes and endothelia expressed MMP-2, TIMP-2 and -3. In groups 2-4 expression of MMP-1 and -2 was increased in cardiomyocytes. In group 2 percentage of fibrocytes expressing TIMP-3 was decreased, but MMP-2 positive fibrocytes were increased. Medium injection (group 3) resulted in increased myocardial fibrosis and TIMP-3 expression. Stem cell injection (group 4) led to an increased MMP-1 and MMP-2 expression in cardiomyocytes.

mRNA levels of MMPs were higher in group 3 than in group 4. In contrast mRNA levels of TIMPs were not different.

ORAL PRESENTATIONS

Conclusion: Autologous MSCs injection into the LV wall improved clinical heart function, minimized myocardial fibrosis and varied MMP and TIMP expression. The pathogenetic meaning of these findings is still unclear, but corresponding to the literature paracrine mechanisms have to be discussed.

SESSION 2B
**ALIMENTARY/
LIVER DISEASE (2)**

COCCIDIOSIS IN BRITISH ALPACA (VICUGNA PACOS)

*Alex Schock, Cornelia Bidewell, Paul Duff, Sandra Scholes, Robert Higgins
VLA Lasswade, UK*

INTRODUCTION

Severe intestinal disease associated with *Eimeria punoensis*, *E. lamae*, *E. alpaca* and *E. macusaniensis* has been observed in South American camelids in the Americas and Australia 1-4. However, there is conflicting information about the pathogenicity of the various camelid *Eimeria* species in alpacas. The objective of this retrospective study was to investigate the importance and character of coccidial infection in British Alpaca.

MATERIAL AND METHODS

Review of VIDA data and material received at VLA Lasswade between 1999 and 2004 revealed 54 recorded cases of coccidial infection in alpacas. In 28 cases, specification of *Eimeria* was carried out based on the morphological features of faecal oocysts and /or coccidial forms in tissue sections. Detailed histopathology was carried out in 22 cases.

RESULTS

On the available evidence of gross lesions, microscopic lesions and faecal analysis, a diagnosis of coccidiosis could be supported in 40/54 cases of which 13 were adults. In a further 9 alpaca, the data suggested that the coccidial infection was a significant contributory factor in the disease presentation. In the remaining 5 alpacas, low numbers of coccidial structures without significant damage to the small intestinal mucosa were detected. *E. macusaniensis* and *E. punoensis* were the most frequently identified species in the UK. *E. ivitaensis* was not detected in any of the samples.

CONCLUSIONS

This investigation confirms that coccidial infection, particularly with *E. macusaniensis* and *E. punoensis*, is of clinical significance in British alpacas over a wide age range.

REFERENCES

1.Rosadio RH, Ameghino EF.(1994). *Vet Rec* 135: 459-460. 2.Guerrero CA, Hernandez J, Bazalar H, Alva J.(1971). *J Protozool* 18: 162-163. 3. Lenghaus C, O'Callaghan MG, Rogers C.(2004). *Aust Vet J* 82: 711-712. 4. Cebra CK, Mattson

LESIONS ASSOCIATED WITH CLOSTRIDIUM PERFRINGENS INFECTION IN A CHICKEN INTESTINAL LOOP MODEL

Richard Ducatelle, Leen Timbermont, Anouk Lanckriet, Ahmad Gholamiandekhordi, Freddy Haesebrouck, Filip Van Immersee
Gent University, Belgium

Clostridium perfringens is the most widely distributed pathogen in nature. It is the causative agent of several important animal diseases, including necrotic enteritis in broiler chickens. The purpose of the present studies was to molecularly characterize *Clostridium perfringens* isolates from healthy and diseased poultry flocks and to study the effects of some of these isolates on the mucosa in a chicken intestinal loop model. Therefore 32 isolates were obtained from 7 flocks with necrotic enteritis and 27 isolates were obtained from 23 flocks without clinical problems. Toxinotyping was done using PCR tests. All isolates from diseased as well as from healthy flocks belonged to toxinotype A, harbouring the alpha toxin gene, but not beta, iota and epsilon toxin genes. Two isolates harboured enterotoxin gene and 4 had beta2 toxin gene. Alpha toxin quantification using an ELISA test on the supernatant of stationary phase cultures revealed high and low toxin producing isolates from diseased as well as from healthy flocks. A selection of high and low alpha toxin producers were injected in ligated small intestinal loops in adult layer type chickens under general anaesthesia. After 10h incubation the animals were euthanized and the intestinal wall was examined histologically. Lesions were characterized by leakage of proteinaceous material from the intestinal mucosa into the lumen. Numerous *Clostridium* bacteria were adhering to this protein precipitate in the lumen. There was multifocal micro-ulceration and necrosis of the mucosa, bordered by a rim of heterophilic granulocytes. These lesions are similar to those observed in field cases. The virulence mechanisms involved however are unclear. This loop model may be a valuable tool to further investigate the virulence mechanisms involved in necrotic enteritis.

Session 2B

CONTRIBUTION OF THE LIVER TO CLINICAL AND PATHOLOGICAL CHANGES IN FELINE INFECTIOUS PERITONITIS

Anja Kipar, Alexandra Malbon, Marina L. Meli, Anne Vaughan-Thomas, Manfred Reinacher, Hans Lutz,
University of Liverpool, UK

Feline infectious peritonitis (FIP) is a well-known coronavirus (FCoV)-induced systemic disease, characterised by fibrinous-granulomatous serositis with

ORAL PRESENTATIONS

protein-rich effusions into body cavities, granulomatous-necrotising phlebitis and periphlebitis and granulomatous inflammatory lesions in several organs. The disease is clinically characterised by recurrent fever and the development of FIP lesions is triggered by activated, virus infected monocytes. Many systemic changes indicative of excessive cytokine release are seen in cats with FIP, but cytokine production in haemolymphatic tissues and virus infected monocytes was shown to be only limited. Therefore, other organs, among them the liver, must contribute to the pathogenesis of FIP.

This study investigated the possible contribution of the liver by examining it for the transcription and translation of cytokines. Real time RT-PCR for feline cytokines interleukin-1 β (IL-1 β), IL-6, IL-10, IL-12p40, tumour necrosis factor- α (TNF- α), granulocyte colony stimulating factor (G-CSF), macrophage-CSF (M-CSF) and GM-CSF was carried out on the livers of experimentally serotype I and naturally infected cats with FIP, experimentally FCoV serotype I infected cats without FIP and specific pathogen free (SPF) control cats. There was generally no transcription of these cytokines in SPF cats whereas all cytokines were transcribed both in cats with FIP and FCoV-infected cats without FIP. However, mRNA levels of all cytokines except GM-CSF were significantly higher in the cats with FIP. Immunohistology for IL-1 β , IL-6, IL-10, IL-12p40 and TNF- α was performed to demonstrate cytokine translation and identify the cells producing the cytokines. This confirmed that translation does occur and that the hepatocytes are contributing to the cytokine production. Other cell types in the liver, such as bile duct epithelia, smooth muscle cells, Kupffer cells and leukocytes also expressed these cytokines, both constitutively and in FIP. These results suggest a substantial contribution by hepatocytes to systemic effects such as fever and vasculitis in the development of FIP.

Session 2B

CHRONIC INTESTINAL PSEUDO-OBSTRUCTION IN A BERNESE MOUNTAIN DOG

Valerie Vandenberge, D. Paepe, R. Ducatelle, K. Chiens

University of Ghent, Belgium

Pseudo-obstruction is a rare syndrome in which ineffective intestinal propulsion causes clinical and radiographic signs of obstructive bowel disease without physical obstruction. Chronic intestinal pseudo-obstruction (CIPO) has been described in several dogs, without breed, age or sex predilection. Aetiology is unknown but an immune-mediated, unknown toxic or infectious aetiology have been proposed.

A 2.5 year old male Bernese Mountain Dog was presented at the Faculty of Veterinary Medicine with a history of vomiting, inappetence, diarrhea for 2

weeks, weight loss and no response to antibiotics, anti-emetics and prokinetic therapy. On physical examination the abdomen was painful and distended. Blood and fecal analysis revealed no abnormalities. Abdominal radiographs and ultrasonography revealed hypomotile and severely distended small intestinal loops. A celiotomy was performed and the small intestine was found to be distended, aperistaltic and obstructed. Because of the poor prognosis the dog was euthanized and presented for necropsy.

Macroscopically a severe dilation of the duodenum, a hemorrhagic necrotising enteritis and impaction of jejunum and ileum was found. Mesenteric lymph nodes were enlarged.

Samples of the duodenum, jejunum, ileum, colon, oesophagus and ganglion trigeminale were taken for histological examination.

The sections of the duodenum, jejunum and ileum revealed atrophy of the tunica muscularis and infiltration with lymphocytes, plasmacells and histiocytes. The lamina muscularis mucosae was hyperthrophic and the mucosa as well as the submucosa were infiltrated with lymphocytes, plasmacells and few eosinophils. No abnormalities were found in the sections of the colon, oesophagus and ganglion trigeminale. The lesions in the small intestine are suggestive for CIPO.

This case is the second report of impaction of the intestine, associated with CIPO. The histopathologic and clinical findings are similar to the other cases. No vacuolar degeneration of the ganglia of the myenteric plexus was found.

Session 2B

LONGTIME PATHOLOGICAL SURVEY OF A COLONY OF CAPTIVE HELD LESSER HEDGEHOG TENRECS (ECHINOPS TELFAIRI)

Denise Thaller¹, Heinz Künzle², Marcus Clauss³, Franz Schwarzenberger⁴, Peter Schmidt¹

¹Department of Pathobiology, Institute of Pathology and Forensic Medicine, University of Veterinary Medicine, Vienna, Austria, ²Department of Anatomy, Ludwig Maximilians University of Munich, Munich, Germany, ³Division of Zoo Animals, Exotic Pets and Wildlife, University of Zurich, Zurich, Switzerland, and ⁴Department of Natural Science, Institute of Biochemistry, University of Veterinary Medicine, Vienna, Austria

Appropriate feeding and keeping of animals under laboratory conditions can be a challenge. Therefore a continuous monitoring of the health status and the cause of death is essential. The lesser hedgehog tenrec (*Echinops telfairi*), a member of the newly recognised Afrotheria group, living in Madagascar, is especially interesting in terms of phylogeny, reproduction, thermoregulation and neurobiology. In their natural habitat they are feeding on insects and invertebrates. In a colony of these animals, deaths for unknown reasons occurred

over several years. Hence a longtime survey was started on frozen or routinely formalin fixed animals or organs.

Within two years, necropsies on 87 tenrecs were performed. In the majority of the animals the predominantly pathological findings included a moderate to high fatty liver degeneration and hepatic siderosis. The nutritional status was very good with large fat deposits but a poorly filled or empty gastrointestinal tract. Therefore the fatty liver degeneration was most likely caused by lipomobilisation following a deficient food intake. Sporadic skin and tongue lesions as well as pneumonia were evident.

Excessive iron storage in animals is reported in mammals and birds. A study in common marmosets for example showed that a diet with higher iron levels led to an increase in liver iron content and that those animals had a higher mortality than those on a low iron diet. On the basis of these results a feeding trial was started where the so far used cat or dog food, containing mostly heme iron with a higher availability, is compared to two foods with lower (non- heme) iron levels. Future investigations will show if the changes in the feeding regime had any effect on the occurrence of hepatic siderosis in this study group.

Session 2B

IMMUNOHISTOCHEMICAL DETECTION AND DISTRIBUTION OF THE VIRAL GP55 ANTIGEN IN PIGS NATURALLY INFECTED WITH CLASSICAL SWINE FEVER VIRUS

Trpe Ristoski, Milijana Knezevic, Librado Carrasco, Francisco Javier Salguero, Alejandro Nunez, Josif Tosevski

Faculty of Veterinary Medicine, Ss. Cyril and Methodius, Republic of Macedonia

Introduction. In this study we investigated the distribution of viral glycoprotein (Gp55) in pig tissues naturally infected with CSF virus. Apart of retrospective analyses, during this study we detect a tissue alterations and correlation of viral antibodies with certain cells or tissue types.

Material and Methods. Tissue samples (tonsils, mesenterial lymph nodes, spleen, colon and pancreas) were formalin fixed and embedded in paraffin wax. The immunohistochemical (ABC) method was performed by using monoclonal antibody (Monoclonal antibody to Pest viruses - WH303, CVL, UK) in 1:100 dilution.

Results. Gp55 antibody labeled numerous cell types in the examined tissues. In tonsils, Gp55 labelled cells were epithelial cells on the surface and in the tonsil crypts, lymphocytes between lymph follicles, macrophages and endothelial cells of the blood vessels. In tissue sections of the mesenterial lymph nodes, the highest accumulation of positive cells was demonstrated in trabecular and subcapsular dilatated sinuses and smooth muscle cells in trabecules and follicles.

ORAL PRESENTATIONS

In the spleen, Gp55 labeled cells were present between follicles. In the pancreatic tissue, positive immunoreactivity was demonstrated in single acinar cells. Gp55 labeled cells in the colon were endothelial cells, numerous macrophages, lymphocytes, and cells of the submucosal and subserosal plexus.

The highest immunoreactivity for Gp55 antibody was expressed in cells of tonsils, mesenteric lymph nodes, spleen and colon. In these organs, the most reactive cells were endothelial, epithelial cells and also macrophages. This finding confirms that they are target cells for CSF virus.

Viral antigen presence in the above mentioned organs was accompanied by alterations of various character as hyperemia, hemorrhages, fibrinoid necrosis, various degenerative alterations, apoptosis, necrosis and inflammations.

SESSION 3A
NEOPLASIA (1)

IMMUNOLOGICAL DIVERSITY BETWEEN CLASSICAL AND ATYPICAL OVINE PULMONARY ADENOCARCINOMA.

Marcelo De las Heras, Christina Summers, Alfredo Benito, Aurora Ortín, Jose Antonio García de Jalón, Mary Norval¹, James Michael Sharp²

Universidad de Zaragoza, Spain, ¹The University of Edinburgh, ²VLA Penicuik

Ovine pulmonary adenocarcinoma (OPA), characterized by tumour cell growth in the lungs is a consequence of transformation of the type II pneumocytes and non-ciliated Clara cells by Jaagsiekte sheep retrovirus (JSRV). Two pathologically distinct forms of OPA have been identified. In the classical form the neoplastic lesions are firm, confluent masses composed of a single layer of neoplastic epithelial cells lining the alveolar lumina or bronchioles. Atypical OPA lesions are seen as individual, small nodules with a distinctive differentiation between the neoplastic region and the surrounding parenchyma. Notwithstanding the pathological differences, no detectable systemic immune responses have been detected in either form. One of the main clinical distinctions between the two is that there is a substantial increase in the production of alveolar surfactant in classical OPA, with no increase in lung fluid in the atypical form. Surfactant proteins are synthesised in type II pneumocytes and proteins A and D, known to be immunoregulatory have also been shown to be quantitatively immunosuppressive. Previously we revealed that the predominant immune response in classical OPA was an influx of macrophages and high levels of IFN-gamma expression. However, no immune cells infiltrating the tumour nodules were detected. In this present study we have investigated local immune responses in early atypical lesions and we identified infiltration of CD4+, CD8+ and gamma-delta T cells. Less intense levels of IFN-gamma expression compared with the classical form were detected also. Unexpectedly the intensity of surfactant protein staining is comparable for both forms of the disease, despite the fact that increased levels of surfactant are particular only to classical OPA. This study has provided the baseline for future examination and comparison with the microenvironment of extensive lesions. Furthermore, this study has shown that not only are classical and atypical early OPA lesions distinctive pathologically but also that there are major local immunological differences.

BETARETROVIRUSES-RELATED ANTIGENS IN HUMAN LUNG ADENOCARCINOMAS.

Marcelo De las Heras, Antonio Marchetti, Pablo Murcia, Sohail Jahid, Aurora Ortin, Javier Azua, Luis Borderias, Alfredo Benito, Ramiro Alvarez, Jose Antonio Jiménez-Mas, Hung Fan and Massimo Palmarini
Universidad de Zaragoza, Spain

About 30% of human lung adenocarcinomas (LAC) express an antigen that is detected by immunohistochemistry with an antiserum raised against the major capsid protein (CA) of Jaagsiekte sheep retrovirus (JSRV), the etiological agent of a transmissible lung adenocarcinoma of sheep (De las Heras et al, 2000, Eur Respir J 16:330). In this study we have further investigated whether JSRV or JSRV-related virus antigens are present in a proportion of human lung adenocarcinomas. We have analysed LAC by immunohistochemistry with antisera raised against different JSRV Gag proteins and against Gag proteins of other phylogenetically related retroviruses, including Mouse mammary tumour virus (MMTV), Mason-Pfizer monkey retrovirus (MPMV) and the human endogenous retrovirus HERV-K. Results obtained showed that 13/43 human LAC tested express antigens that cross-react with two different polyclonal antisera raised against different domains of the JSRV Gag polyprotein while they do not cross-react against a control pre-immune serum. However, we ruled that JSRV itself is associated with LAC, as the same Gag-positive LAC samples were negative when tested with an antiserum against the surface domain of the JSRV envelope. In addition, a monoclonal antibody crossreacting by ELISA or immunoblotting with JSRV and HERV-K CA was also used. 8/42 human LAC were positive using this monoclonal antibody and 7/8 also resulted positive by using antisera towards the JSRV-CA/MA. Non of the Gag-positive LAC samples cross-reacted with an anti-MMTV Gag serum, a phylogenetically related Betaretrovirus that has recently controversially associated with human breast cancer. Analysis in a set of 70 human LAC where data on patient gender, smoking habits, tumour histological subtype and EGFR mutations were available, showed a significant association between Betaretrovirus Gag-positive LAC and tumor onset in women. Further molecular analysis will be necessary in order to determine the nature of retrovirus-related Gag antigens in human LAC.

CYCLOOXYGENASE-2 EXPRESSION IN CANINE AND FELINE SQUAMOUS CELL CARCINOMAS AND ITS CORRELATION TO VEGF EXPRESSION

Alessandro Poli, Francesca Millanta, Michele Corazza, Davide Lorenzi, Ranieri Verin*

*Department of Animal Pathology and *Veterinary Clinic, School of Veterinary Medicine, University of Pisa - Italy*

Cyclooxygenase-2 (COX-2) plays an important role in the tumorigenesis of a variety of human cancers. Recent evidence suggests that COX-2 over-expression is involved in tumour growth and spread by modulating the production of several angiogenic factors, including Vascular Endothelial Growth Factor (VEGF). The role of COX-2 has also been recently investigated in a series of small animal tumours. However, the expression of COX-2 in canine and feline squamous cell carcinomas (SCCs) remains not well defined yet. The over-expression of COX-2 has been investigated by immunohistochemistry in 51 specimens of feline and canine cutaneous and non cutaneous SCCs and the correlation between COX-2 over-expression, VEGF expression and clinicopathologic variables evaluated. The immunoreactivity of COX-2 was cytoplasmic. COX-2 over-expression was observed in 60% of the canine and 64% of the feline carcinomas and in both species it was significantly higher than that in healthy tissues used as controls ($P < 0.01$). VEGF expression was not observed in normal skin and mucosae. The VEGF expression was significantly higher in feline than in canine SCCs ($P = 0.02$). In both species an increased VEGF expression was not correlated to COX-2 over-expression. In the dog VEGF expression was significantly correlated to tumour grade, according to the Broder's scheme. Our study suggests that either dogs and cats may be a suitable animal model to investigate the role of COX-2 in human SCCs and the reliability of the use of COX-2 antagonists for the therapy of these tumours also in the small animal practice. On the other hand, further study will be required to investigate the role of COX-2 pathway in SCC angiogenesis.

SESSION 3B
WILDLIFE PATHOLOGY

THE NATURAL HISTORY OF AN OUTBREAK OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS (LCMV) IN A CAPTIVE COLONY OF GEOFFROY'S MARMOSETS (CALLITHRIX GEOFFROYI) IN THE UNITED KINGDOM.

Mark F. Stidworthy, Nic J. Masters, Robin Gopal, John C.M. Lewis, Andrew G. Greenwood

International Zoo Veterinary Group, UK

Lymphocytic Choriomeningitis Virus (LCMV) is a rodent-borne zoonotic arenavirus and the aetiology of "Callitrichid hepatitis". During six weeks from January 2006, a U.K. zoo experienced three deaths in a colony of six Geoffroy's marmosets (*Callithrix geoffroyi*). These roamed within a recently-established naturalistic walk-through tropical house shared with birds, iguanas, two-toed sloth (*Choloepus didactylus*) and fruit bats (*Carollia perspicillata*). Rodent (mouse and rat) infestation was a recognised problem. Clinically, a two day history of severe depression, rapid weight loss and irreversible collapse with seizures preceded death. Clinicopathological findings included leucopaenia, anaemia, azotaemia and hypercholesterolaemia. Gross necropsy findings in the three animals included necrotising hepatitis, serosanguinous effusion and nephrosis. Histologically, multifocal necrotising hepatitis was present in all cases, with marked non-suppurative phlebitis with thrombosis in two. Acute tubular necrosis or tubulointerstitial nephritis with thrombosis were present. Interstitial pneumonia and non-suppurative vasculitis in other tissues were variable features. Ultra-frozen tissues were submitted for PCR testing and virus isolation. All three animals were PCR-positive using primers designed from published sequence data from a Callitrichid hepatitis outbreak in Germany. Arenavirus was isolated from one of the animals. Serological screening against LCMV was possible from four of the six animals, including one fatality. The latter was seronegative both 14 days and 1 day before death. Two of the three surviving animals (which were removed from the exhibit) were seropositive, one was seronegative. All retained this status two months after the last death, at which time no viral shedding could be detected by clinical sampling. Unexpectedly, a two-toed sloth was also found to be seropositive. Subsequently, rigorous rodent control was coupled with ongoing surveillance of captured rodents for LCMV. This fatal disease outbreak in Callitrichidae highlights the importance of robust control of wild rodent reservoirs of infectious disease, including zoonotic arenaviruses, within enclosed naturalistic zoo exhibits.

CHEETAH MYELOPATHY - PATHOLOGICAL FINDINGS AND IMMUNOHISTOCHEMICAL INVESTIGATION

V Café Marçal, C Botteron, A Url, C Walzer, N Robert

Institute of Animal Pathology Vetsuisse Faculty Bern (Department of Zoo and Wildlife Pathology), Switzerland

Numerous cases of ataxia due to a degenerative myelopathy of unknown etiology has been observed in the past twenty years in the EEP captive cheetah population. Several causes for the disease have been considered, including genetic, environmental / toxic, nutritional and viral factors, but to date no definitive etiology could be found.

Histopathologically, the degenerative lesions are restricted to the white matter of the spinal cord, especially the distal cervical to mid-thoracic segments. The changes are bilateral symmetric and often affect the lateral and ventral spinal cord funiculi. The lesions are characterized by ballooning of myelin sheaths, which are either devoid of axons, or contain intact or fragmented axons, or macrophages, associated with varying degrees of astrogliosis/-cytosis, and with macrophage infiltration. Considering the presence of intact axons within dilated myelin sheaths, the lack of features typical for early axonal degeneration, and the excess of myelin loss compared to axonal degeneration, the lesion was considered as a primary disorder of myelin or myelin-axon interaction.

In order to better characterize the spinal cord lesions, immunohistochemical investigations were performed using following antibodies: CD68; MAC 387; myelin oligodendrocyte specific protein (MOSP), myelin basic protein (MBP), 2',3'-Cyclic Nucleotide 3'Phosphodiesterase (CNPase), GFAP, amyloid precursor protein (APP), and neurofilaments (NF200 Ph_n and NF 68,200).

This study shows that establishing immunohistochemical protocols in a new species, i.e. the cheetah, may represent a challenging task and that the results may be confusing. Preliminary results, however, do not indicate changes typical of early axonal degeneration. MOSP labelled cells with aberrant cytoplasmic processes and did not allow to differentiate between reactive oligodendrocytes or astrocytes. Although these initial trials using immunohistochemical methods don't clearly answer the questions about the primary process, they represent important steps in the clarification of the underlying pathomechanisms and help to focus on appropriate subsequent investigations.

**SCUTICOCILIOSIS IN SEA DRAGONS IN BASEL ZOO,
SWITZERLAND**

*Stephanie Rossteuscher, Christian Wenker, Thomas Jermann, Thomas Wahli,
Heike Schmidt-Posthaus*

Center for Fish and Wildlife Health, Switzerland

Until now, scuticociliosis has been rarely described in sea horses, flounders and turbot. Here we present a case study of a population of sea dragons in a zoological aquarium chronically infected with scuticociliates.

Beginning in 2004, over a period of 19 months, 9 sea dragons (*Phycodurus eques*, *Phyllopteryx taeniolatus*) were found dead in an aquarium of Basel Zoo, Switzerland. All animals showed only faint clinical symptoms over a short period before dying. Macroscopic examination revealed mainly skin lesions with multiple, often hemorrhagic, ulcerations. At histology, epidermal lesions were associated with necrosis of underlying dermis and musculature. In the lesions, multiple ciliates, consistent with scuticociliates, were found. In several animals these ciliates had invaded blood vessels and could also be demonstrated in internal organs i.e. gills, kidney, endocrine system and CNS. In these organs, only mild alterations and inflammatory reactions were evident.

To date two other cases of ciliate infections in seahorses, the family sea dragons belong to, are documented. Skin lesions predominate in one case whereas in the other case systemic infections without skin alterations were observed.

In contrast to these findings, our sea dragons presented with both pronounced skin changes and a systemic infestation with the parasites. With the presence of these two features in combination with a minimal inflammatory reaction, there appear to exist different pathogenic events in the scuticociliate infection.

SESSION 4A
NEOPLASIA (2)

**ROLE OF CYCLOOXYGENASE-2, EP2 RECEPTOR, AND
MICROSOMAL PGE SYNTHASE-1 EXPRESSION IN FELINE
INVASIVE MAMMARY CARCINOMAS**

Francesca Millanta, Simona Citi, Daniele Della Santa*, Lorenzo Ressel,
and Alessandro Poli*

*Department of Animal Pathology and *Veterinary Clinic, School of Veterinary
Medicine, University of Pisa, Italy.*

Cyclooxygenase- 2 (COX-2) over-expression is involved in the progression of feline mammary carcinomas (FMCs) and in affecting the overall survival (OS). PGE₂ is a major metabolite of the COX pathway in the mammary gland and has been shown to have tumour-promoting activity and to induce angiogenesis. EP2, a subtype of the PGE₂ receptor is highly expressed in breast tumours and correlates with the tumorigenic and angiogenic phenotype. Microsomal PGE synthase-1 is an enzyme downstream to COX-2 and affects PGE₂ production only. Induced expression of mPGES-1 is observed in several types of human cancers. The immunohistochemical expression of EP2 receptor and mPGES-1 has been analysed in invasive FMCs and correlated to COX-2 and VEGF expression, clinico-pathologic parameters, and to the OS. EP2 expression was found in 25 of the 47 carcinomas (53%), while mPGES-1 expression was observed in 31 cases (66%). The expression of both markers in the healthy mammary tissues and in the hyperplastic lobules in the adjacent mammary tissue was significantly lower than in carcinomas ($P < 0.05$). No differences in mPGES-1 and COX-2 overexpression were observed ($P > 0.05$) and COX-2 overexpression did not significantly induce an increased EP2 receptor expression. No statistical differences were observed between EP2 receptor and mPGES-1 expression, with 22/31 mPGES-1 over-expressing tumours also EP2 receptor positive. No correlation was found between EP2 and mPGES-1, and VEGF expression. Our study suggests that the expression of mPGES-1 is upregulated in COX-2 over-expressing FMCs. The EP2 receptor positivity, however, seems to be only related to mPGES-1 over-expression. The increased expression of EP2 receptor in mPGES-1 positive tumors may suggest that the inhibition of the EP2 pathway could be evaluated as a novel approach in the prevention and/or treatment of FMCs.

TELOMERASE ACTIVITY AND IMMUNOHISTOCHEMICAL EXPRESSION OF PCNA, P53 AND KI67 IN TESTICULAR TUMOURS OF DOGS

Nikos Papaioannou, Dimitra Psalla, Panayiotis Loukopoulos, Ioannis Vlemmas

Department of Pathology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece

Testicular tumours are common in aged dogs and include germ cell tumours (seminoma, teratoma, embryonal carcinoma), sex cord-stromal tumours (Sertoli cell tumours and interstitial cell tumours) and mixed germ cell-sex cord stromal tumours. Seminoma, Sertoli cell tumours (SCTs) and interstitial cell tumours are the most common tumour types observed and occur with approximately equal frequency. The aim of this study was to examine the immunohistochemical expression of telomerase and certain established tumour markers (PCNA, p53 and ki-67) in a range of canine testicular tumour types and to examine the interrelations between them.

Serial sections from 36 formalin-fixed testicular tumours (20 seminomas, 12 SCTs, 3 interstitial cell tumours and 1 mixed germ cell-cord stromal tumour) were either stained with hematoxylin and eosin or were stained immunohistochemically using primary antibodies against PCNA (Oncogene), p53 and ki-67 (both Dako), and telomerase (h-TERT, Novocastra). Immunohistochemical analysis was also performed in four normal canine testes.

15/20 seminomas and 6/12 SCTs were positive for p53. All seminomas (20/20) and SCTs (12/12) showed strong nuclear activity with PCNA while only 2/20 and 3/12 were stained with ki-67 respectively. h-TERT protein was detected in 15 seminomas and 6 SCTs either intracytoplasmically or intranuclearly 15 of 20 seminomas were positive for p53 and the ki-67 immunostaining was expressed in two of them. The diffuse type of the above tumours were strongly expressed h TERT. The interstitial cell tumors were immunoreactive to all examined antibodies. The mixed tumor was only positive for PCNA.

Our results support the hypothesis that p53 inactivation is involved in the malignant progression of seminomas. Moreover, they suggest that quantitation of PCNA-positive nuclei provides an objective method for assessing proliferative activity in testicular tumours, while immunohistochemical examination for ki-67 may be of limited value. Telomerase activity was demonstrated in both seminomas and SCTs. Telomerase activation appears to be a rather early event in canine testicular cancer progression. Examination of the relationship of these results with telomerase expression levels in testicular tumours, a study currently under way, may further elucidate its role in testicular cancer progression.

**PATHOLOGICAL AND IMMUNOHISTOCHEMICAL
CHARACTERISATION OF CANINE VISCERAL
HAEMANGIOSARCOMAS**

*Adrian W Philbey, Duncan S Russell, Colin S Nixon, Michael J Stear, Hal
Thompson*

University of Glasgow Veterinary School, UK

This study characterised the pathological findings in dogs with visceral haemangiosarcoma diagnosed at the University of Glasgow Veterinary School from 1988 to 2003. The mean age of 277 affected dogs was 9.4 years and 58.3% were males. The breeds most frequently represented were German Shepherds, Retrievers, Crossbreeds, Collies and Terriers, in decreasing order. In 78 cases submitted for postmortem examination, the spleen, right atrium (specifically right auricle) of the heart, lungs and liver were the most frequent sites of involvement. Haemangiosarcomas were identified at two or more sites in 74/78 cases (95.0%). The spleen and right atrium appeared to be the most frequent sites of primary origin, with 62/78 cases (79.5%) having tumours in at least one of these two sites and 27/78 cases (34.6%) having involvement at both sites. The lungs and liver frequently contained multiple discrete nodules and were considered to be sites of metastasis. The liver was the main site of dissemination of splenic haemangiosarcomas, via the splenic vein and portal circulation, whereas the relationship between splenic and pulmonary tumours was relatively weak. There was a strong association between tumours occurring in the right atrium and lungs, consistent with dissemination via the pulmonary artery to the lungs. Metastatic tumours were identified in skeletal muscle, connective tissue, omentum, peritoneum, lymph nodes, adrenal glands and the central nervous system. Tumours in the liver and lungs were usually well-differentiated, even though most were considered to be secondary tumours derived from haemangiosarcomas of higher grade. CD31 was detected immunohistochemically in neoplastic endothelial cells of 83/85 (97.6%) tumours and von Willebrand's factor (vWf) was detected in 92/93 (98.9%) tumours from 78 cases. These findings suggest a biclonal origin for canine haemangiosarcomas and demonstrate the utility of vWf and CD31 as markers for neoplastic endothelial cells.

DIFFERENT SITES ARE INVOLVED IN THE METASTATIC INFLAMMATORY MAMMARY CARCINOMA RESPECT TO OTHER METASTATIC NON-INFLAMMATORY MAMMARY TUMORS.

Laura Peña, Mónica Clemente, Dolores Pérez-Alenza,

Dpto. Medicina y Cirugía Animal (Anatomía Patológica). Facultad de Veterinaria. Ciudad Universitaria. Madrid, Spain

Inflammatory mammary carcinoma (IMC) is the most aggressive spontaneous type of mammary cancer both in women and in dogs. Several epidemiological, clinical, pathological, and genetic alterations have been specifically associated with inflammatory mammary carcinomas compared with other non-inflammatory mammary carcinomas. The objective of this study was to compare necropsy findings in dogs that had died by IMC respect to those found in dogs that died by metastatic-non inflammatory mammary cancer. Only complete necropsies (with macroscopic description and histopathology) performed between 1995-2005 from cases examined and followed-up in the Veterinary Teaching Hospital of Madrid were considered. A total of 72 necropsies of dogs with metastatic malignant mammary tumors (39 with IMC and 33 with non-inflammatory metastatic malignant mammary tumors (M-MMT)) were included in the study. Among the intact females (n= 67), 22.4% presented polycystic ovaries (PO) and either cystic endometrial hyperplasia (CEH) (n=13) or pyometra (n=2) without association with the inflammatory/non-inflammatory types. There were significant differences in the internal organs affected with metastases between the two groups: IMC cases presented less involvement in lungs (p=0.031), liver (p= 0.009), kidneys (p=0.02) and bones (p= 0.04). Interestingly, IMC cases showed distant metastases in urinary bladder (17.9%, p=0.013) and genital tract (10.2%) while M-MMT did not. The different metastatic pattern observed in IMC cases might be related with the distinct pathogenic mechanisms involved in this type of cancer, such us invasiveness and angiogenesis.

EXPERIMENTAL PROCEDURE FOR RS-1 ALVEOLAR-TYPE HEPATOMA

Eugenia Tirifon¹, Rodica Anghel¹, Nicolae Manolescu¹, Irinel Popescu³, Georgeta Dinescu², Manuella Militaru², Corneliu Mateescu¹, Ana-Maria Burduşel¹, Iolanda Dumitrescu¹, Dana Breslaşu², Marieta Panait¹, Patricia Boeţi³, Crina Tirifon⁴

¹Institute of Oncology, Prof.dr.Alex.Trestioreanu Bucharest, Romania

²Faculty of Veterinary Medicine, Bucharest, Romania

³Fundeni, Clinical Hospital, Bucharest, Romania

⁴University of Medicine and Pharmacy Carol Davila, Bucharest, Romania

A method to obtain a RS-1 alveolar hepatoma, under the hepatic capsule, from inbred immune-suppressed R rats, was developed. The subcutaneous hepatoma suspension was inoculated directly in the hepatic lobes, after the peritoneum incision, or indirectly, through the peritoneum. After inoculation, at certain time intervals, the rats were sacrificed, and the presence, development, morphologic, macroscopic and histological aspects of the tumor were investigated. In the transperitoneal inoculation case, the tumoral process evolved in 32.0% of the rats, beginning with the 15th day after the inoculation; 18.7% rats developed tumors both on the walls and inside of the organs from the abdominal cavity, starting from the firsts days of the tumor evolution, but without liver localization; in 8.2% of the cases, it was a strictly hepatic localization, and 5.1% cases presented concomitant localization and evolution – hepatic and abdominal. In direct inoculation in the hepatic lobes case, the hepatic tumoral process developed in 46.0% of the inoculated rats and was observed starting in the same 15th day after inoculation. Lately, after 33-50 days from inoculation, a tumor invasiveness in the abdominal walls and some organs (spleen, gastro-intestinal tract) was observed, in 95.5% cases. Hepatoma, under the Glisson's capsule were detected as little, white, encapsulated, compact and greasy nodules, placed on the liver surface or in the hill neighborhood or between the different hepatic lobes. Histologically, both hepatic hepatoma and subcutaneous hepatoma exhibited the same pattern, of alveolar type, with different degrees of differentiation. Until the 22nd day after the inoculation, the degree of differentiation was poor, but lately it was observed a large tumor with a great differentiation degree. Now, the hepatoma is maintained as experimental lesion with subcutaneous localization.

SESSION 4B
VARIETAL PATHOLOGY (1)

THE PATHOLOGY OF CHRONIC EROSION DERMATOPATHY IN MURRAY COD, MACCULLOCHELLA PEELII PEELII (MITCHELL).

James E Baily, Michael J Bretherton, Fiona M Gavine, Hugh W Ferguson, Jimmy F Turnbull

Faculty of Veterinary Medicine, University of Glasgow, UK

Chronic erosive dermatopathy (CED) is a disease of intensively farmed Murray cod in Australia that has been reported in association with the use of groundwater supplies. CED results in focal ulceration of the skin overlying sensory canals of the head and flanks. Trials were conducted at an affected fish farm to macroscopically and microscopically study the development of the condition, both in Murray cod and in goldfish, and to assess the reported recovery of lesions when affected fish were transferred to river water. Grossly, lesions began after 2–3 weeks with degeneration of tissue at the periphery of pores communicating with the sensory canals. Widening of these pores along the axis of the canals resulted from a loss of tissue covering the canal. Hyperplasia of the canal epithelial lining was seen after 3 weeks in borehole water and subsequent necrosis and sloughing of this tissue resulted in the loss of the canal roof. Canal regeneration occurred when fish were transferred from borehole water into river water. The lack of lesions in other organs and the pattern of lesion development support exposure to waterborne factors as the most likely aetiology.

Session 4B

PATHOMORPHOLOGICAL PATTERN OF CARP LIVER CAUSED BY THE HERBICIDE AVANS 330 SL IN FISH CLINICALLY HEALTHY AND WITH ICHTHYOPHTHIRIASIS

Jozef Szarek, Izabella Babinska, Monika Truszczynska, Ryszard Kolman, Andrzej K. Siwicki, Joanna Wojtacka, Halina Kolman, Krystyna A. Skibniewska, Izabella Zmyslowska**, Dagmara Chelstowska*

*University of Warmia and Mazury in Olsztyn, Faculty of Veterinary Medicine, 10-719 Olsztyn, Poland; *The Stanisław Sakowicz Inland Fisheries Institute, 10-719 Olsztyn, Poland; **Faculty of Environmental Sciences and Fisheries, 10-719 Olsztyn, Poland*

Introduction: Avans 330 SL (AV) has recently become a popular herbicide used for the control of aquatic vegetation. However, less is known about its toxicity to aquatic ecosystems, including fish. The aim of this study was to examine the impact of AV on the pathomorphology of liver in clinically healthy carps and the ones infected with *Ichthyophthirius multifiliis*, at concentration in the so called toxically safe level.

Material and methods: The experiment was performed during 96 h on fingerling of clinically healthy carp (*Cyprinus carpio* L.) divided into 4 groups (n = 10):

ORAL PRESENTATIONS

A1 and B1 – control fish with no herbicide exposure, A2 and B2 - the carp exposed to AV, (a product of Zeneca, Agro-Chemicals, Great Britain) at the concentration of 2 mg trimethylsulphonium glyphosate per l-l of water. Fish from the group A were clinically healthy and carp from the group B became infected with *Ichthyophthirius multifiliis* (natural invasion). Carp were examined clinically and macroscopically, and liver was investigated microscopically and ultrastructurally (Opton 900 TEM).

Results: It was stated that the most frequent morphological changes were observed particularly in fish infected with *Ichthyophthirius multifiliis* and bathed in water with AV, and they included regressive lesions, such as parenchymatous and vacuolar degeneration and focal necrosis. Less often in these cases, and even less often in carp with *Ichthyophthiriasis* or only exposed to AV, circulatory disorders in the form of hyperaemia and minor extravasations were noted. The ultrastructural alterations were quite often observed in mitochondria and in endoplasmic reticulum.

Conclusion: The analyses of the morphological lesions observed in liver showed that Avans in 2 mg of trimethylsulphonium glyphosate per l-l exerted pathogenic effects on carp which led to morphological changes and generated intensification of these lesions in fish infected with *Ichthyophthirius multifiliis*.

Session 4B

PATHOLOGICAL CHANGES IN THE BRAIN AND EXCRETORY KIDNEY OF RAINBOW TROUT FRY EXPERIMENTALLY INFECTED WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS.

Michal Reichert, Jerzy Antychowicz, Marek Matras

National Veterinary Research Institute, Poland

Pathogen free rainbow trout fry, with remnants of jolk sac, produced in virus free laboratory environment was experimentally infected via water with Infectious haematopoietic necrosis virus (IHNV) strain received from Community Reference Laboratory for Fish Diseases in Aarhus, Denmark. During the experiment clinical symptoms, mortality as well as gross and histopathological changes were recorded. Disease incubation period ranged from 9 to 27 days depending on individual fishes. Mortality was 100% after 28 days. Some virus infected fishes were dying without any visible symptoms or gross pathological lesions. The most prevalent changes were exophthalmia, pseudofaeces, gill anemia, darkening of the skin and petechiae on the fin base. The most spectacular change observed grossly was hydrocephalus together with swelling of the dorsal part of the cranium behind the eyes, which occurred usually in conjunction with exophthalmia. Presence of hydrocephalus is diagnostic feature of rhabdovirus induced disease of pike fry. Relevance of our

finding is strengthened by the lack of information in the available literature on the occurrence of this symptom in IHNV infected trout fry. Histopathological examination revealed that the most affected organs were brain and excretory kidney. In the brain there was accumulation of fluid in the ventricle of the mesencephalon. Pathological changes in the kidney consisted of disruption of the normal structure of tubules, tubular necrosis and displacement of normal haematopoietic elements by histiocytic aggregates.

Poland is IHN free country and this is the reason why field veterinarians and fish farmers lack experience regarding the IHNV. Therefore there is a special need for information concerning the IHN induced pathology. The present study makes an effort to meet those needs.

Session 4B

PATHOLOGIC STUDY OF THE PROTECTIVE EFFECTS OF THIAMINE ON EXPERIMENTAL LEAD POISONING IN RABBIT

*Ahmad Reza Movassaghi, Mohammad Reza Aslani, Atefeh Sarafan Sadeghi
School of Veterinary Medicine, Ferdowsi University of Mashhad, Iran*

Lead is an important toxic agent in nature which affects various tissues and organs in human beings and animals. Lead intoxication has been known as a health threatening disease in recent decades. The protective effects of thiamine were evaluated histopathologically in experimental lead poisoning in rabbits in this study. Twenty white Newzealand rabbits were divided into two groups (10 animal in each group). Rabbits in group 1 received one dose of 40 mg/kg lead acetate solution subcutaneously (every day and for 15 days). Rabbits in group 2 received lead acetate solution in the same manner associated with intramuscular administration of thiamine at dose 10 mg/ kg. All the rabbits were euthanased after 15 days. Tissue samples from the heart, brain, liver, kidney and spleen were fixed in 10 per cent buffered formalin, and sections were prepared for histological examination and stained with haematoxylin and eosin. Histopathological examination of specimens in group 1 revealed severe hyperemia, perivascular and perineuronal edema, laminar neuronal necrosis and perivascular hemorrhage in the brain. There were hyperemia, muscle fiber necrosis and infiltration of mononuclear inflammatory cells in the heart. Kidneys showed various degrees of hyperemia, acute tubular necrosis and intratubular proteinaceous casts. There were also hyperemia, hepatocellular degeneration and necrosis, acid -fast intranuclear inclusion body and mild infiltration of mononuclear inflammatory cells in the liver. All the spleens showed severe hemosiderosis. In group 2, the cerebral lesions were similar to the lesions of group 1. Lesions in other organs were confined to mild hyperemia and degenerative changes. In conclusion, the results of this study indicate that thiamine could reduce the severity of lesions induced by lead poisoning except for cerebral lesions.

SESSION 5A
CNS/MUSCLE

CORRELATION BETWEEN BAX OVEREXPRESSION AND PRION DEPOSITION IN MEDULLA OBLONGATA FROM NATURAL SCRAPIE WITHOUT EVIDENCE OF APOPTOSIS

Rosa Bolea, Jaber Lyahyai, Carmen Serrano, Eva Monleón, Carlos Moreno, Rosario Osta, Pilar Zaragoza, Juan J. Badiola, Inmaculada Martín-Burriell
University of Zaragoza, Spain

Although apoptosis has been implicated in the neuronal loss observed in prion diseases, the participation of apoptosis related factors, like the Bcl-2 family of proteins, is still not clear. Moreover, there are conflicting data against the main role of apoptosis in the neuropathology associated with TSEs. Many studies have been developed in vitro or in experimentally infected animal models but, at present, little is known about this process in natural spontaneous and acquired prion diseases. In this work, the implication of Bax and Bcl-2 has been investigated through the analysis of their expression and protein distribution in medulla oblongata of naturally scrapie infected sheep. Moreover, their spatial relationship with PrPSc deposition, neuronal vacuolation and neuropile spongiosis has also been analysed and finally, the possible induction of neuronal apoptosis in this model. Real time RT-PCR showed an overexpression of the pro-apoptotic gene Bax in scrapie medullas and immunohistochemistry confirmed its accumulation. On the other hand, no variation was observed for Bcl-2 neither at transcript nor at protein level. Bax distribution, PrPSc deposition, neuronal vacuolation and spongiosis were quantified in different medulla oblongata nuclei and their spatial relationship was evaluated. Bax staining showed a positive correlation with prion deposition, suggesting that this factor is involved in prion neurotoxicity in our natural model. Despite Bax overexpression, neuronal apoptosis was neither revealed by TUNEL nor by immunohistochemical detection of the activated form of caspase-3. Absence of apoptosis in medulla oblongata of naturally scrapie infected sheep is in the process of being discussed.

THE PATTERNS OF ACCUMULATION OF PRION PROTEIN DURING PRECLINICAL SHEEP SCRAPIE AND BSE SUGGEST DIFFERENT PATHWAYS OF NEUROINVASION

Silvia Sisó, Martin Jeffrey, Hazel Baird, Stuart Martin, Sue Bellworthy, Francesca Chianini, Lorenzo González
VLA Lasswade, UK

Clinical signs in sheep scrapie usually appear after widespread accumulation

of the disease-associated isoform of the prion protein (PrP^d) in the brain. It is generally believed that, after oral exposure -often followed by lymphoid tissue replication-, neuroinvasion occurs via the autonomic nervous system and, as a result, the dorsal nucleus of the vagus nerve (DMNV) is the initial point of PrP^d accumulation in the brain. Nevertheless, the topographical and temporal spread of PrP^d to other brain areas during the preclinical period in relation to the route of exposure and other factors is not properly documented.

We examined by immunohistochemistry the brains of 35 sheep either exposed to natural infection (Shetland and Suffolk sheep from two flocks) or to experimental challenge; these last included scrapie by the oral, subcutaneous and intravenous routes in Cheviot and Suffolk sheep, and BSE in Suffolk, Cheviot and Romney sheep by the oral or intracerebral routes. All animals were studied at preclinical stage of scrapie either because they died from intercurrent conditions, were part of a sequential killing strategy or were culled after confirmation of infection by biopsy.

We found that initial PrP^d accumulation occurred in most cases in the DMNV and in the hypothalamus, regardless of the breed of sheep, PrP genotype, TSE source and, more surprisingly, route of infection. Moreover, the topographical distribution and magnitude of PrP^d deposition, with consistent involvement of the periventricular organs, suggest that the pathways of entry of the TSE agents in the brain might be different from those arising from the autonomic nervous system. Also, the pattern of PrP^d spread within the brain seemed to be TSE strain-dependent.

These findings, and the apparently route-related susceptibility to TSE infection in sheep of some genotypes, argue for a review of the current hypothesis of TSE neuroinvasion route.

Session 5A

CNS LESIONS IN SMALL RUMINANTS: A HISTOPATHOLOGICAL ANALYSIS OF FALLEN STOCK BRAINS COLLECTED IN A ONE YEAR TSE SURVEY

Anna Oevermann, Alexandra Nicolier, Martina Friess, Marc Vandeveldel, Monika Hilbe, Andreas Zurbriggen, Marcus Doherr, Torsten Seuberlich, Hervé Brünisholz, Reto Wyss, Ulrich Kihm, Heinzpeter Schwermer, Dagmar Heim, and Catherine Botteron

Neurocenter, Switzerland

During 2004/2005, a one year survey for transmissible spongiform encephalopathies (TSEs) in small ruminants was conducted at the Swiss National Reference Laboratory for animal TSEs. 3184 brains of fallen stock - perished

or not regularly slaughtered animals – (2404 sheep, 788 goats) were examined at different anatomical regions (brainstem, cerebellum, midbrain, hippocampus, thalamus, basal ganglia, parietal and frontal cortex) by histopathology and immunohistochemistry.

This report describes the microscopical CNS lesions that were observed during the survey. In 252 brains (7.9%) lesions were identified that were classified into four different categories. 1) Inflammatory/infectious diseases comprised the largest group (147 sheep, 43 goats). Of these, 82 sheep and goats (2.6%) had listerial meningoencephalitis, this being the most prevalent CNS disease in this study. In 24 cases (22 sheep, 2 goats), a suppurative meningitis/meningoencephalitis compatible with bacterial infection was diagnosed. Histopathological CNS lesions consistent with Visna and Borna disease were observed in 4 and 3 sheep, respectively. In 12 animals (4 goats, 8 sheep), migrating nematodes were suspected. Three cases of atypical scrapie were identified (1 goat, 2 sheep). 2) Toxic/metabolic diseases (35 sheep, 22 goats): Polioencephalomalacia was the most common disease of this category (19 sheep, 6 goats). Furthermore, spongy degeneration (5 sheep, 11 goats), metabolic encephalopathy (7 sheep, 2 goats) and 6 cases of suspected enterotoxemia (all sheep) were observed. 3) Neoplasms (4 sheep) included two lymphomas, one intracranial lipoma and one pituitary adenoma. 4) One case (1 sheep) of a chronic infarct was assigned to the category Vascular disorders.

This study of fallen stock points out that CNS lesions are common in small ruminants in Switzerland. The prevalence of listerial meningoencephalitis (2.6%), a disease of zoonotic potential that is reportable in Switzerland, was unexpectedly high indicating that its occurrence has been underestimated in the past.

SESSION 5B
CLINICAL PATHOLOGY

**DIAGNOSIS OF BOID INCLUSION BODY DISEASE BY
EXAMINATION OF BLOOD SMEARS AND PULMONARY LAVAGE**

Adrian W Philbey, Alistair M Lawrie

University of Glasgow Veterinary School, UK

Boid inclusion body disease (IBD) is a transmissible disease of snakes seen most commonly in boids and pythons (Family Boidae), as well as being reported in colubrids (Family Colubridae) and viperids (Family Viperidae). The disease is characterised clinically by anorexia, regurgitation, weight loss, lethargy and neurological signs. Histopathological findings include degeneration of hepatocytes, pancreatic epithelial cells, renal tubular epithelial cells and lymphoreticular cells, along with interstitial pneumonia and meningoencephalitis. Eosinophilic inclusion bodies are present histologically in the cytoplasm of epithelial cells in the lungs, gastrointestinal tract, kidneys and pancreas, as well as in hepatocytes, neuroglial cells in the brain and splenic lymphoreticular cells. Boid IBD is usually diagnosed on the basis of clinical signs and detection of inclusion bodies by histopathology at postmortem examination, or antemortem in biopsies of liver, kidney, pancreas, oesophageal tonsil or skin. Inclusion bodies may also be detected in leucocytes and erythrocytes in blood smears. Boid IBD is described here in a 3-year-old red-tailed boa constrictor (*Boa constrictor* subspecies *constrictor*) with proliferative pneumonia. We also describe the presumptive antemortem diagnosis of boid IBD in other snakes by examination of blood smears and by pulmonary (bronchofaveolar) lavage. Inclusion bodies in blood smears and cytospin preparations are basophilic when stained with May-Grünwald-Giemsa after methanol fixation and eosinophilic when stained with haematoxylin and eosin after fixation in formalin vapour.

SESSION 6A
CNS/MUSCLE (2)

**IMMUNOHISTOCHEMICAL CHARACTERISATION
OF THE CNS CELL INFILTRATIONS IN GREYHOUND
MENINGOENCEPHALITIS**

*J.J. Callanan, S Coates, J Brady, S Worrell, P Daly, E.J. O'Neill, A Kipar¹,
School of Agriculture, Food Science & Veterinary Medicine, University College
Dublin, Ireland, ¹University of Liverpool, UK*

Greyhound meningoencephalitis is currently classified as a breed-associated idiopathic central nervous system inflammatory disorder. The non-suppurative inflammatory response can be distinguished from other breed-associated disorders and from GME, a non-breed associated idiopathic inflammatory disorder, based on histopathology and lesion topography. Lesions consist of diffuse and focal gliosis with gemistocytosis focused in the region of the caudate nucleus and cerebrocortical grey matter. Accompanying this lesion is extensive perivascular cuffing and cell infiltrations of the meninges. While the clinical and histopathological changes favour a viral aetiology, to date clinical, pathological, serological, tissue culture and molecular studies have failed to identify an agent.

The aims of the present study were to characterise the inflammatory cell infiltrations and compare observations with those known for other CNS inflammatory diseases of the canine.

Formalin-fixed or frozen cerebral brain tissues from twelve 5 to 18 month old greyhounds (4 female, 8 male) with histopathological confirmation of meningoencephalitis were selected. Immunohistochemistry was performed using anti-human antibodies to CD3 (Serotec) and CD79a (Dako) antigens, anti-canine antibodies to CD4 and CD8 antigens (Serotec) and anti-human antibodies to Lysozyme, the myeloid/histiocyte antigen (m/h Ag) and MHC II.

Perivascular cuffs, meningeal infiltrations and gliotic regions were rich in CD3+ T Cells. The majority of these cells (approx 50%) were CD8+ and particularly prominent in regions of gliosis. CD4+ T cells were in smaller numbers (<25%) within perivascular cuffs, meningeal tissue and even in lower numbers in regions of gliosis. CD79a+ B cells represented the second major cell population within perivascular cuffs and meningeal infiltrates, with smaller numbers of B cells scattered within the gliotic regions.

Lysozyme expression was seen in monocytes within the vascular lumen and attached to endothelial cells. They were relatively rare in the perivascular cuffs, but often more numerous within the parenchymal infiltrates (gliotic lesions). Monocytes were also found to express m/h Ag, although the perivascular or

gliotic cell populations contained very few positive cells. MHC II expression was prominent in regions of gliosis, on lymphocytes of the perivascular cuffs and on vascular endothelial cells.

In all greyhound meningoencephalitis cases, the immunophenotype of the infiltrating cell population was similar. In contrast to GME, the lymphocyte cell infiltrations were more mixed, being rich in CD8 and CD4+ T and in B cells while devoid of significant numbers of macrophages. The reduced numbers of these latter cells suggesting minimal recruitment of peripheral blood monocytes. In many respects the cell infiltration resembled that expected in necrotizing meningoencephalitis (Pug dog encephalitis) and could also be related to the limited data available on cell infiltrates associated with viral infections.

Session 6A

VIRAL SPREAD IN BRAINS OF BORNA DISEASE VIRUS-INFECTED TNF-TRANSGENIC MICE

Katharina Kramer, Doris Porombka, Dirk Schaudien, Ulrich Eisel, Wolfgang Baumgärtner, Christiane Herden

University of Veterinary Medicine Hannover, Germany

Experimental BDV-infection of TNF-transgenic mice was carried out to study the effect of neuronal TNF-overexpression on viral spread and persistence in the brain.

Neonatal TNF-transgenic (homozygous and heterozygous) and non-transgenic mice were infected intracerebrally with a mouse-adapted BDV-strain. Non-infected animals served as controls. Mice were killed between 14 and 42 days post infectionem (dpi). Brains were fixed in formalin and embedded in paraffin or embedded in OCT® and stored at -80°C. Viral RNAs specific for the BDV-nucleoprotein (BDV-N) and BDV-glycoprotein (BDV-GP) gene were demonstrated by in situ hybridization (ISH) at 14, 28 and 42 dpi and quantified by real time RT-PCR at 21 and 42 dpi. For normalization of these viral RNA species, BDV-Intron I and the BDV-antigenome were also analyzed.

By ISH, BDV-N and BDV-GP specific mRNA was found in all brain regions at 14 dpi and persisted until 42 dpi in all infected mice groups. BDV-N mRNA was mainly present in the cytoplasm, whereas BDV-GP mRNA was found in the nucleus and cytoplasm. Quantification revealed that cDNA copy numbers specific for BDV-N was 10 to 100-fold higher in all mice groups compared to other BDV-genes at both time points investigated. BDV-GP cDNA copy numbers decreased in all BDV-infected mice groups during the investigation period, whereas the BDV-intron I specific cDNA was present in nearly constant amounts in all groups at all time points. The increasing amount of BDV-antigenome specific cDNA during the investigation period indicates active

viral replication. Viral cDNA copy numbers of all BDV-genes investigated were often lower in homozygous transgenic animals.

Expression of viral RNA indicates a similar mode of transcription independent of the transgenic status of the animals. However, it remains to be determined if the lower copy numbers of BDV-genes in homozygous animals might be induced by high TNF levels in the brain.

Session 6A

REGIONAL TROPISM OF BORNA DISEASE VIRUS TRANSCRIPTION AND REPLICATION IN DIFFERENT BRAIN AREAS OF EXPERIMENTALLY INFECTED LEWIS RATS

*Doris Porombka, Eickmann Markus, Wolfgang Garten, Wolfgang
Baumgaertner, Christiane Herden*

University of Veterinary Medicine Hannover, Germany

Borna disease virus (BDV) is a highly neurotropic, nonsegmented, negative and single-stranded (-ss) RNA virus. Typically, BDV-infection causes a severe neurological disorder, characterized by nonpurulent meningoencephalomyelitis and viral persistence. However, the *in vivo* strategies of BDV to control its spread, replication and persistence are incompletely understood. There is evidence that BDV utilizes tightly regulated mechanisms to control its transcription and viral protein expression including limited BDV glycoprotein synthesis. Initial *in vivo* studies indicated a restricted glycoprotein (BDV-GP) expression in certain brain areas, such as hippocampus, cerebral cortex, thalamus and amygdala.

To investigate essential strategies of viral replication and persistence, the expression of BDV-specific transcripts in different brain areas of experimentally infected Lewis rats were analyzed.

Therefore, BDV-transcripts specific for the nucleoprotein (+ssBDV-N), glycoprotein (+ssBDV-GP), intron I-specific +ssRNA (+ssBDV-Intron I) and full length antigenome (+ssBDV-AG) as a marker for active virus replication were quantified in two different brain areas with or without immunohistochemically detectable BDV glycoprotein expression (hippocampus and striatum). Serial sections of shock frozen rat brains were transversally cut, mounted on PEN-MembraneSlides and stained by H&E. From each animal, the hippocampal area and striatum were harvested after 14, 24, 42, and 90 days post infection (dpi) using laser microdissection and +ssRNA was measured by real time RT-PCR.

In general, up to 42 dpi, the hippocampus showed higher copy numbers of all BDV-transcripts compared to the striatum, most obviously for +ssBDV-AG. Interestingly, the ratio of BDV-specific transcripts was comparable in either hippocampus or striatum, but varied slightly between different time points.

In summary, detailing the BDV-specific expression profile of viral transcripts

and of the viral antigenome in selected brain areas represents a key step for understanding the *in vivo* strategies of BDV to control its replication and persistence in the CNS.

Session 6A

NEUROLOGICAL CHANGES AND BRAIN COPPER ELEVATION IN NORTH RONALDSAY SHEEP: A MODEL FOR WILSON'S DISEASE?

Catherine Botteron, Jasmin Paris, Richard Ryvar, Michael Loughran and Susan Haywood

University of Liverpool, UK

Wilson's disease is a genetic disorder of copper (Cu) metabolism in man characterised by accumulation of Cu in many organs, notably in the liver, causing chronic hepatitis with progression to macronodular cirrhosis. In the brain, findings may include degenerative changes in the basal ganglia and cortico-subcortical areas. Cerebral Cu concentration is consistently increased.

North Ronaldsay (NR) sheep have adapted to a Cu-impooverished environment and display an abnormal susceptibility to Cu toxicosis when transferred to a Cu-replete habitat. Affected sheep develop a progressive chronic hepatitis with fibrosis, together with excessive Cu deposition in hepatocytes and Kupffer cells.

A study was performed to establish whether Cu accumulates in the brain of NR sheep and to determine if there are neuropathological parallels with Cu-related brain lesions in human beings. Six adult NR sheep suffering or having died from Cu toxicosis, were compared with 3 immature sheep of the same breed. Brain and liver samples were analysed for dry matter Cu content. Histopathological examination, including chemical localisation of Cu and metallothionein (MT) immunostaining were performed.

When compared to the young sheep (group 0), all adult NR sheep exhibited a markedly increased level of Cu accumulation in both liver and brain (up to 10 fold). There was a positive correlation ($r= 0.95$) between liver and brain Cu accumulation.

Sheep which had the most elevated Cu concentrations (Group 3), displayed liver fibrosis with incipient cirrhosis and histopathological changes in the brain characterised by multifocal vacuolation of the white matter and presence of Alzheimer type II astrocytes. Rhodanine stain revealed Cu accumulation in astrocytes and increased immunolabelling for metallothionein (MT) was detected. These findings suggest that the blood-brain barrier of NR sheep presents unique features and that this breed could be used as animal model for Wilson's disease and, in particular, for the study of abnormal Cu transport and metabolism in the brain.

**CHARACTERIZATION OF THE CENTRAL NERVOUS SYSTEM
INFLAMMATORY RESPONSE IN NATURAL CASES OF OVINE
VISNA**

Julio Benavides, Carlos García-Pariente, Miguel Fuertes, M. Carmen Ferreras, J. Francisco García-Marín, Valentin Pérez

Universidad de León, Spain

Maedi-Visna is a lentiviral disease of sheep characterized by a non suppurative inflammatory infiltrate of the affected organs. In the central nervous system, different lesional patterns can be observed: areas showing a predominance of perivascular cuffs, intense inflammatory infiltrates formed by lymphocytes and macrophages in different proportion, or lesions characterized mainly by malacia and liquefaction. The main objective of this study was the characterization of the inflammatory response in lesions showing the three mentioned patterns. Immunohistochemical methods, using antibodies against CD3, CD79 or CD45R lymphocyte receptors, VMP-32 against macrophages and GFAP for astrocytes recognition were applied, as well as luxol-fast blue-PAS and oil red 0 methods for demyelination demonstration. Perivascular cuffs were formed by T-lymphocytes and macrophages located mainly at the edges of the cuffs. In the adjacent neuropil, normal GFAP staining was observed. Inflammatory pattern was seen predominantly in the encephalon, characterized by T-lymphocytes and/or macrophage infiltration of the neuropil. When the latter predominate, T lymphocytes were located mainly at the periphery of the lesion, whereas macrophages, filled with degenerated myelin, were the main cell located in the centre, where liquefaction was frequently seen. In the periphery of lesions, an increase in the number of reactive astrocytes was observed, their number decreasing towards the centre of the lesion, and absent in areas with liquefaction. Lesions characterized by a predominance of malacia were seen mainly in the spinal cord. Inflammatory cells, mostly T lymphocytes and macrophages, appeared in the perivascular cuffs or in the periphery of the malacic zones. An increase in the number of reactive astrocytes was related to the presence of inflammatory cells and, in this pattern, GFAP positive fibres appeared in the vacuolated areas. Gitter cells, seen in the malacic zones, were negative to VPM32 marker. Astrocytes, T lymphocytes and macrophages are the main cells involved in the inflammatory response seen in Maedi-Visna nervous lesions, with differences in their arrangement according to the lesional pattern.

NEUROPATHOLOGICAL CHANGES IN APPARENTLY HEALTHY MINK ON ALEUTIAN DISEASE VIRUS-INFECTED IRISH FUR FARMS AND SEQUENCE VARIATIONS IN THE VP2 REGION OF THE VIRUS.

Hanne Jahns, Paul Daly, Hugh F. Bassett, Daniel J. Sammin, Maire C. McElroy, John J. Callanan

Central Veterinary Research Laboratories, Ireland

The objective of the present study was to detail the spectrum of neuropathology associated with ADV infection in Irish farmed mink and to characterise the viral isolates associated with both different patterns of neuropathological changes and with different farms.

Brains were obtained from apparently healthy mink, which were killed for pelting on five Irish fur farms. Using PCR, ADV was detected in pooled mink brains (minimum 10 pooled brains per farm) from animals on four of the farms. No virus was found in brains collected from the farm which its owners claimed to be free from AD.

Non-suppurative meningoencephalitis was seen in 38 of 138 mink brains from four Irish fur farms. Fibrinoid necrotising arteritis was apparent in 11 of the mink brains, all of which were sourced from the same farm. No significant histopathological changes were seen in the animals from the AD-free farm.

When the PCR products (ADV-VP2 gene) obtained from 11 mink brains with and without histopathological lesions were partially sequenced, the nucleotide sequences of all resembled one or other of the three types recognised internationally. The predicted amino acid residues for all of these sequences included those believed to confer pathogenicity. Interestingly, sequencing data revealed multiple virus types only on the farm in which necrotising arteritis was present as the predominant neuropathological feature in brains of sampled mink.

SESSION 6B
VARIETAL PATHOLOGY (2)

DIGITAL MICROSCOPY EQA IN VETERINARY PATHOLOGY – AN RCVS TRUST FUND PROJECT.

Peter Brown¹, Dan Johnson², Sean Callanan³, Kevin Isaacs⁴

¹University of Nottingham, School of Veterinary Medicine and Science UK,

²Slidepath, Dublin, ³University College, Dublin, Ireland, ⁴RCVS Trust Fund

Continuing Professional Development is a requirement of both the UK Royal College of Veterinary Surgeons, the Royal College of Pathologists and the European College of Veterinary Pathologists that is not currently provided by any particular group or organisation. One aspect of CPD would be the establishment of external quality assurance (EQA) in Veterinary Pathology.

Digitisation of microscope slides is now an established method of distributing and viewing histological sections and is already employed by SlidePath in conjunction with various EQA schemes for specialist medical pathology groups in the UK and Europe. Digital histology overcomes problems of preparation and distribution of duplicate slides and facilitates rapid submission, collation and analysis of participants' responses.

A digital microscopy EQA scheme is being launched, funded by a small grant from the RCVS Trust Fund. The slides used would be contributed by the various likely participants in the EQA exercise including pathologists in Veterinary Schools, private diagnostic laboratories, the Veterinary Laboratory Agency and the Pharmaceutical Industry. Participant Veterinary Pathologists will be provided by a convenient form of EQA exercises that would act as one form of CPD and could help provide the basis of a core of material for post-graduate education in Veterinary Pathology.

A series of digitised slides will become available at a rate of three per month. Participants will be provided with secure log-on access and invited to submit a diagnosis and comment on features of interest that they note, or features highlighted by the project management group.

The results of the EQA process will be reported to participants at regular intervals and a final report prepared covering the entire series of cases. A review meeting will be held to discuss the process, provide feedback and to consider continuation on a self-funding basis.

ANAPLASMA PHAGOCYTOPHILUM: SITES OF PRIMARY INFECTION

Udo Hetzel, Anja Kipar, Zerai Woldehiwet

Faculty of Veterinary Science, University of Liverpool, UK

Anaplasma phagocytophilum (AP; formerly known as *Ehrlichia phagocytophila*) is the aetiological agent of tick-borne fever in sheep and cattle. The rickettsial agent invades polymorphonuclear phagocytes (PMN) and monocytes. After experimental intravenous infection, however, a lag phase of approximately 2 days is observed in which the agent cannot be demonstrated in the blood by conventional PCR and cytology.

The aim of this study was to identify the sites of primary multiplication of AP. We infected susceptible sheep by intravenous inoculation with a blood stabilate of AP and humanely killed 1-2 animals after various intervals (10 min to 96 h post-inoculation (pi)). Haemolymphatic tissues, liver, lungs and peripheral blood leukocytes (PBL) were examined for the presence of AP by transmission electron microscopy (TEM), immunohistology (IH), PCR and in situ hybridisation (ISH).

AP-specific DNA was first identified by PCR in each one animal in the bone marrow and liver at 8 h pi and in the spleen at 36 h pi. By 48 h pi, lungs and spleens were positive. PBL samples were positive from 72 h pi (also by IH and ISH), and at 96 h pi all organs tested positive. However, immediately after infection (10 min), AP was detected within pulmonary intravascular macrophages (PIM), hepatic Kupffer cells and the haemolymphatic tissues by IH and/or TEM. The PIM remained positive throughout, whereas the detection of AP in Kupffer cells and haemolymphatic tissues was transient and only recurred with bacteraemia.

Our results indicate that PIM may be important targets of primary multiplication of AP before the development of bacteraemia, which results in heavy infection of PMN and monocytes. Different from other (specialised) macrophages, the PIM seem not to destroy the agent but could instead represent the primary site of infection, ensuring low-level multiplication before PMNs, the major targets of AP are infected.

**STUDY OF THE PATHOGENESIS OF THE HPAI H5N1
EXPERIMENTAL INFECTION IN PECKIN DUCKS, BASED ON
IMMUNOHISTOCHEMISTRY AND IN SITU HYBRIDIZATION**

Franco Mutinelli, Marta Vascellari, Anna Granato, Lara Basilicata, Laura Trevisan,

Istituto Zooprofilattico Sperimentale Delle Venezie, Italy

The ongoing H5N1 Asian epidemic is currently affecting a number of avian species including ducks. These birds are an important part of the poultry industry in the affected countries, and it is likely that they are acting as reservoirs of infection. The lack of knowledge on the pathogenesis of HPAI H5N1 infection and the unavailability of information on the efficacy of vaccination in these species have stimulated the following investigation. Five day-old ducklings were vaccinated with 0.5 ml/bird by SC route in the neck and subsequently boosted at 4 weeks of age with 1 ml/bird by the same route with an inactivated AI H5N2 virus vaccine. The challenge of vaccinated and unvaccinated (n=14) Peckin ducks has been carried out with 100 µl containing 10⁷ EID₅₀ of the HPAI H5N1 virus (A/Duck/Vietnam/12/05), administered to all the ducks by intra-nasal and oral route. Clinical symptoms were recorded twice a day up to 10 days post-infection. All the vaccinated ducks showed to be healthy throughout the period of the clinical monitoring. All unvaccinated ducks showed signs of disease (conjunctivitis and slight depression) starting from day 2 PI. On day 3 PI, six birds died, and serious nervous signs (torticollis, incoordination, tremors, seizures), were recorded in five birds. On post-mortem examination haemorrhages were observed along the whole intestine tract and in the stomach, gizzard, trachea, pancreas and brain. No histological lesions were observed in tissues of vaccinated ducks, as well as no viral antigen was detected by IHC and ISH. Histological lesions, as well as IHC positivity, were recorded in pancreas and brain of unvaccinated ducks. The ISH revealed viral antigen associated with acinar pancreatic cells and with nervous cells of the CNS as well as of the Meissner submucosal plexus of the intestine. Experimental findings agree with those previously observed in naturally infected ducks with HPAI virus.

SESSION 7A
VARIETAL PATHOLOGY (3)

THE HIGH INCIDENCE OF BILATERAL THYROID LESIONS IN HYPERTHYROID CATS SUPPORTS BILATERAL THYROIDECTOMY AS THE SURGICAL TREATMENT OF CHOICE IN FELINE HYPERTHYROIDISM.

James O'Donovan, Carmel Mooney, Adrian Philbey, Hal Thompson, John Callanan.

School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Ireland

Thyroid glands were collected from 170 hyperthyroid cats. Bilateral thyroidectomy was performed in 45 cases (26.5%). Unilateral thyroidectomy was performed in 125 (73.5%) cases.

The median age for all thyroidectomised cats was 13 years [Range: 5.4- 18 years] and for the bilateral thyroidectomy group was 13.8 years [Range: 9.0, 17.5 years]. Approximately half the cats had been treated with either methimazole or carbimazole prior to thyroidectomy.

Bilateral adenoma and/or hyperplastic follicular lesions were present in 42 of the 45 cats (93.3%) that underwent bilateral thyroidectomy. In each of the remaining three cases an adenoma was present unilaterally, with the contralateral lobe composed of variably sized involuted follicles. Of those cases with lesions in both thyroid lobes six (14%) had bilateral diffuse follicular hyperplasia, 20 (48%) had bilateral multifocal nodular hyperplasia, five (12%) had bilateral thyroid adenomas and nine (21%) had unilateral adenomas with contralateral multifocal nodular hyperplasia. Coalescing nodules forming small adenomas were present in 46% of thyroids classed as multifocal nodular hyperplasia.

Bilateral hyperfunctional thyroid lesions have previously been recognised in hyperthyroid cats by other workers. The high prevalence of bilateral lesions in hyperthyroid cats found in this study supports routine bilateral thyroidectomy as the surgical treatment of choice for feline hyperthyroidism.

IMMUNOHISTOLOGICAL DEMONSTRATION OF FELINE INFECTIOUS PERITONITIS VIRUS STRUCTURAL PROTEINS

Bianca Weber, Kernt Koehler, Manfred Reinacher

Department of Veterinary Pathology of Justus Liebig University Giessen, Germany

Introduction: Immunohistological detection of coronavirus antigen within the lesions is a sensitive method to confirm Feline Infectious Peritonitis.

The current study was undertaken to investigate differences in amount and cellular distribution and of three structural proteins: membrane glycoprotein (M), nucleocapsid protein (N) and spike glycoprotein (S). Monoclonal

ORAL PRESENTATIONS

antibodies from different private and commercial sources have been employed (Hohdatsu1, Custom Monoclonals2, IDEXX3). Usability of combinations of the monoclonal antibodies for improvement of the diagnostic signal have been tested additionally as well as influence of postmortal tissue deterioration on the signal.

Materials and Methods: FIP virus structural proteins were demonstrated using monoclonal antibodies on formalin fixed and paraffin embedded kidneys of 24 cats using various buffer-based antigen retrieval protocols and the peroxidase anti-peroxidase method (PAP method). Granulomas were classified into three different categories according to the amount of coronavirus antigen present. Tissues were classified into four different categories according to their state of postmortal deterioration.

Results: The nucleocapsid protein was identified throughout the cytoplasm of infected macrophages, the membrane protein and the spike protein were mostly observed as granular precipitates near the nucleus, most probably located in the Golgi apparatus.

The spike glycoprotein gave the lowest signal intensity. Combination of the different monoclonal antibodies resulted in a higher signal intensity than each monoclonal antibody on its own.

Post mortem tissue deterioration had no significant influence on the identification of FIP virus structural proteins.

Conclusions: These findings support the hypothesis that the N protein is only translated in the cytosol whereas mature M and S glycoproteins assemble mostly in the Golgi apparatus⁴.

Regarding immunohistological diagnosis of FIP, monoclonal antibodies demonstrating the nucleocapsid or the membrane glycoprotein (or both as a cocktail) are best for verification of the diagnosis of Feline Infectious Peritonitis.

Session 7A

DETECTION OF FELINE INFECTIOUS PERITONITIS VIRUS-LIKE ANTIGEN IN FERRETS

Jorge Martínez, Manfred Reinacher, David Perpiñán, Antonio José Ramis

Universitat Autònoma de Barcelona, Spain

Feline infectious peritonitis (FIP) is caused by the feline coronavirus (FCoV). It is a well-known and widely distributed coronavirus-induced systemic disease in cats and non-domestic felids. The disease is characterized by fibrinous to granulomatous serositis with protein-rich effusions in body cavities and granulomatous inflammatory lesions in several organs. In the last three years some clinicians, based on symptoms and serology, have suspected the possibility of infection of FCoV in ferrets. In fact microscopic granulomatous lesions

observed by us were consistent with FCoV infection.

We selected 9 of those ferret cases submitted to our Pathology Diagnostic Service corresponding to the period 2004-2005. The nine ferrets were of different origin and presented very unspecific symptoms. These animals were all necropsied and submitted to us by the same clinician. He sent several organs and tissues (lymph node, kidney, mesentery, intestine, spleen, liver, lung, pancreas, heart and adrenal glands) in 10% formalin for histopathological examination.

The histopathological picture consisted of a granulomatous inflammatory reaction in nearly all the tissues evaluated. Lymph nodes and mesentery, the most affected tissues in frequency and severity, showed severe multifocal granulomas affecting the normal structure of the tissues. Granulomas showed a variable large central area of macrophages, surrounded by a broad rim of lymphoplasmocytic infiltrate; in some of them, a central area of necrosis was detected. Occasionally single to few neutrophils scattered between macrophages were observed. In other organs, granulomas were detected with different frequencies depending on the animal evaluated. Lastly, inflammatory angiocentric pattern usually described in cats was only observed in some small granulomas located in mesentery and liver.

In granulomas of 7 of the ferrets a specific immunohistological reaction using the monoclonal antibody directed towards feline coronavirus could be seen in macrophages similar to the well known reaction which is to be found in feline granulomas in FIP. Therefore we conclude that coronavirus can induce in ferrets a disease with a high similarity to feline infectious peritonitis.

Session 7A

FELINE MYCOBACTERIAL KERATITIS

Christina McCowan, Janet Fyfe, Anu O'Reilly

University of Melbourne, Australia

Two cats from unrelated households were presented to an ophthalmologist with slowly progressive central corneal opacities. Histological identification of the infecting organisms as mycobacteria was supported by PCR amplification and sequence analysis. The same, previously unidentified species of mycobacterium was identified in both cases. Laboratory culture was unsuccessful despite the myriad organisms present. Surgical excision of the lesions was followed by recurrence within 12 months in both cases.

Infection of the cornea with mycobacteria is a recognised sequel to LASIK surgery in humans, but only two previous reports, both from cats, exist in the veterinary literature. Mycobacterial disease in cats, traditionally titled “feline leprosy” and regarded as infection with *M.lepraemium*, is most commonly associated with infection of the skin or subcutis. Recent reassessment of

infections using molecular probes has shown that *M.lepraemium* is not the only culprit in such cases, and has identified two syndromes of dermatitis in Australian cats, both resulting from infection with mycobacteria which are refractory to culture. The novel organism described here is also fastidious in the laboratory and identified only by molecular characterisation.

This report discusses identification and treatment of feline mycobacterial keratitis.

Session 7A

PORCINE CIRCOVIRUS TYPE 2 INFECTIONS IN HEALTHY PIGS AT SLAUGHTER

M.Rütten, R. Stephan, A. Pospischil, T. Sydler

Institute of Veterinary Pathology of Vetsuisse faculty of University of Zürich, Switzerland

Porcine circovirus type 2 (PCV-2) infection and the associated postweaning multisystemic wasting syndrome (PMWS) has become epidemic in the porcine population in Switzerland since 2003. The question we wish to address is to what extent the virus might be present within the pig population at slaughter, without overt signs of disease. In particular, we selected for “white spotted” kidneys, an abnormality which until now was only described in animals suffering from PMWS. The object of this study was to establish whether PCV-2 is present in “white spotted” kidneys, even in apparently healthy pigs. To this end, we investigated kidneys of pigs, considered at the live animal inspection prior to slaughter to be healthy animals with the required weight (100, ± 5kg), but which following slaughter, were rejected on the basis of abnormalities. A total of 86 kidneys were investigated. 9/86 (10%) kidneys showed acute embolic nephritis, all the others 77/86 (90%) had a disseminated, fine, poorly demarcated, white colouration similar to white spotted kidneys of ruminants. Histologically, chronic multifocally interstitial, peritubular, periglomerular and perivascular lympho-histiocytic nephritis with some plasma cells and sometimes with primary lymphfollicle formation were found. In 29/77 (38%) of the white spotted kidneys PCV-2 positive reaction was recorded by immunohistochemistry using a monoclonal antibody (F217) specific for PCV-2 capsid antigen encoded by ORF2. The antigen was mainly found in the tubular epithelial cells and occasionally in histiocytes and macrophages. The positive signal of the immunohistochemistry correlated well with the inflammatory lesions. Although the PCV-2 labelling signal was in the majority of the cases low, in 2 cases with severe interstitial lympho-histiocytic nephritis and numerous multinucleated giant cells, a very strong positive signal for PCV-2 was evident.

ORAL PRESENTATIONS

This study shows that animals older than the typically affected age group of PMWS can carry a burden of PCV-2 without showing any obvious clinical signs of disease. In addition, a significant part of fine “white spotted” kidneys seem to be associated with PCV-2 infections.

SESSION 7B
REPRODUCTIVE
PATHOLOGY

CHLAMYDIA-RELATED ABORTIONS IN CATTLE FROM GRAUBUNDEN, SWITZERLAND

Nicole Borel, Ruedi Thoma, Patrick Spaeni, Roseline Weilenmann, Komkrich Teankum, Enrico Brugnera, Dieter R. Zimmermann, Lloyd Vaughan, Andreas Pospischil

Institute of Veterinary Pathology, Switzerland

In 2001, the first case of bovine chlamydial abortion was reported in canton Graubunden, Switzerland. In this region, *Chlamydomphila* (Cp.) *abortus* is endemic in small ruminants. Hence, we aimed to investigate the incidence of chlamydia-related abortions in cattle from Graubunden. During breeding seasons of 2003/2004, formalin-fixed and paraffin-embedded placenta specimens (n=235) from late term abortions in cattle were analyzed by histopathology, immunohistochemistry with a Chlamydiaceae-specific monoclonal antibody against chlamydial lipopolysaccharide (LPS) and two different polymerase chain reaction (PCR) methods (16S rRNA PCR, IGS-S PCR), followed by PCR product sequencing. In 149 out of 235 cases (63.4%) histopathological lesions such as purulent and/or necrotizing placentitis were observed. Chlamydial antigen was clearly demonstrated in immunohistochemistry in only 1/235 case (0.4%). Cp. *abortus* or Cp. *psittaci* was found in 12/235 (5.1%) and 10/235 cases (4.2%) by 16S rRNA PCR and IGS-S PCR, respectively. However, we detected by 16S rRNA PCR 43/235 cases (18.3%) to be positive for „Chlamydia-like“ organisms. In contrast to the situation in small ruminants in the canton Graubunden, bovine abortion due to Cp. *abortus* seems not to play an important role. Nevertheless, zoonotic potential should be taken into account when handling abortion material from cattle. The significance of „Chlamydia-like“ isolates other than *Waddlia chondrophila* remains an open question in abortion and needs further investigation.

STAPHYLOCOCCAL ABORTION STORM ASSOCIATED WITH INDWELLING CATHETERS

John Edwards, Arantza Lassala, Thomas Spencer

Texas A&M University, USA

An experiment on nutritional stress included forty-two ewes divided into three groups. Beginning at 60 days of gestation (dog), each ewe had a catheter sewn in place into a prepared area of the jugular vein and received an injection three times daily. The injection consisted of sterile saline or a solution of sterile saline containing arginine. Twenty-one of the first twenty-six ewes that lambd aborted

between 81 day and term. After the first three abortions, each ewe received a once-weekly, prophylactic intramuscular injection of oxytetracycline (20mg/kg); however, abortions continued. Abortions occurred in all groups-including controls. Ewes showed no clinical signs of illness. Fetuses from ten, aborted ewes were examined. Fetuses were expelled in their membranes, and most had early mummification. Macroscopically, placentitis and pneumonia were noted, and infectious abortion was diagnosed. Large numbers of coagulase-positive Staphylococcus (C-pS) were isolated from the abomasum and lung, and C-pS were among isolates from the placentas. It was hypothesized that contamination from the catheters or the solutions had caused the abortions. Three ewes were killed and examined 1-3 days after abortion. All ewes were judged to not be significantly stressed nutritionally and had no indication of ketoacidosis. Each ewe had endometritis with some placental retention; however, all had severe, chronic, jugular vein phlebitis and thrombosis from which C-pS were isolated. Previously used solutions were not available for culture. Twice weekly, oxytetracycline injections were begun by the investigator, and sanitation efforts were redoubled. Eight days after increasing the antibiotic regime, the first of 5 normal births were recorded; however, occasional abortions continued. The outbreak was considered similar to Staphylococcal pyemia of lambs, a condition associated with C-pS-induced abortion and septicemia/pyemia in neonatal lambs.

Session 7B

PATHOMORPHOLOGICAL INVESTIGATIONS OF THE HYPOPHYSIS-OVARIAN-UTERUS-AXIS DO NOT CONTRIBUTE TO THE DIAGNOSIS “ZEARELENONE INTOXICATION” IN 70-DAY-OLD FEMALE PIGS!

*Anne Reischauer, Christin Ellenberger, Susanne Döll *, Sven Dänicke **, Heinz-Adolf Schoon*

Institut für Veterinär-Pathologie, Veterinärmedizinische Fakultät, Universität Leipzig, An den Tierkliniken 33, 04103 Leipzig, Deutschland

** Universiteit Utrecht, Faculteit Diergeneeskunde, Veterinaire Farmacie, Farmacologie en Toxicologie, Yalelaan 16, 3584 CM, Utrecht, Nederland*

*** Institut für Tierernährung, Bundesforschungsanstalt für Landwirtschaft (FAL), Bundeallee 50, 38116 Braunschweig, Deutschland*

Infections of feedstuff with the mycotoxin zearalenone (ZON) and its derivatives are considered to be important reasons for fertility problems in pigs due to the estrogenic properties of the toxin. Although pathognomonic alterations caused by a ZON-intoxication in pigs have not yet been found, increasing demands for pathomorphological investigations had to be satisfied in recent

years in order to confirm the diagnosis of a ZON-mycotoxicosis. Therefore, the objective of this study was to investigate the effect of orally administered ZON on the pituitary, ovaries and uteri of 70-day-old female piglets. In order to characterize pathomorphological parameters which reflect the influence of ZON on the pituitary-ovary-uterus-axis, (immuno-) histological and morphometrical methods were used.

After weaning 100 piglets were equally allotted in 5 treatment groups receiving diets containing increasing amounts of naturally contaminated maize (0.01 (control group, I) to 0.46 (group V) mg ZON per kg diet) for 35 days.

Sections of the formalin fixed and paraplast embedded pituitaries, ovaries and uteri were stained with hematoxylin eosin for routine light microscopy. Immunohistological investigations were performed using antibodies against estrogen receptor, progesterone receptor, follicle stimulating hormone, luteinising hormone, gonadotropin-releasing hormone receptor, and proliferating cell nuclear antigen. Furthermore, morphometrical examinations were carried out.

Compared to the control group, the histological and immunohistochemical findings in pituitaries, ovaries and uteri of the ZON-treated groups did not provide evidence for parameters which can be regarded as specific for a ZON-intoxication. Not until statistical analyses single morphometrical parameters in the uterus (e.g. calibre) showed high-dose-ZON-related alterations. Summing up, routine diagnostic pathology can not contribute to the confirmation of the diagnosis of a ZON-mycotoxicosis in 70-day-old female piglets. The only reliable methods, so far, represent analyses of diets and of mycotoxin residues in the animal (especially in bile fluid).

Session 8, which takes place on 1 September 2006 between 1600 and 1730 hours, is the Poster Session. It will take place in Faculty Room North and the Conference Room on the ground floor of David Hume Tower. Please refer to the section on Poster Presentations to see the full list of posters on display.

SESSION 9A
VARIETAL PATHOLOGY (4)

LAFORA'S BODIES CHARACTERIZATION IN CANINE LAFORA DISEASE: TOPOGRAPHICAL, HISTOPATHOLOGICAL, IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDIES.

Merce Márquez, Lola Pérez, Susana Teijeira, Carmen Navarro, Marti Pumarola

Autonomous University of Barcelona (UAB), Spain

Canine Lafora disease is a hereditary autosomal recessive fatal disorder of the carbohydrate metabolism characterized by the presence of numerous intraneuronal PAS (+) Lafora bodies (LB) mainly in the nervous tissue. The main clinical signs include seizures, jerky head movements, body tremors, intermitent myoclonic contractions of the head and neck muscles and hindquarter ataxia. Other PAS (+) polyglucosan bodies (PGB) such as Corpora amylacea (CA) are present in healthy aged dogs and can be confused with LB.

We present the study of two canine Lafora's Disease cases compared to a healthy aged dog. Both cases were old animals presenting progressive tremors, ataxia and paraplegia. At necropsy, no gross changes were observed. Microscopical examination showed high PGB density throughout the brain, principally in the Cerebellar cortex Thalamus and Midbrain, and with less intensity in the Pons, and Medulla Oblonga. PGB showed different appearance depending on their localization: homogeneous, in the neuropil; radial, in the perikaria; or concentric, inside neuronal processes. On routine stains (HE, PAS, Alcian blue, LFB, Masson, Crystal violet...) and silver impregnation (Bielschowsky), PGB showed a similar appearance. Some PGB, corresponding to CA, had a stronger alcohol resistance Toluidine Blue metachromasia. Immunohistochemical studies showed that all the PGB were positive to Ubiquitin, Heat Shock Proteins 70 (HSP70) and 25 (HSP25), and 200 KDa Neurofilaments. However, only CA were also labelled against Neuron-Specific Enolase, S-100 protein, Glial Fibrillary Acid Protein, and Tau-protein. Ultrastructurally LB were easily identified in the neuropil and some within the neuronal cytoplasm. They were not membrane-bound and measured from 3 to 7 microns in diameter. Some of them disclosed an electron-dense core but most were homogeneous and formed by intermingled fine and short branching filaments of about 70 to 80 Angstroms in diameter.

These results indicate that, in Canine Lafora disease, LB are mainly located in the cerebellar cortex. In other brain areas there is a mixture of LB and CA. Only concentric cerebellar PGB correspond to LB whereas extra-cerebellar concentric, radial and homogeneous PGB are a mixture of both LB and CA. Our study reveals a different composition of LB compared to CA, indicating their

possible neuronal and glial origin respectively. The cytoskeleton components and HSPs composition of PGB would suggest a cellular sustained stress phenomenon as the responsible for the polyglucosan accumulation.

Session 9A

PRION-INDUCED NEURODEGENERATION - A TALE OF RAFTS AND ANCHORS

Alun Williams, Clive Bate

The Royal Veterinary College, UK

The conversion of the normal cellular PrPC to the disease-associated PrPSc is a key feature in the pathogenesis of Transmissible Spongiform Encephalopathies (TSEs, or prion diseases). Such PrPSc is a major component of the infectious agent (prion) of TSEs and is neurotoxic via a mechanism that has been shown to be dependent on lipid rafts, specific intracellular trafficking pathways and activation of phospholipase A2. Lipid rafts are specialised membrane microdomains in which GPI-anchored proteins, such as PrPC, are localised. In this study, the process by which aggregated PrPSc molecules cause neuronal damage was addressed by incubating neurones partial GPI analogues to compete with PrP-GPI for second messenger signalling systems. These analogues inhibited the neurotoxic effect of defined PrP peptides and reduced PLA2 activation. In contrast, high concentrations of GPI anchors isolated from PrPC, but not from two other GPI-anchored proteins, Thy-1 or Decay Accelerating Factor (CD55), mimicked two of the effects of prions / PrP peptides on neurones in that the PrP-GPI anchors activated PLA2 and promoted caspase-3 activation in neurones. Collectively, these studies support the concept that PrPSc is neurotoxic as a consequence of the self aggregation of PrPSc in lipid rafts and that the resultant concentration of PrP GPI triggers activation of specific cell signalling pathways associated with prion-induced neuronal death.

MORPHOLOGICAL CHARACTERISATION OF EOSINOPHILIC MYOSITIS IN BOVINES IN BELGIUM

*Lieve Vangeel, Koen Chiers, Kurt Houf, Jozef Vercruyse, Richard Ducatelle
Ghent University, Faculty of Veterinary Medicine, Merelbeke, Belgium*

Eosinophilic myositis (EM) is a rare pathological entity that appears worldwide in clinical healthy bovines. It is characterized by multifocal grey-green lesions in striated muscles which results in condemnation if observed in slaughterhouses. The etiology and pathogenesis are unknown, however, it has been suggested that *Sarcocystis* spp. may be involved. The objective of the present study is the morphological characterisation of EM-lesions to obtain better insight in the pathogenesis.

Samples from 79 condemned bovine carcasses were collected. They were stained with H&E, Giemsa and immunolabelled for CD3+ and CD20+ cell phenotypes.

On histology three types of lesions could be differentiated, namely a diffuse inflammation, a multifocal inflammation and an eosinophilic granuloma. In all types eosinophilic granulocytes, lymphocytes, and mast cells were observed. In the eosinophilic granuloma the centre of the lesion was lined with a rim of epitheloid macrophages and there were multinucleated giant cells. These three types of lesions occurred frequently in the same animal. Thick walled sarcocysts (*S. hominis* or *S. hirsuta*) were detected in the centre of lesions from 11 animals (14%) and remnants were found in 6 animals (7,6%). Immunohistochemically all samples were positive for CD3+ and CD20+ cells. The CD3+ cells showed a diffuse distribution in all types of lesions, whereas the CD20+ cells were mostly organised in a more follicular pattern in the granulomas.

In conclusion the presence of three types of lesions in the same animal may indicate different stages of the lesion. If sarcocysts play a role in the pathogenesis, our results suggest that in 14% *S. hirsuta* and/or *S. hominis* are involved. Considering the inflammatory response, the lesions consist of a mixed lymphocytic population, which has a specific distribution in the granulomatous type.

This work was supported by a Ph.D. grant of the Institute for the Promotion of Innovation by Science and Technology in Flanders.

MICROVASCULATURE IN SKELETAL MUSCLES OF GRMD (GOLDEN RETRIEVER MUSCULAR DYSTROPHY) DOGS

Frédérique Nguyen, Marie-Thérèse Masson, Lydie Guigand, Isabelle Goubault-Leroux, Marie-Thérèse Lavault, Roselyne Primaut, Monique Wyers, Yan ChereL

UMR 703 INRA - National Veterinary School of Nantes, France

Introduction. As blood vessels represent a promising route to deliver therapeutic genes or cells to dystrophic muscle, the question arises whether dystrophin deficiency is associated with impaired muscular vascularization, as previously shown in the X-linked muscular dystrophy mouse. The study was performed in the closest animal model of Duchenne Muscular Dystrophy, the GRMD (golden retriever muscular dystrophy) dog.

Objectives. To determine the nature and severity of microvessel anomalies in GRMD muscles, and their correlations with muscle lesions.

Methods. The gracilis and sartorius cranialis muscles were sampled in 1- to 3-month-old GRMD (n=4) and control (n=6) dogs. Automated morphometry was used on von-Willebrand-factor immunostained sections to quantify microvessel density. Manual morphometry was used on semi-thin sections to quantify muscle fibrosis and myofibre size; and on ultra-thin sections to assess the ultrastructure of blood capillaries.

Results. Microvessel density was unaltered in GRMD gracilis muscle (758 ± 198 /mm²) compared to control one (764 ± 54 /mm²). There was capillary hypertrophy (microvessel diameter, $3.9 \mu\text{m}$ vs 3.2 in controls), with a normal endothelium area ($64 \pm 18\%$ vs $70 \pm 17\%$ in controls), and normal lumen area ($29 \pm 18\%$ versus $23 \pm 18\%$ in controls). GRMD microvessels showed thickened basement membrane (81 ± 16 nm versus 65 ± 11 nm in controls). Fibrosis in GRMD dogs ($14 \pm 4\%$ versus $7 \pm 3\%$ in controls) correlated with increased frequency of basement membrane duplication (56% versus 14% in controls), decreased number of myofibres adjacent to a microvessel, and increased distance from microvessel to myofibre (541 ± 551 nm versus 158 ± 168 in controls). Similar results were found in the sartorius cranialis muscle ($P < 0.05$ for all results).

Conclusion. Microvasculature is impaired in GRMD skeletal muscles, as soon as three months of age. The increased capillary-to-myofibre distance correlates with endomysial fibrosis and may impede intravascular therapeutic trials.

BILATERAL JUGULAR PHLEBECTASIA IN A PEDIGREE BELTEX TUP

Henny Martineau , Jill Thomson

Scottish Agricultural College, UK

This is a case report on a two year old pedigree Beltex tup, which was presented for post mortem at the Scottish Agricultural college with a history of progressive respiratory signs and death. Following multiple blood sampling for Maedi Visna accreditation, the farmer reported the appearance of a soft swelling in the middle of the neck, left of midline. Post mortem examination identified this as a focal area of jugular distension, and the lesion was found to be bilateral. Additional observations included marked swelling of the head, mild occlusion of the laryngeal lumen due to proliferative keratinised squamous tissue overlying the arytenoid cartilages and pulmonary oedema and congestion. Histological examination of the jugular veins revealed areas of architectural disorganisation within the tunica media and tunica adventitia.

This tup was diagnosed as having bilateral jugular phlebectasia, a previously unreported disease in sheep. The literature sites reports of a similar condition in children, sometimes following periods of excessive straining. However, there is no mention of life threatening consequences and surgical correction is said to be curative in most instances. The author will end the presentation with a full discussion on possible aetiologies of this disease.

POSTERS

CHRONIC ENZOOTIC OR ATYPICAL PNEUMONIA IN SHEEP*Farhang Sasani, Hemmatzadeh Farhid, Reza Mirzaie**Department of veterinary Pathology, Faculty of Veterinary Medicine, Tehran University- Iran.***Summary**

In an abattoir study on 2000 sheep during 2004 – 2005, just 56 lungs with pathologic findings were obtained. Lungs were examined for histopathologic, bacteriologic, mycoplasmal studies and so for culture and serologic test (FA) for PI-3 virus.

The virological study was done on 53 cases in connected with culture of PI-3 which there were 12 positive CPE and 41 Negative. Also in viral culture of PI-3, five cases were positive that all these cases had positive response to FA test. 20 cases or 35.7% of pneumonic lungs had composed of chronic lesions that many of them revealed interstitial pneumonia or lymphoid interstitial pneumonia that eight cases of them had positive CPE and PI-3 virus was isolated from four cases (50%) .

In pathologic and microbiologic study of cases, there were 30 cases (53.5%) with purulent bronchopneumonia (P.Br) , one case (1.7%) with fibrinous bronchopneumonia (F.Br) five cases (8.9%) with Lymphoid Interstitial Pneumonia (LIP), eight cases (14.2%) combination of LIP and P.Br, seven Cases (12.5%) combination of IP and P.Br. In 6 cases mycoplasma was isolated that in 50% of them IP was observed. In bacteriological cultures, in cranioventral consolidated lungs,

3 cases of E.coli, 2 cases of Mannheimia hemolytica and 2 cases of clostridium were isolated.

In this survey, it was observed that during slaughtering, most pneumonic lungs, showed purulent bronchopneumonia and there were many cases of interstitial pneumonia , These findings revealed the important role of viral specially

PI-3 , mycoplasmal and bacterial agents in producing pulmonary lesions such as IP.

P2

PRELIMINARY STUDY OF PASTEURILLOSIS FROM OVINE AND CAPRINE ABATTOIR LUNG SPECIMENS*A.Ezzi *, S Moradi beedehendi, A. R. Jabbari***Pathology Department of Razi Institute, Karadj, Iran*

Aim of study :Collecting pneumonic tissue from abattoir and isolating Pasteurella spp.

Materials and Methods : 60 ovine pneumonic lung tissue out of 4946 and 8

caprine out of 969 were collected weekly from Ziaran abattoir during fall 2005 to winter 2006 .They were precisely considered at the autopsy hall at the Pathology Department of Razi Institute The shape, consistency and the predilection of lesions to the lobes of the tissue were taken a note in the Research Forms. Samples were divided equally to Bacteriology and Histopathology Department The former was cultured at the blood agar .Then the positive one again culturing at the special culture media and using chemical tests . Isolates were maintained frozen at -70C in a 60:40 phosphate buffered saline (ph.7.2):glycerol . Molecular tool including polymerase chain reaction (PCR) procedure was carried out for confirming of *P.multocida* (Townsend et al 2001) .The latter were fixed in 10% formalin saline .Then the embedded sections were trimmed 5 micron by rotary microtom and stained by H&E,PAS,Gram and Giemsa methods

Results: Totally 10 *Pasteurella* spp were identified from 5915 slaughtered sheep and goat in Ziaran abattoir .*Pasteurella multocida* was isolated from 4 ovine (13.33%) and 1 caprine pneumoniac lung tissue (25%) that had been shown grossly in cranial and middle in 3 and cranial and caudal lobes in 2 consolidated lung tissue. Histopathology sections revealed purulent broncho-pneumonia ,bronchitis and bronchiolitis and also purulent abscessation . In one sample (goat) isolation of *P.caballi* was mixed with *P.dagmatis* . In 4 cases of pneumonic lung tissue *P. caballi* was isolated as well . *P. multocida* samples were confirmed by PCR assay in this study .

Conclusion : *P.multocida* can be a condidate for producing vaccine in prospective studies.

P3

STREPTOBACILLUS MONILIFORMIS (“RAT BITE FEVER”) ENDOCARDITIS IN TWO BARBARY MACAQUES (*MACACA SYLVANUS*).

*Mark F. Stidworthy, Martin L. Whitehead, Peter S. Aylmer, Susan G. Keane,
Henry Malnick*

International Zoo Veterinary Group, UK

Streptobacillus moniliformis is a zoonotic pathogen colonising the oropharyngeal flora of rats. Human infections result from rat contamination of bites (“rat bite fever”), scratches or foodstuffs, leading to pyrexia, rash, polyarthritis and rarely endocarditis. Infection is poorly documented in non-human primates but descriptions of spontaneous disease include one case of endocarditis in a Rhesus macaque (*Macaca mulatta*). Here, naturally occurring disease is described in a pair of sub-adult Barbary macaques (*Macaca sylvanus*) sharing an enclosure at a U.K. zoo with historical rodent problems. Early in January 2006, the female,

which had sustained a cut foot two weeks earlier, developed mild foot swelling and rapidly progressive neurological signs. It died despite enrofloxacin therapy. Necropsy identified vegetative endocarditis of the left atrioventricular valve, fibrinous pericarditis, haemorrhagic interstitial pneumonia and focal haemorrhage in the thalamic cut surfaces of the brain. The male, which appeared clinically normal and received no treatment, was unexpectedly found dead two days later. Necropsy revealed vegetative endocarditis of left and right atrioventricular valves, fibrinous pericarditis, haemorrhagic interstitial pneumonia and a splenic infarct. In both cases, histological examination confirmed vegetative endocarditis with valvular colonisation by gram-negative pleomorphic bacilli. Multifocal necrotising to suppurative myocarditis, haemorrhagic interstitial pneumonia and subacute infarctions in spleen and kidney were present. In addition, the first case had multifocal thromboembolic lesions with ischaemic necrosis in the thalamus and internal capsule white matter of the brain. No bacteria were isolated from this animal. Post mortem heart blood cultures from the second yielded a pure growth of slow growing, biochemically unreactive, facultatively anaerobic gram-negative pleomorphic bacilli. Partial sequencing of 16S ribosomal DNA from subcultures identified the organism as *Streptobacillus moniliformis*. Rat bite fever is rarely described but should be considered a cause of fatal valvular endocarditis and thromboembolic disease in macaques exposed to rodents or their excreta.

P4

EXPRESSION OF CLASS II MAJOR HISTOCOMPATIBILITY COMPLEX MOLECULES IN BOVINE CHRONIC PNEUMONIA ASSOCIATED WITH DIFFERENT BACTERIAL INFECTIONS

Enrico Radaelli, Fausto Antonioli, Mario Luini, Guido Ruggero Loria, Eugenio Scanziani

Igiene e Sanità Pubblica Veterinari, Italy

In normal lung parenchyma type II pneumocytes and dendritic cells are the principal antigen presenting cells (APC) expressing major histocompatibility class II complex (MHCII). Cytokines and antigenic stimuli can evoke overexpression of MHCII by activated monocytes-macrophages and reactive bronchus-associated lymphoid tissue (BALT). Persistent inflammatory conditions are also supposed to trigger aberrant MHCII expressions in airway epithelium and endothelium.

In this study the expression of MHCII in 18 spontaneous cases of bovine chronic bacterial pneumonia was investigated by immunohistochemistry using a mouse monoclonal antibody against α -chain of human leukocyte antigen (HLA)-DR. The bacteria associated with pneumonia were *Mycoplasma bovis* (6 cases),

M. bovis and Pasteurella multocida coinfection (3 cases), Arcanobacterium pyogenes (3 cases), P multocida (3 cases), Mannheimia haemolytica (3 cases). Three lung samples without any pathological changes were introduced as controls.

In all pneumonic lesions type II pneumocytes, intralveolar macrophages, infiltrating histiocytes and lymphoid cells were immunopositive for MHCII. Prominent MHCII expression was observed in BALT cells and airway epithelium mostly during P. multocida and M. haemolytica infection. Strong bronchiolar epithelium MHCII expression also occurred in A. pyogenes-induced pneumonia. M. bovis and A. pyogenes-induced fibrino-necrotizing pneumonia were characterized by consistent endothelial MHCII signal. Necrotic foci associated with A. pyogenes and M. bovis-induced pneumonia did not display any peculiar association with MHCII immunoreactivity.

Our results strongly suggest that the immune mechanisms participating in the pathogenesis of chronic bacterial pneumonic processes involve both MHCII overexpression by classic APC and MHCII aberrant expression by non professional-APC such as bronchiolar epithelium and endothelial cells. The aberrant expression of MHCII in respiratory epithelium and the overexpression of MHCII in BALT cells indicate a consistent enhancement of MHCII signal pathway during follicular peribronchiolitis. The strong endothelial MHCII immunoreactivity observed in fibrino-necrotizing pneumonia could be associated with the severe vascular changes occurring in such lesion.

P5

SIMULTANEOUS CANINE DISTEMPER VIRUS, CANINE ADENOVIRUS TYPE-2 AND MYCOPLASMA CYNOS INFECTION IN A PUPPY WITH PNEUMONIA

Sonja Chvala (1), Florian Zeugswetter (2), Viviane Benetka (3), Karin Möstl (3), Joachim Spargser (4), Herbert Weissenböck (1)

(1)Department of Pathobiology, Institute of Pathology and Forensic Veterinary Medicine, (2)Clinical Department of Small Animals and Horses, Clinic for Internal Medicine and Infectious Diseases, (3)Clinical Department of Diagnostic Imaging, Infectious Diseases and Clinical Pathology, Clinical Virology, Zoonoses and Emerging Infections Group, and (4)Department of Pathobiology, Institute of Bacteriology, Mycology and Hygiene, University of Veterinary Medicine, A-1210 Vienna, Austria.

The case of a 5-month-old, female pinscher with pneumonia is reported. The puppy was bought in a pet shop in France and brought in to Austria. She developed signs of oculonasal discharge and coughing after one week. In spite of intensive treatment over a period of two weeks the dog's condition deteriorated to pumping breathing with croaking lung sound, fever, severe lymphopenia and weight loss. Because of the severe clinical signs the dog was euthanatized. At necropsy, the

lung lobes presented compact, dark red and edematous with slight serous effusion into the pleural sac. Histologically, severe multifocal fibrinous-necrotizing pneumonia with accompanying exsudation of neutrophils and macrophages, and large, amphophilic, intranuclear inclusion bodies predominantly in alveolar and bronchial epithelial cells and alveolar macrophages were present. Additionally, acidophilic inclusion bodies in the cytoplasm of alveolar epithelial cells and in the epithelial cells of the stomach were found. Other tissues, including brain, had no apparent pathological lesions.

Immunohistochemically, canine distemper virus (CDV) – antigen was present in high amounts in lung, cerebellum and stomach. In situ hybridization for detection of canine adenovirus (CAV) nucleic acid gave positive signals in alveolar cells and macrophages of the lung, no signal was found in the liver. Virological examination of lung samples by PCR and sequencing of the PCR amplification product (626 bp) revealed the presence of CAV type 2 (100% identical to reference strain Toronto A26/61). Bacteriological examination of the lung yielded large amounts of *Mycoplasma cynos*. *Mycoplasma cynos* infection was confirmed by immunohistochemistry with detection of abundant positive signals in the lung tissue.

CDV and CAV-2 are common causes for pneumonia in dogs. Sometimes both infections can occur simultaneously. While *Mycoplasma cynos* is frequently considered an opportunistic agent, recent work identified its potency to cause pneumonia in young dogs. The present case is the first description of a triple infection with the above mentioned pathogens, each of which might have facilitated the invasion by the others.

P6

THE ADDED VALUE OF CARBON MONOXIDE UPTAKE CAPACITY IN ASSESSING SEVERITY OF EXPERIMENTAL RESPIRATORY DISEASE IN MOUSE

Adélie Habyarimana, Daniel Desmecht

University of Liège, Belgium

The laboratory mouse offers many practical advantages for in vivo research as a broad range of genetically characterized stocks are available, maintenance is easy, costs are low and a large array of mouse-adapted reagents is available. For the follow-up of experimental respiratory diseases, single- and double-chamber plethysmography is available, which permits daily noninvasive monitoring of respiratory function. A specific equipment was developed by the industry to measure the carbon monoxide uptake of mice, which is supposed to give a global quantitation of the gaz exchange capacity of the whole respiratory system. Here, we have implemented this equipment, along

with double chamber plethysmography, in a model of experimental influenza disease. Plethysmographic and carbon monoxide values were collected along with, body weight, histopathology and lung viral titers. The added value of CO uptake measurements was established by comparison with the results yielded by other approaches. It mainly consisted in its ability to predict deep pulmonary involvement in the early course of the experimental disease. It also proves to be the technique of choice whenever the severity of the experimental disease has to be compared between different cohorts of mice.

P7

PATHOMORPHOLOGICAL AND IMMUNOHISTOCHEMICAL FINDINGS OF CARDIAC AMYLOIDOSIS IN THE GERIATRIC DOG

T Stassen , R. P. Linke , W Hermanns

Ludwig-Maximilians-University, Germany

Amyloid deposition is common in geriatric dogs and involve, among other organs, the heart. In contrast to the beta-precursor protein-derived cerebral amyloidosis, the type of cardiac amyloid occurring in the wall of myocardial blood vessels is still unknown.

During the last four years 287 hearts of aged dogs were collected for detailed investigations. The necropsy findings included degeneration of the mitral valve and left-ventricular hypertrophy in about half of the cases. However, no immediate signs for a vascular disease were found macroscopically. Tissue samples of three defined locations of each heart were taken for histological evaluation. The presence of amyloid was verified in Congo red-stained sections by the characteristic green birefringence upon polarisation. Amyloid deposits are located predominantly in intima and media of small and medium-sized intramural blood vessels. Altogether, 21 dogs showed cardiac amyloidosis. Dogs over ten years of age and of large breeds, as the German shepherd and Rottweiler, were affected mostly.

Further immunohistochemical investigations were performed to identify the precursor protein of the cardiac vascular amyloid.

P8

EXPRESSION OF INDUCIBLE NITRIC OXIDE AND NITROTYROSINE IN LUNGS OF CALVES WITH SEVERE NECROTISING BRONCHOPNEUMONIA AFTER EXPERIMENTAL MYCOPLASMA BOVIS INFECTION

Kathrin Hermeyer, Björn Jacobsen, Joachim Spargser, Renate Rosengarten, Marion Hewicker-Trautwein

University of Veterinary Medicine, Germany

Mycoplasma (M.) bovis is one of the most pathogenic bovine mycoplasmas and can cause a variety of diseases, e.g. pneumonia, arthritis and mastitis. The most severe type of pulmonary lesions described in calves with natural or experimental *M. bovis* infection is necrotising bronchopneumonia. In these cases, focal areas of necrosis are demarcated by numerous neutrophils, macrophages and fibrotic tissue. The mechanisms by which *M. bovis* causes necrotising lung lesions are not understood. Inducible nitric oxide synthase (iNOS) is an isoenzyme expressed in vitro by macrophages upon activation by bacterial constituents and/or cytokines. Once expressed, iNOS allows large quantities of nitric oxide (NO) to be synthesised. NO produced by iNOS-expressing macrophages is an essential mediator of killing microorganisms and is also known to contribute to tissue damage. The purpose of this study was to investigate the expression of iNOS and its product nitrotyrosine (NT) in necrotising lung lesions of calves with chronic experimental *M. bovis* infection. Paraffin sections of formalin-fixed lung samples with necrotic lesions from 11 calves originating from different *M. bovis* infection experiments were examined by applying the ABC method and antibodies to iNOS, NT and macrophages (CD68, S100A8, S100A9). Sections from lungs of non-infected calves served as control tissues. *M. bovis* DNA was detected by in situ hybridization (ISH) with digoxigenin-labelled probes with specificity for *M. bovis* variable surface lipoprotein A (VspA). In infected calves, ISH revealed strong and abundant hybridization signals within necrotic lung areas. Presence of *M. bovis* DNA was associated with marked expression of iNOS and NT being largely restricted to macrophages surrounding the necrotic lung foci. Our findings suggest that iNOS and its products synthesized by activated macrophages may play a role in the pathogenesis of necrotic lung lesions and may also be involved in the persistence of *M. bovis* in these necrotic areas.

P9

IDENTIFICATION OF MYCOBACTERIA IN FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUE: STILL A DIAGNOSTIC DILEMMA!

Anja Kipar, Claire Dixon, Gareth W. Thomas, Udo Hetzel, Richard Birtles
University of Liverpool, UK

The literature provides variable information on the molecular identification of mycobacteria in routinely formalin-fixed and paraffin-embedded (FFPE) tissue samples. Some reports suggest that PCR is more sensitive than the Ziehl-Neelsen (ZN) stain, but this is not consistently so. Also, species identification of the mycobacterium is often not attempted.

We aimed to develop a PCR system that can reliably amplify mycobacterial DNA, suitable for sequencing to allow (sub)species identification in diagnostic FFPE tissue samples. Several PCR systems to amplify regions of 111-660 bp length in

the 65kd heat shock protein gene (hsp65) with BCG-DNA as positive control, as well as a PCR system to identify *M. avium* were established. DNA extracted from FPPA tissue sections originating from cases where culture had identified *Mycobacterium (M.) bovis*, *M. avium* sp. paratuberculosis or *M. microti*, and from eight diagnostic pluri- and paucibacillary samples of (non)tuberculous lesions where the diagnosis had relied on the ZN stain, was amplified.

Amplification products could be visualised from some samples with a (nested) PCR for a 477bp fragment. Sequences allowing for mycobacterial species identification were obtained from the pluribacillary tuberculous lesions of a *M. microti*-infected vole and a focal severe granulomatous steatitis (panniculitis) with myriads of intracellular acid-fast bacilli in a cat, which represents the first definite case of feline *M. malmoeense* infection. *M. malmoeense* is a slow-growing mycobacterium found infrequently in the environment which is pathogenic in humans (respiratory disease, lymphadenitis, arthritis) but has also been found in cattle.

All other samples failed to provide definite results, either due to insufficient DNA extraction (n=1), too low yield of amplification product to allow sequencing (n=4) or due to *Propionibacterium acnes* contamination (n=3), particularly of the dermatitis cases.

Results confirm that mycobacteria-induced lesions still represent a diagnostic dilemma, when culture is not possible, as species identification becomes increasingly important.

P10

CHRONIC RESPIRATORY MYCOPLASMA BOVIS INFECTION IN CALVES INDUCES INFLUX OF DENDRITIC CELLS INTO AIRWAY MUCOSA AND STIMULATES PROLIFERATION OF BRONCHUS-ASSOCIATED LYMPHOID TISSUE

Anne Thomasmeyer¹, Joachim Spergser², Renate Rosengarten², Marion Hewicker-Trautwein¹

¹*Department of Pathology, University of Veterinary Medicine, Hannover, Germany*

²*Department of Bacteriology, Mycology and Animal Hygiene, University of Veterinary Sciences, Vienna, Austria*

Mycoplasma (M.) bovis is an important cause of severe pneumonic lung lesions in calves. Studies in lungs of different mammalian species have demonstrated that a dense network of dendritic cells (DCs) specialised for antigen presentation and activation of T lymphocytes exists and that these cells participate in the immunoregulation and pathogenesis of acute and chronic infectious airway diseases. So far, data about the occurrence of DCs in *M. bovis* induced pneumonia in cattle are not available. The aim of this study was to examine the distribution

and numbers of DCs and the proliferation of bronchus-associated lymphoid tissue (BALT) in lungs of *M. bovis* infected calves. Ten calves were immunised intramuscularly (i.m.) with recombinant *M. bovis* variable surface lipoprotein A (VspA) and challenged intratracheally with *M. bovis* strain 1067. Four calves were given a placebo i.m. and were inoculated intratracheally with *M. bovis* strain 1067. Four non-inoculated calves served as controls. Paraffin sections of lung samples were stained immunohistochemically by using the ABC method and antibodies to MHC class II and *M. bovis* Vsp antigens. Numbers of MHC class II+ DCs were determined by counting them in 5 randomly selected high power fields in the mucosa of bronchi and bronchioli. At necropsy, all infected animals had pneumonic lesions and *M. bovis* was detected by PCR in samples from the respiratory tract in 13/14 infected calves. Statistical analysis of dendritic cell counts revealed that in all inoculated animals, in comparison to control calves, significantly increased numbers of MHC class II+ DCs were present. Furthermore, a marked increase of BALT was noted in infected animals. In conclusion, the results of this study indicate that persistent pulmonary infection of calves with *M. bovis* provides a potent immunological stimulus inducing marked proliferation of BALT and influx of MHC class II-expressing DCs into the airway mucosa.

P11

GASTRIC DILATATION-VOLVULUS (GDV) ASSOCIATED WITH CHRONIC PANCREATITIS IN A GUINEA PIG

Sarina Shibly, Frank Künzel

Institute of Pathology, University of Veterinary Medicine, Austria

Guinea pigs are rarely affected by GDV. To the authors' knowledge, this is the first report of GDV associated with chronic pancreatitis in a guinea pig.

A 4-year-old male short haired guinea pig was presented with a one day history of anorexia, obstipation and listlessness. Clinical examination revealed painful abdominal palpation, apathy and slightly anaemic mucous membranes.

Urine analysis was performed and showed keton-, protein- und haematuria. On the basis of these results the working diagnosis was cystitis and ketoacidosis, and therapy consisted of antibiotics (enrofloxacin, Baytril®) and forceful feeding. A few hours later the guinea pig was found dead in its cage and taken to the institute of pathology for dissection.

Macroscopic evaluation revealed an extremely dilated stomach filled with gas and fluid contorted clockwise at 180 degrees. A hyperaemic small intestinal loop was wound tightly around the caudal part of the esophagus and the duodenum causing mechanical obstruction. Furthermore, multiple miliary renal cysts and small amounts of urinary gravel in the otherwise unaltered bladder

were found.

For the histologic examination tissues were fixed in formalin, embedded in paraffin wax, sectioned and stained with H & E. Besides moderate fatty degeneration of liver and kidneys multifocal inflammatory infiltrates consisting mostly of small lymphocytes could be demonstrated in immediate proximity to the pancreas suggesting chronic pancreatitis.

We conclude that although guinea pigs rarely suffer from GDV, this disease should be taken into account if a sudden onset of gastrointestinal symptoms is reported. As chronic pancreatitis is known to alter digestive functions, it may have been involved in the development of GDV in this patient. The ketonuria noticed could be linked to a possible diabetic disease, although due to a lack of diagnostic data this assumption remains highly speculative.

P12

EXPRESSION OF TFF2 IN CARCINOGENESIS OF CANINE GASTRIC MUCOSA

Simonetta Appino, Antonella Vercelli, Daniele Gribaldo, Maria Teresa Capucchio, Alessandra Sereno, Federico Valenza

Università degli studi di Sassari, Italy

Trefoil factor 2 (TFF2), a spasmodic polypeptide (SP), is one of the three known mammalian trefoil peptides. Trefoil peptides are small proteins secreted by the gastrointestinal mucosa. TFF2 is expressed and secreted preferentially by gastric mucous and regulated in different pathological conditions of gastrointestinal tract, which involve regeneration and restitution of epithelial cell, mucosal protection and healing of ulcer. In this study the expression of TFF2 was immunohistochemically analyzed in paraffin-embedded samples from 25 dogs with either endoscopic biopsy or subtotal gastrectomy specimens of gastric mucosal lesions, including 5 cases of chronic superficial gastritis (CSG), 1 case of chronic hyperplastic gastritis (CHG), 1 acute necrotizing gastritis (ANG), 4 gastric carcinoma (GCA) and 9 helicobacter + (H+) samples with no significant lesion and 3 non pathological samples (NP). TFF2 was located in the cytoplasm of gastric mucous neck cell. The expression of TFF2 was positive (+) in all NP; TFF2 positive (++) cell density in H+ was higher than in H- infection and showed an higher positivity (+++) in CGS. Analyzed carcinomas showed a different TFF2 expression. Increase of TFF2 expression in CSG may be associated with the protective mechanism after gastric mucosal injury. Different degree of TFF2 expression in GCA is probably due to different stages of differentiation of GCA and related to the decrease in the number of gastric gland cells expressing TFF2. The effect of Helicobacter on the expression of

TFF2 may depend on the status of gastric mucosa. Further investigation will allow to better evaluate the promising possibility to apply TFF2 as a prognostic marker in gastric carcinogenesis.

P13

HISTOLOGICAL ASPECTS IN TCR-HA INS-HA DOUBLE TRANSGENIC MICE TREATED WITH POTATO BUDS LECTIN

Emilia Ciobotaru, Manuella Militaru, Aneta Pop, Dorel Lucian Radu, Georgeta Dinescu, Teodoru Soare

Faculty of Veterinary Medicine Bucharest, Romania

TCR-HA Ins-HA double transgenic mice, with hyperglycemia and hypoinsulinemia were treated daily and orally with 100 µg/animal potato buds lectin (non-genetic modified potatoes by affinity chromatography on chitin) for 10 days. After the first administration and every 2 days of the experiment the mice glycemia was measured. Mice euthanasia was performed 12 days after the first administration of lectin. Pancreas, liver and kidney were harvested for histological investigation. Histological section were Masson trichromic stained.

All animals presented an important decrease of glycemia (68.4% from initial value).

The lesions of pancreas were typical for diabetes mellitus type 1. Histological features were gradual, beginning with small lymphocyte infiltration around the pancreatic islets and finishing with massive mononuclear infiltrates within the islets. The cells of islets presented nuclear picnosis and vacuolar cytoplasm, being isolated in small groups in a large, diffuse lymphocyte population. There were no morphological differences between control and treated animals.

Diffuse vacuolar hepatitis and fat liver were diagnosed in control group. Treated animals presented same focal and discrete lesions of liver in a reversible stage or normal histological aspect.

Renal injuries are represented by incipient diabetic glomerulosclerosis (focal or diffuse fibrosis of basement membrane and mesangium), interstitial arteries and tubes being normal. There are no significant differences between control and treated animals.

The findings of this experiment proved an important decrease of glycemia and protective effect of potato buds lectin on liver morphology in TCR-HA Ins-HA double transgenic treated mice.

USEFULNESS OF A PCR METHOD FOR THE DETECTION OF MYCOBACTERIUM AVIUM SUBSP PARATUBERCULOSIS IN PARAFFIN-EMBEDDED TISSUE SAMPLES.

Valentín Pérez, Oscar Moreno, Marta Fernández, Carlos García-Pariente, María Muñoz, M. Carmen Ferreras, J. Francisco García-Marín.

Universidad de León, Spain

Paratuberculosis, an infectious disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map), is characterized by a granulomatous enteritis and lymphadenitis. Animals showing initial or latent infections have focal granulomas located in the intestinal Peyer's patches or lymph nodes. Ziehl-Neelsen (ZN) or immunohistochemical techniques applied to these samples for the demonstration of Map are usually negative. This work evaluates the efficacy of a PCR technique for the detection of Map nucleic acids in paraffin-embedded ileocecal lymph node tissue samples from 101 slaughtered calves. Samples were fixed in 10% buffered formalin and embedded in paraffin wax in a period shorter than 7 days. A PCR technique using primers amplifying a 217 bp DNA fragment was employed in two, 7 μ -thick sections from each sample. Lesions found were classified as diffuse, characterized by a severe granulomatous lymphadenitis that appeared in one case; multifocal, in 5 samples, formed by 10-15 well-defined granulomas negative or scarcely positive to ZN; focal, seen in 24 animals, composed of small granulomas showing Langhan's giant cells and macrophages; doubtful lesions, formed by 20-50 macrophages containing brown pigment in 41 cases; unspecific, composed of groups of 3-5 macrophages filled with pigment, in 15 samples, and 9 calves with no lesions. The latter types were always negative to ZN. Nine animals (37.5%) of those with focal lesions, 16 (39%) with doubtful forms and 100% of those showing multifocal or diffuse lesions were positive by PCR. All animals with unspecific or without lesions were negative. PCR method employed has demonstrated to be useful and efficient in the demonstration of the presence of the aetiologic agent in focal granulomatous lesions, suggestive to be caused by Map but negative to ZN and immunohistochemical methods.

P15

ROLE OF MACROPHAGES AND CYTOKINES IN LYMPHOCYTES APOPTOSIS IN BOVINE VIRAL DIARRHOEA

PJ Sánchez-Cordón, M Pedrera, JL Romero-Trejejo, MJ Bautista, JC Gómez-Villamandos

Department of Comparative Pathology, Veterinary Faculty, University of Córdoba, Spain

The purpose of this work was to explore the spatial and temporal relationship between lymphoid depletion, quantitative changes of macrophages and release of cytokines by these cells in the ileum of colostrum deprived calves inoculated with a noncytopathic bovine viral diarrhoea (BVD) virus strain.

10 Ten colostrum-deprived Friesian calves of 6-8 weeks old were used. Two animals were used as uninfected controls, while the other eight calves each received an intranasal inoculation of noncytopathic BVD virus genotype-1 strain 7443 and slaughtered in groups of 2 animals at 3, 6, 9 and 14 post-inoculation days (pid). Samples of ileum were fixed in 10% buffered formalin, Bouin's solution and 2.5%glutaraldehyde, and routinely processed for structural, immunohistochemical and ultrastructural studies. The avidin-biotin peroxidase method in combination with different antigen unmasking techniques was used for immunolabeling macrophages (MAC387) and cells expressing cytokines (TNFalpha, IL-1alpha and IL-6). Positive cells were counted and tested for significance ($P < 0.05$) by Mann-Whitney's U-test.

Myeloid cells, mainly monocytes-macrophages and some neutrophils were immunolabelled against MAC387 antibody in both control and infected animals. A significant increase of macrophages population in follicles, interfollicular areas, lamina propria and epithelium of ileum were evident from 6 pid, peaking at 9 pid inside the lymphoid follicles; the number of positive cells in the ileum compartments subsequently decreased slightly. In addition to these quantitative changes, the macrophages population, displayed, from 3 dpi, ultrastructural changes indicative of phagocyte and, occasionally, secretory activation; phagocytized cell debris (mainly apoptotic bodies), an increase in cell size and an increase in lysosomes, characteristics of phagocyte activation, were observed. From 3 pid onwards, a significant increase in the number of cells, mainly macrophages expressing TNFalpha and IL-1alpha was observed in the lamina propria of ileum. However, the presence of cells releasing these chemical mediators in follicles and interfollicular areas was scarce, peaking at 9 pid. The presence of cells marked against IL-6 in the ileum was not significant.

The massive lymphocytes apoptosis induce the increase of cell debris and the appearance of macrophages with changes indicative of phagocytic activation. However, the presence of a low number of macrophages releasing cytokines in the follicles and interfollicular areas of ileum, ruled out the hypothesis that apoptosis could be associated with the quantitative and qualitative changes observed in macrophages.

PRESSURE-RELATED ABDOMINAL CHANGES IN PIGS WITH ‘WHEY BLOAT’ - A CASE REPORT.

Jill Thomson, Walker Miller, Nigel Wolfenden, Dorothy Thomson
Scottish Agricultural College, UK

Introduction

‘Whey bloat’ (intestinal haemorrhage syndrome or ‘bloody gut’) is a cause of sudden deaths in whey-fed pigs. The pathogenesis is uncertain but theories include effects of fermentation, intestinal volvulus, acute allergy to milk proteins, and infection with enteropathogenic or enterotoxigenic agents including *Lawsonia intracellularis*. High mortalities on two whey-feeding units enabled further investigations into the pathogenesis of ‘whey bloat’.

Materials and Methods.

Post mortem examinations and microbiology were carried out on 26 cases. Intra-gastric pressure measurement were made shortly after death on six pigs affected with whey bloat and on six pigs that had died from unrelated causes.

Results

All pigs with ‘whey bloat’ had distended, intensely congested and haemorrhagic intestines containing bloody-stained watery fluid. Other intra-abdominal organs were generally pale. The lungs showed congestion, oedema and collapse. No enteropathogenic agents or Clostridial alpha, beta or epsilon toxins were detected in any cases. Histopathology showed severe congestion of all layers of the intestinal wall with widespread haemorrhage, but no enteritis, proliferative enteropathy, intestinal necrosis or invasion of Clostridial-type organisms. All veins were markedly distended and blood-filled. Intra-gastric pressure measurements in 6 affected pigs ranged from 36 - 48 mmHg; those in unaffected pigs ranged from 2 - 6 mmHg.

Discussion.

The intra-gastric pressure values in ‘whey bloat’ cases were high, consistent with pig model fatalities during simulated human ‘abdominal compartment syndrome’ experiments. There, pressures in excess of 30 mmHg were fatal for pigs if sustained for approximately 30 minutes. Such pressure obstructs venous return via the mesenteric veins and/or the caudal vena cava resulting in massive intestinal blood pooling, major organ failure, shock and death. These findings explain the intestinal changes in the absence of volvulus. Prevention of ‘whey bloat’ involves restriction of whey intake to no more than 20% of the total ration on a dry matter basis and feeding the whey plus dry feed as a complete ration.

THE ROLE OF PATHOLOGICAL INVESTIGATION IN THE COMPLEX DIAGNOSIS OF LIVER LESIONS IN SLAUGHTERED LAMBS

Manuella Militaru, Emilia Ciobotaru, Dumitru Militaru, Beatrice Stirbu, Carmen Petcu, Georgeta Dinescu, Teodoru Soare
Faculty of Veterinary Medicine Bucharest, Romania

The aims of this research are to estimate the importance of cytological investigation in establishing the etiology of the hepatic lesions in abattoir slaughtered lambs and the morphology of these lesions according to the importance of foodstuff biosecurity.

Animals: 50 abattoir slaughtered lambs, clinically healthy, examined in the abattoir.

Methods: gross investigation of the condemned liver; cytological investigation (May Grünwald Giemsa stain); histological investigation (Masson trichromic stain on fragments fixed in 10% solution of formaldehyde and paraffin embedded); serological investigation for IgG antibodies anti-Toxoplasma gondii (ELISA, indirect method).

Gross lesions as hepatitis associated with unspecific vascular injuries, necrotizing hepatitis (foci with 2 - 20 mm diameter), different stages of parasitic traumatic hepatitis (produced by migratory larvae), cystic parasitic granulomas of hepatic capsule, focal acute or chronic perihepatitis determined condemnation of the liver. Hepatoperitoneal cisticercosis (*Cysticercus tenuicollis*) was diagnosed and visceral necrobacillosis was suspected.

Cytologically, different pathological patterns were identified according to the type of diagnosed lesion. A dominant blood cell population, macrophages and few *T. gondii* trophozoites were correlated with miliary necrotizing hepatitis. In necrotizing hepatitis with 5-20 mm diameter foci, degenerated hepatocytes and a granuloma cell population (macrophages, epithelioid cells, multinucleated giant cells) were cytologically identified. Traumatic hepatitis induced by larvae migration was correlated with the abundance of eosinophils (recent lesions) or with a dominant population including lymphocytes, macrophages and fibroblasts (old lesions). Histological investigation confirmed parasitic etiology of hepatic injuries, improving the data about tissue reactivity against parasites.

Serology proved that 62% of investigated cases had positive reaction to IgG antibodies anti-*T. gondii*.

In conclusion, hepatic lesions in abattoir slaughtered lambs had parasitic etiology. Cytological exam correlated with gross morphology is helpful in diagnosis of liver injuries and in suspicion/establishing of etiologic diagnosis. Infection with *T. gondii* was diagnosed in lambs with ELISA (indirect method) and cytology (liver).

BALANTIDIASIS ASSOCIATED WITH PMWS

Oscar Quesada, Francisco Rodríguez, Antonio Fernández, Antonio Espinosa de los Monteros, Marisa Andrada

University of Las Palmas de Gran Canaria, Spain

In a 130 breeding sows pig farm of Gran Canaria (Spain), an increase in the mortality rate occurred in 8-week-old piglets. Clinical signs were growth retardation, wasting, respiratory distress and, in some cases, diarrhoea. One piglet was submitted to the Veterinary School where it was euthanized and routinely necropsy performed. Tissue samples were taken for histological and immunohistochemical studies. Histopathological lesions were characterized by severe lymphocyte depletion and histiocytic proliferation in lymph nodes, tonsils, Peyer's patches and spleen. Scattered multinucleated giant cells were also seen in tonsils and lymph nodes. The intestine showed a diffuse epithelial necrosis with abundant lymphocytes, macrophages and eosinophils infiltrating the lamina propria. Numerous oval parasitic structures with a single large nucleus and covered with a row of short cilia, identified as *Balantidium* spp., were located at the surface of the villi, within crypts and invading the intestinal mucosa, submucosa and lymph vessels. Immunohistochemically, lymphoid tissues and mononuclear cells of the intestinal lamina propria were positive to porcine circovirus 2 (PCV-2). PMWS affected animals have an increased susceptibility to secondary infections as the result of an immunosuppression status. The probable association between PCV-2 and *Balantidium* spp. infection is discussed.

P19

HISTOPATHOLOGICAL EFFECT OF CIPROFLOXACIN ON FETAL LIVER & KIDNEY DEVELOPMENT IN RAT.

Arash Khaki¹, Nahedeh Nilforoshan², A Afhin Khaki³, Ali Rezaie¹

¹Department of Veterinary Pathology, Islamic Azad university, Tabriz/Iran,

²Dept of Biotechnology University of Portland (PCC), Portland/USA.

³Department of Anatomical Sciences, Medicine College Tabriz/Iran.

Introduction: Ciprofloxacin is an antibiotic from fluoroquinolone with a wide range and widely used in various infection diseases. Since there is little information about ciprofloxacin side effect on kidney and liver of rat fetal, this preliminary study was planned. The aim of present study was to determine the histopathological effects of ciprofloxacin on kidney and liver of rat fetal.

Material & methods: Thirty Wistar rats were selected and randomly divided into two groups; control (n=15) and test (n=15). The test group has been received

14mg/kg (PO) ciprofloxacin daily during pregnancy. However, the control group just received plate. After delivery, liver tissues of neonatal in both groups were taken and prepared for light microscopy; staining method was H&E.

Resulted: Microscopic study of liver tissue slices in test group showed hepatocysts were vesiculated & degenerated & vacuolated in compared to control group. Weight of fetal in test groups were reduced in compared to control group ($P<0.05$).

Conclusion: Since Ciprofloxacin had side effect on liver of rat fetal. It is not suggested that Ciprofloxacin has been used during pregnancy in human.

Key words: Ciprofloxacin, fetal kidney and liver development, histopathological effects, Rat.

P20

FIBROUS EPULIS (GINGIVAL HYPERPLASIA) IN A HORSE

Enrico Vezzali, Fabio Rodolfi, Giuseppe Sarli, Debora Stefanile, Cinzia Benazzi

University of Bologna, Italy

Epulis is the generic and clinical term for tumor-like masses on the gingiva, including hyperplastic as well as neoplastic lesions. Fibrous epulis is uncommon in horses, and its rarity has induced us to write this case report.

A 18-year-old Italian saddle horse gelding was referred for examination and surgical treatment of an intraoral pedunculated mass of 5 cm in diameter, located in the mucosa in the vestibular portion of the incisor bone of the upper jaw. The radiographic examination excluded any bone involvement. Six months after surgical removal, there was no recurrency development.

The mass was firm, smooth and grey-pink in colour and connected to the gingiva with a peduncle of fibrous tissue. Histologically, an intact multilayered squamous epithelium bordered well vascularized fibrous connective tissue with only a few foci of inflammatory cells. Squamous epithelium formed long fronds originatig from the surface, which assumed a net shape if trasversally sectioned. The mass showed moderately dense cellularity represented by small stellate to spindle-shaped fibroblasts, regularly disposed in a dense fibrillar collagen background. No deposition of either collagen or bone matrix was seen. Some small blood vessels were spread in the stroma. The above reported inflammatory foci showed typical features of chronic inflammation, with lymphoid cells but no granulocytes.

The discrete mass here described can be defined as localized gingival hyperplasia or fibrous epulis for its features, i.e. mature fibrous tissue accompanied by chronic-active inflammatory reaction.

CORRELATING NECROTIZING ENTERITIS TO TOXIGENIC CLOSTRIDIUM PERFRINGENS IN THE INTESTINE OF NEWBORN PIGLETS

Horst Posthaus, Marco Jaeggi, Chiara Brachelente, Nicole Wollschlaeger, Marianne Wyder, Sarah Albini, Edy Viley and Carlos Abril

University of Bern, Switzerland

Clostridium perfringens is an important pathogen and the cause of different clinical diseases in a variety of hosts. Different strains are known to secrete a large array of potential virulence factors, however which of these are responsible for disease development is unresolved for many of the observed clinical syndromes. *Clostridium perfringens* Type C strains are the causative agents of a fatal necrotizing enteritis in neonatal piglets. The main virulence factor responsible for lesion development is thought to be the beta-toxin, which is secreted by beta toxigenic *C. perfringens* Type C. In 1997 an additional exotoxin, called beta-2 toxin, was identified. Subsequent studies demonstrated that it is an important virulence factor in clostridial enteric diseases in humans and several animal species. Epidemiological evidence indicates that the beta-2 toxin also plays a key role in the development of enteric disease in newborn piglets. However it is currently unknown whether secretion of this toxin occurs in the intestinal tract of piglets and whether this correlates to the development of necrotizing lesions in the intestinal mucosa. Our project is designed to i) evaluate the prevalence of beta- and beta-2 toxigenic *C. perfringens* strains in newborn piglets, ii) investigate the expression of both toxins in the intestine of diseased and non-diseased piglets and iii) correlate this to the presence of pathological lesions and disease outbreaks. Our approach is to perform systematic clinical, bacteriological, pathological, immunohistochemical, and molecular biological investigations on diseased and non diseased animals from selected pig herds in Switzerland. The goal is to define those clostridial exotoxins, which are secreted in the intestinal tract and are associated with pathologic lesions. This is important for the understanding of the basis of clostridial virulence and the improvement of preventive strategies.

P22

LYMPHOCYTES APOPTOSIS ON GUT-ASSOCIATED LYMPHOID TISSUE OF CALVES EXPERIMENTALLY INOCULATED WITH A NONCYTOPATHIC STRAIN OF BOVINE VIRAL DIARRHOEA VIRUS

M Pedrera, PJ Sánchez-Cordón, JL Romero-Trejejo, MA Risalde, JC Gómez-Villamandos

University of Córdoba, Spain

The aim of this work was to identify lesions on gut-associated lymphoid tissue (GALT), to ascertain the nature and distribution of infected cells as well as the distribution and rates of apoptotic cells, in colostrum deprived calves inoculated with a noncytopathic bovine viral diarrhoea (BVD) virus strain.

10 Ten colostrum-deprived Friesian calves of 6-8 weeks old were used. Two animals were used as uninfected controls, while the other eight calves each received an intranasal inoculation of noncytopathic BVD virus genotype-1 strain 7443 and slaughtered in groups of 2 animals at 3, 6, 9 and 14 post-inoculation days (pid). Samples of ileum were fixed in 10% buffered formalin, Bouin's solution and 2.5 % glutaraldehyde and routinely processed for structural, immunohistochemical and ultrastructural studies. The avidin-biotin peroxidase complex method was used to immunolabel infected cells using the monoclonal antibody (MoAb) 15C5. TUNEL and cleaved caspase-3 were used to study apoptotic cells. Positive-cells were counted and tested for significance ($P < 0.05$) by Mann-Whitney's U-test.

Histopathologic study displayed a progressive lymphoid depletion, mainly in lymphoid follicles of Peyer's patches, from 3 pid onwards, together with pyknosis and karyorrhexis characteristic of apoptosis. In addition, a cellular infiltrate of large mononuclear cells, resembling macrophages, became more prominent over time. Some of these cells with abundant cytoplasm containing phagocytized cells debris (tingible bodies). Ultrastructural examination exhibited changes consistent with lymphocyte apoptosis as well as phagocyte and secretory activation of macrophages. Viral infection was confirmed by immunohistochemical methods and ultrastructural examination from 3 pid. Monocytes-macrophages and lymphocytes, mainly located in lymphoid follicles of Peyer's patches, were recognized as the main host cells increasing in number until 9 pid. From 3 pid, TUNEL and cleaved caspase-3 positive-cells showed a significant increase, mainly in lymphoid follicles of Peyer's patches, peaking at 6 pid. These cells included pyknotic lymphocytes and macrophages.

The results obtained here would suggest that direct action of BVD virus on lymphocytes plays a minor role in the massive lymphoid depletion. The quantitative and qualitative changes observed in macrophages, supporter the hypothesis that indirect mechanisms may induce apoptosis.

EXPRESSION OF KIT RECEPTOR IN FELINE CUTANEOUS MAST CELL TUMORS

C Rodriguez-Carino, D Fondevila, J Segales, R Rabanal

Universitat Autònoma de Barcelona, Spain

KIT is a transmembrane protein, also called CD117 or stem cell factor receptor. The ligand of KIT, stem cell factor, is a cytokine that stimulates mast cell growth and differentiation. Mast cell tumours (MCTs) are common in dogs and less frequent in cats. The aim of this study was to investigate any relationship between KIT expression and the different histopathological types of feline MCTs, as it has been previously described in dogs. Twenty-seven feline MCTs were selected to perform this retrospective study. The samples were routinely processed and stained with Toluidine blue. The tumours were classified in well-differentiated (20), pleomorphic (4) and atypical or poorly granulate (3). An immunohistochemistry to detect CD117 was applied on all samples. The immunoreactivity was recorded by intensity, cellular location and distribution within the tumour. Well-differentiated MCTs were predominantly characterized by moderate intensity (25,93%), cytoplasmatic location (44,44%) and diffuse distribution (22,62%). Pleomorphic MCTs expressed moderate intensity (7,40%), cytoplasmatic (14,81%) and paranuclear (11,11%) stain and displayed diffuse or focal reaction (7,44% each-one). One of the atypical MCTs was positive and showed weak stain intensity, located in the cytoplasm with a multifocal distribution. According to the results, there was no clear correlation between the type of feline cutaneous MCTs and KIT expression. However, the use of KIT antibody may be of value for the diagnosis of pleomorphic and atypical MCTs and to establish a differential diagnosis of round cell tumours in cats.

P24

AMYLOID-PRODUCING ODONTOGENIC TUMOUR IN A CAT

Nicola Parry

Tufts University, USA

Neoplasia of the oral cavity is relatively common in older cats. Most types are malignant, tend to be locally aggressive and have variable metastatic potential, with squamous cell carcinoma and fibrosarcoma seen most commonly. Odontogenic tumours are low-grade malignancies with minimal metastatic potential and are rare in cats. This report documents an amyloid-producing odontogenic tumour in a cat.

A gingival mass from a fourteen year old domestic shorthaired cat with a history of a nodular swelling in the region of the left mandibular second premolar

tooth was surgically excised for microscopic evaluation. Grossly the mass was 5 x 4 x 3mm, pink-tan and firm. Histologically it was a well demarcated, unencapsulated neoplasm composed of epithelial cells separated by lakes of homogenous eosinophilic material within a fibrovascular stroma. Mitotic figures were not seen. The homogenous eosinophilic material was Congo red positive and produced apple-green birefringence under polarised light, suggestive of amyloid. These microscopic features are consistent with an amyloid-producing odontogenic tumor.

Amongst the domestic species, few reports of this neoplasm are documented in small animals, with slightly more reports in dogs than cats. These tumours have microscopic features similar to the calcifying epithelial odontogenic tumour first described in humans by Jens Pindborg in 1955 and eponymously known as the "Pindborg tumour". Despite the similarities, however, this lesion in domestic animals is not considered to be a direct counterpart of the human tumour. The term amyloid-producing odontogenic tumour has therefore been suggested as more appropriate for this lesion in dogs and cats.

P25

CYTOMETRIC DNA PLOIDY DETERMINATION IN BLOOD AND MILK LYMPHOCYTES OF ANIMALS INFECTED WITH BOVINE LEUKEMIA VIRUS.

Maria Szczotka, Piotr Kubiś

National Veterinary Research Institute, Poland

Cytometric study can rapidly quantitate the DNA content of isolated cells or nuclei by measuring their density after staining with special dyes. The nuclear DNA content of 1 to 5 x 10000 nuclei is typically measured to generate a DNA histogram. Two characteristics of the tumour cell population, ploidy and proliferative activity, can be determined from this histogram. Tumor ploidy is determined by the DNA content of tumor cells in G₀ 1 phase relative to the non malignant G₀-1 cells. Tumors in which the stemline DNA content is not measurably different from nonmalignant reference cells are referred to as diploid, and those with altered stemline DNA content as aneuploid. The numerical ratio of the mean DNA content of phase G₀-1 tumor cells to that of normal cells is the DNA index. Investigations were performed on blood, milk and tissues samples taken from animals infected with bovine leukemia virus and control group. The presence of specific bovine leukemia virus antibodies in sera of infected animals was detected in ELISA test. Proviral DNA was detected with PCR, nested-PCR and PCR in situ. Dual-colour flow cytometry with the use of specific monoclonal antibodies (for lymphocyte CD markers and BCL-2 protein) and conjugates labelled with FITC or PE was performed.

Milk and blood lymphocytes were isolated on Histopaque gradient. For DNA determination lymphocytes smears and tissues sections were stained according to the method of Feulgen and cells were analysed in microscope equipped with LUCIA Cytogenetic system. The results demonstrated much stronger expression of BCL-2 protein in blood lymphocytes of infected animals than in healthy group. This expression was very high especially in cells isolated from leukemic tumours. Their vitality was prolonged due to Bcl-2 protein, which physiologically blocks apoptosis. The BLV infection caused depletion of CD4 lymphocytes in infected animals and proliferation of IgM+CD19+ cells. These cells had immature character without tendency to differentiation. In nuclei of blood, milk lymphocytes and tumor cells the mean density of DNA content was much higher than in healthy group, and we observed aneuploidy. In blood lymphocytes of leukotic animals mean density of DNA content was 1,0889 and in control group -0,275. Similar values were observed in milk and tumor cells of animals in the both groups.

Determination of tumor ploidy and proliferative activity with the use of cytometry is useful tool in oncology, as it is an indicator of tumor growth and its heterogeneity, which is essential for the further prognosis and therapy.

P26

NUMEROUS HELICOBACTER-LIKE ORGANISMS WITH GASTRIC TUBULAR ADENOMA IN A DOG

Eléni Pavlidou Casto, Alberto Crisciotti, Carlo Maurelli, Beatrice Diaz, Roberta Graca

Italy

Association of Helicobacter species with gastric tumours is not yet established in dogs. This study presents some histopathological features and the follow-up of a Helicobacter-like organisms accumulation combined with gastric adenoma in a dog to better understand the role of these bacteria in canine gastric pathology. In a 10-year-old female spayed breed mixed dog with chronic intermittent vomiting a pyloric antral mass was identified and removed. The histological preparations were evaluated through H&E and Steiner's method. A basophilic adenomatous proliferation of gastric mucous glands with tall columnar epithelium, basal, irregularly crowded nuclei and low mitotic rate was observed. Branched tubules, focal polipoid and papillary proliferation supported by dense fibrovascular stroma and mild lympho-plasmacytic infiltration were present. The gastric epithelial cells had round to oval, hyperchromatic nuclei, 2 to 3 red cells in diameter, coarse granular chromatin pattern, 1 to 2 small nucleoli and abundant, clear cytoplasm. A heavy accumulation of filamentous spiral bacteria within the abnormal glandular pits and surrounding the papillary structures stained

dark brown to black with Steiner's method. The histopathological diagnosis was: numerous *Helicobacter*-like organisms with gastric tubular adenoma. The dog treated with antibiotics to eradicate bacteria is still alive, in good body conditions, without relapses and with no more evidence of *Helicobacter* species. To our knowledge this is the first report of canine gastric tubular adenoma combined with numerous *Helicobacter*-like organisms. Our current hypothesis is that there may be some correlation between the gastric mass or the clinical signs of our dog and *Helicobacter* species accumulated in the area of the lesion. Nevertheless one case is not enough to found valid conclusions. Thus it has to be combined with others to give useful evidence and reliable answers. Much still remains to be studied about the role of *Helicobacter* species in canine gastritis and gastric cancer.

P27

MAMMARY CARCINOMA IN A MARE: PATHOLOGIC AND IMMUNOHISTOCHEMICAL FINDINGS.

E. Mozos, R. Zafra, C.R. Jiménez, M.J. Ruiz, F. Pardiñas, C. Riber
University of Córdoba, Spain

Mammary gland tumors are uncommon in mare and its pathological characteristics have been occasionally reported (Prendergast et al., 1999, Hirayama et al., 2003). This study described the macroscopic, histopathologic and immunohistochemical features of a ductal carcinoma.

Case Report

A 20-year-old, nonlactating, nonpregnant thoroughbred mare was diagnosed of chronic suppurative mastitis and submitted to the Hospital of Veterinary Faculty of Córdoba. After clinical evaluation, a large tumor mass (44x35x27cm) was excised with the right mammary gland. Metastases were not found. The cut surface was multilobulated with redish-yellow-grey color and thick branches of fibrous connective tissue. Histopathologic examination revealed an intraductal and intralobular adenocarcinoma with areas of solid carcinoma and focal infiltration of the stroma and lymphatic vessels. Moreover, the parenquima showed both hyperplastic and chronic inflammatory lesions. Serial sections of the tumor were used for the immunohistochemical study; a panel of nine antibodies were analyzed: AE1/AE3, CK1, CK19, Vimentin, GFAP, S100, Smooth muscle actin, MCH-class II y Factor VIII-AgR.

Tumor cells showed variable reactivity with AE1/AE3 antibody and were negative for CK19; ductal cells with squamous differentiation were positive for CK1, as well as groups of infiltrating neoplastic cells. Neoplastic cells were negative for vimentin, GFAP, Smooth muscle actin and MCH-class II. MCH-class II reacted with lymphocytes and mononuclear infiltrating the intra and interlobullar septa.

Conclusion

Neoplastic cells of mammary carcinoma in mare were AE1/AE3 positive (heterogeneous pattern) and negative for CK 19, vimentin, S100 and GFAP and differed with the previous studies (Hirayama et al., 2003).

References:

Prendergast M, Bassett H, Larkin HA. (1999). Mammary carcinoma in three mares. *Vet Rec* 144: 731-732.

K. Hirayama, Y. Honda, T. Sako, M. Okamoto, N. Tsunoda, M. Tagami, and H. Taniyama. (2003). Invasive ductal Carcinoma of the mammary Gland in a Mare. *Vet Pathol* 40: 86-91.

This study has been supported by the Research Group PAI Agr 137. Junta de Andalucía. Spain.

P28

IMMUNOHISTOCHEMICAL EXPRESSION OF VERSICAN IN CANINE MELANOCYTIC LESIONS

Rosa Rabanal, María José Docampo, Anna Bassols

Universitat Autònoma de Barcelona, Spain

Melanocytic lesions are relatively common in dogs. They account for up to 20% of all cutaneous neoplasms and constitute the most common malignant neoplasia of the oral cavity in this specie. The extracellular matrix (ECM) influences cellular behaviour and therefore plays an active role during tumour development and progression. Versican is a proteoglycan of the ECM which is expressed during embryogenesis and has a restricted expression pattern in normal adult tissues. Versican is known to be abnormally expressed in several types of neoplasia and has been shown to modulate several cellular functions such as proliferation, adhesion and migration in vitro, promoting the malignant phenotype. We have analyzed the immunolocalization of versican in 35 canine melanocytic lesions with both benign (n=14) and malignant (n=21) features, using the antibody 2B1, against the core protein of versican. Most of the benign lesions were devoid of versican (9/14; 64.3%) or showed expression only associated with intratumoral hair follicles (1/14) or at points of the dermo-epithelial junction (2 dermal melanocytomas with junctional activity), and only two cases presented mild positivity. Conversely, 16 of the malignant melanomas (76.2%) displayed immunoreactivity in the stroma between the tumoral cells and/or at the border between tumour and peritumoral stroma, with variable intensity and distribution of the staining, whereas 5 of them (23.8%) were negative. In normal tissue adjacent to tumours, versican was unconstantly found below the epithelium, surrounding hair follicles and blood vessels, in both benign and malignant lesions. Our results suggest that versican may play a role in canine

malignant melanoma progression and that could be therefore useful as marker of malignancy in canine melanocytic tumours.

P30

PROGNOSTIC SIGNIFICANCE OF SURGICAL MARGIN, KI-67 AND CYCLIN D1 PROTEIN EXPRESSION IN GRADE-II CANINE CUTANEOUS MAST CELL TUMOR.

*Kiyokazu Ozaki, Tetsushi Yamagami, Koji Nomura, Isao Narama
Setsunan University, Japan*

Introduction

Prognosis of canine cutaneous mast cell tumor (CCMT) thought to be significantly correlates with histopathological grading. However, wide variety of histopathologic types from the cases of good prognosis resembling grade-I CCMT to those of bad prognosis similar to grade-III CCMT were seen among the grade- II CCMT accounting for about 50% of CCMT. Therefore, it is important to assess the other prognostic factors to evaluate the malignancy of grade-II CCMT than histopathological grading. The objective of this study is to determine prognostic value of surgical margin, Ki-67 and cyclin D1 protein expression in grade-II CCMT.

Materials and methods: Surgically resected specimen of solitary grade-II CCMT from 48 dogs (complete surgical margin: 11, incomplete surgical margin: 37) with >360 days follow-up periods were used in this study. Histopathologic grading system of CCMT was used according to Patnaik et al. (1984). The expression of cyclin D1 and Ki-67 proteins was determined by morphometrically using the slide stained immunocytochemically and the correlation between the results and survival and recurrence rate of each dog was analyzed statistically.

Results: Recurrence and survival rate of incomplete surgical margin group within 36 months postoperatively was higher than that of complete margin group. In incomplete margin group, dogs with low positive staining of Ki-67 had a significantly better survival, but recurrence rate and ki-67 positivity didn't show significant correlation. The number of cases with cyclin D1 positive tumor cells was small, but most of these cases have a poor outcome with high recurrence rate.

Conclusion: In grade-II CCMT, incomplete excision induces relatively high recurrence and poor prognosis. Ki-67 positivity is a reliable marker for the estimation of prognosis in incomplete margin cases of grade-II CCMT, but is not for recurrence. Cyclin D1 positivity may be a useful predictor, but its sensitivity was very low.

IMMUNOPHENOTYPIC CHARACTERIZATION OF BOVINE LEUKEMIA VIRUS-INDUCED BOVINE LYMPHOMA BY IMMUNOHISTOCHEMICAL METHOD

Soon-Seek Yoon, You-Chan Bae, O-Soo Lee

NVRQS, South Korea

Enzootic bovine leukosis caused by the bovine leukemia virus (BLV) is characterized by the proliferation of neoplastic lymphocytes in peripheral blood and/or various organs. In Korea, since BLV had been detected in 1982, the proportions of BLV-seropositive cattle have been continuously increased. Previous works have shown that most frequently involved organs were intestine, heart, stomach and diaphragm, and 83% was a diffuse large type with multinucleated cells and nuclear cleavages in histopathologic examination. In this study, we examined lymphoma to further define the cellular phenotype using MoAbs by immunohistochemical staining.

Lymphoma tissues were obtained from cattle necropsied and slaughtered from Jan 2002 to Jun 2004 in Korea. Both nested PCR test for BLV and histopathological test were performed on all cattle having lymphoma-suspected gross lesions. Finally, twenty-four BLV-induced lymphoma cases were confirmed and used for this study.

Tissue blocks were embedded in OCT compound, stored -70°C and cut by cryostat. To determine their phenotypes, the tumor cells were labelled with MoAbs against lymphocyte surface antigens(BoCD11b, BoCD2, BoCD5, B, MHC class II-DP and sIgM). Immunohistochemical staining was done by using commercial kit(Dako, LSAB). Concentration of MoAbs were 15µg/ml of immunoglobulin. For quantitative analysis, three areas were selected at random and the proportions of positive cells were calculated by examining at least 200 lymphocytes in 400X field by light microscopy(-: <5% positive, +: 5-20% positive, ++: 20-50% positive, +++: >50% positive cells).

BoCD11b antigens were not detected in all cases. BoCD2 antigens were detected in three cases(+: 3 cases). BoCD5 antigens were in all cases(+: 9, ++: 9, +++: 6 cases). Both B and MHC class II-DP antigens were in all cases(+++: 24 cases). And sIgM antigens were in all cases(++: 8, +++: 16 cases)

Results of this study for B-cell marker(B and MHC class II) and T-cell marker(BoCD2 and BoCD5) are similar to previous reports, on the other hand, that on BoCD11b and sIgM are different from previous reports. However, that most cells consisting bovine lymphoma are B-cell lineage is identical with previous reports. In our previous flow cytometric study, proportions of

BoCD11b antigen-expressing cells in peripheral blood of BLV-seropositive and persistently lymphocytotic cattle were higher(55.7%) than those of BLV-seronegative cattle(46.1%). We can say that there are some differences in characters between lymphocytes in lymphoma tissues and lymphocytes in lymphocytotic peripheral blood.

P32

GRANULOSA CELL TUMOR IN CAT (FIRST CASE REPORT FROM IRAN)

Abbas Tavasoly, Javad Ashrafi Helan

University of Tehran, Iran

Introduction: This is the most common of the sex cord-gonadostromal tumors in all species of animals and is usually a unilateral tumor. In cat prolonged estrus and hair loss with thinning of hair coat was observed.

Materials and Methods: After operation of this cat, Ovary with the tumor mass was removed. Tissue sample was cut at 4 mm thickness and fixed in 10% buffered formalin solution. 7 micron sections were stained with H&E (Hematoxyline and Eosin).

Results: Grossly the size of the tumor mass was 3.5cm in diameter and was smooth nodule. The growth had solid areas. It was firm and grayish yellow in appearance.

Histopathology revealed that the neoplastic cells were predominantly round or ovoid resemble the normal follicular cells , but a few groups of cells were small which contain eccentrically located hyperchromatic nuclei that had microfollicular arrangement with Call-Exner bodies , and tumor cells were radially arranged around eosinophilic material. Mitotic figures and pleomorphism in some areas were prominent.

Conclusion: The classification of these tumors depends on the predominance of the cell type and histological patterns. They may be of three types: The first type is well differentiated and has a uniform population of small cells similar to graafian follicles occasionally with groups of cells surrounding pink proteinaceous or clear fluid forming the so-called Call-Exner bodies. The second type resembles the Sertoli cell tumor of testis. The third type of tumor consisted of ovoid, poorly delineated granulose cell arrangement in a diffuse sarcomatous pattern. A variety of patterns may occur in different areas within the same tumor. Call-Exner bodies are present in some granulose cell tumors and, when present, are the useful diagnostic features. According to the microscopic patterns of this tumor, it was diagnosed as a well differentiated (first type) granulose cell tumor.

IMMUNOHISTOCHEMICAL EVALUATION OF A MULTICENTRIC LYMPHOMA AND AN INTESTINAL CARCINOID IN A DOG

Rui M. Gil da Costa , Célia Lopes , Liliana Martins , Maria A. Pires , Fátima Gärtner

University of Porto, Portugal

A 10-years-old male golden Retriever dog was presented for examination at the "Instituto de Ciências Biomédicas de Abel Salazar", University of Porto, Portugal, showing emaciation and generalized adenomegaly. The animal's condition deteriorated and euthanasia was required. On histopathological analysis a lymphoid malignancy was confirmed. Neoplastic cells formed multiple subcutaneous masses showing no epitheliotropism and multifocal infiltration of the splenic capsule was observed. Immunohistochemically, the neoplastic cells showed positive diffuse membrane immunostaining for CD79 α and negative immunostaining for CD3, being classified as a B cell lymphoma. Additionally, an annular, non-obstructive thickening of the intestinal wall was identified. Histologically, a malignant neoplasm replacing the entire mucosa and submucosa and invading the muscularis was observed. On immunohistochemical analysis, the neoplastic cells showed positive diffuse cytoplasmic granular immunostaining for chromogranin A, synaptophysin, neuron-specific enolase, cytokeratins (AE1,AE3) and c-kit. The tumour was thus diagnosed as an intestinal carcinoid. C-kit expression is commonly found in human carcinoid tumours, but had not yet been reported in the veterinary literature. Mutations of the c-kit proto-oncogene are implicated in the pathogenesis of several human and animal tumours, and our findings may provide further insight into the pathogenesis of canine intestinal carcinoids. Furthermore, c-kit has been demonstrated to be a therapeutic target for tyrosine-kinase inhibitor STI571 (imatinib mesylate) in humans. Positive immunostaining for this protein might be of clinical relevance.

ESTABLISHMENT AND CHARACTERIZATION OF A CELL LINE, MCO-Y4, DERIVED FROM CANINE MAMMARY GLAND OSTEOSARCOMA

Atsushi Kawabata, Kaori Yamamoto, Nguyen Thi Lan, Kazuyuki Uchida, Ryoji Yamaguchi, Tosiharu Hayashi, Susumu Tateyama

University of Miyazaki, Japan

A cell line, MCO-Y4, was established from a mammary gland osteosarcoma of a 16-year-old female mongrel dog. Histopathologically the tumor was composed of osteoblastic cells with an osteoid meshwork and chondroid matrix. The mean doubling time of the cells at the 93rd passage was 32.39 ± 4.66 hr. Immunohistochemically, the osteoblastic and chondroblastic cells were positive for bone morphogenetic protein (BMP)-2/4 and BMP receptor (BMPR) II. The cultured cells were spindle in shape during the growth and the confluent phases. No tumor matrix was detected in the culture dish by alcian blue staining or von-Kossa silver impregnation. MCO-Y4 cells on the chamber slides showed intense immunoreactivity for BMP-2/4 and BMPR II. Noggin, an antagonist for BMP-2/4, showed the growth inhibition on MCO-Y4 cells. In addition, fibronectin might be potential for stimulating growth of MCO-Y4 cells. When transplanted into severe combined immunodeficiency mice, the cells formed tumors consisting of solid proliferation of osteoblastic and fibroblastic cells with woven-bone trabeculae. These tumor cells were intensely positive for BMP-2/4 and BMPR II. Our results suggest that the cell line might be useful for studying the role of BMPs in canine osteosarcoma and the mechanism of ossification.

SERTOLI CELL TUMOR IN PIGEON

Omid Dezfoulian, J. Razmyar, B. Shojadoost, M. Masoudifard, S.M.Peighambari

Faculty of Vet Med, Lorestan Univ-Khorram Abad, Iran

A 12-year-old male pigeon (*Columba livia*) referred to the clinic, demonstrated anorexia, lethargy, emaciation and loose droppings in clinical examinations. The most striking feature, however, was the distention of abdominal region due to the presence of a solid tissue mass, which was detected by palpation. Radiographs and surgery, thereafter, confirmed the presence of the solid tissue mass, which was diagnosed as a Sertoli cell tumor at histopathologic examination. This is the first known report of Sertoli cell tumor in a pigeon.

A PRELIMINARY STUDY ON INCIDENCE OF CUTANEOUS FORM OF MAREK'S DISEASES IN BROILER CHICKENS IN TEHRAN PROVINCE, IRAN.

Mohammad Hassan Hablolvarid, Mohammad Reza Gholami

Razi Vaccine and Serum Resarch Institute, Iran

Introduction. Cutaneous form of Marek's disease produces the most severe losses in broilers. The losses results from high condemnations on postmortem examination in abattoirs. Enlargement of the feather follicles due to accumulation of lymphocytes is the typical lesions. The objective of present study was to achieve a preliminary estimation of incidence of cutaneous form of Marek's disease in broiler chickens in Tehran province, Iran.

Materials and Methods. On post mortem examination in abattoirs, from 27 Oct 2004 to 14 Feb 2006, after recording the gross lesions, samples of the skins of chickens' carcass of 58 broiler flocks, reared in Tehran province- Iran, were collected and examined histopathologically. The age of chickens was between 7-to-8- weeks old.

Results. In 14 flocks (24.1%) enlargement of feather follicles was prominent. But, in 3 flocks (5.1%) only few scattered follicles was involved. Enlarged follicles were more frequent in external crural region. In both instances, 17 flocks (29.3%), in histopathological examination, mild to sever infiltration of lymphocytes around feather follicles and in some cases in dermis were evident.

Conclusions. The results of present study showed that the incidence of cutaneous form of Marek's disease in broiler chickens, in Tehran province, is relatively high. It is probable that further detailed investigations will evoke the need for doing vaccination of broiler flocks, at least, in severely infected region of this province.

P37

MAMMARY GLAND SOLID CARCINOMA IN A MARE

Attilio Corradi, Anna Maria Cantoni, Rosanna Di Lecce, Benedetta Passeri, Massimiliano Paci, Paolo Ravanetti**

Dpt of Animal Heath, Pathology Unit, Faculty of Veterinary Medicine, Parma, Italy

**Equine Practitioners, Parma, Italy*

Mammary gland neoplastic diseases are a rare pathology in the mare. The most frequent histotype described is carcinoma which is locally invasive and highly metastatic. Aim of the study was to investigate histologically and immunohistochemically a case of locally relapse mammary carcinoma in a mare.

At the end of December 2005 a 22 year aged nonpregnant , nonlacting, italian saddle-horse was examined by equine practioners for sudden and progressive thinning. A rough spherical mass, 16x18 cm, was observed in mammary gland. The mmmary gland neofomation was ultrasound examined an described as mass cauliflower shaped. A fine needle aspiration was performed but the smears were non-diagnostic. A tissue biopsy (tru-cut) was then collected and investigated. After tru-cut histological diagnosis the mammary mass was surgically removed. and tissue samples were collected for histological and immunohistochemical investigation. Tru.cut biopsy, samples collected from the mammary gland mass and sentinel node were formalin fixed and paraffin embedded. Sections were histologically (H&E, Van Gieson, Pas-reaction) and immunohistochemically investigated (AE1/AE3, CK19, vimentin, Ki-67).

The results were of an uniform epithelial cell population (AE1/AE3 positive) highly proliferating (Ki67 positive) into and outside ducts, with a typical solid aspect. The same immunohistotype was also observed as metastatic cells in the sentinel node.

Six months later the equine practitioners have informed us of new mass formation in the mammary gland , without clinical evidence of long distance metastasis. A tru-cut biopsies will be performed as soon as possible by the equine practitioners.

In conclusion the mammary gland neoplasia diagnosed was a malignat ductal solid carcinoma metastatic to sentinel node. The follow-up, at six months, indicate, in this case, that the sentinel node removal may be prevent, in the short time, long distance metastasis.

P38

OVEREXPRESSION OF C-MET ONCOGENE IN CANINE OSTEOSARCOMAS AND CELLS LINE

Raffaella De Maria, Silvia Miretti, Selina Iussich, Martina Olivero, Maria Flavia Di Renzo, Paolo Buracco, Roy Levine and Bartolomeo Biolatti

Faculty of Veterinary Medicine of Turin, Italy

c-Met, is one of the most important oncogene belonging to tyrosine kinase receptors. It's involved in several malignant human tumours and specifically in osteosarcomas (OSA) where it is over-expressed in 80% of cases. Canine osteosarcomas show clinical and histopathological findings very similar to human OSA and they could be considered a valid model in comparative oncology. The purpose of this study was to characterize and evaluate Met oncogene expression in 6 canine osteosarcomas cells line and in 30 osteosarcomas. To determine the amount of MET transcript, total RNA was extracted from 6 OSA cells line (D22 D17, CO2, CO3,CO7, CO8) and MDCK, DK, TH canine cells line

and cDNA was subjected to q-PCR using Syber Green method. MET protein expression was evaluated by western blot analysis on cells lines lysates and by immunohistochemistry on 30 cases of canine OSA from paraffin embedded tissues. In this study we characterized MET protein in canine osteosarcomas cells line as a protein of 180 KD co-migrating with human MET of GTL16 cells line that are our positive control. Quantitative studies revealed that D17, D22, CO2, CO3 and CO7 have over-expressed MET cDNA. Indeed, we found by immunohistochemical studies that MET expression is present in 70% of cases of osteosarcomas. These data showed that expression and distribution of the c-MET in canine osteosarcomas are similar to those studied in human and suggested that these tumours could be a suitable model to test innovative approaches to therapy of human osteosarcomas.

P39

A CASE REPORT OF HEPATOCELLULAR CARCINOMA IN A CAMEL (CAMELUS DROMEDARIUS) FROM IRAN

Abbas Javaheri , Abbas Tavasoli , Seyed Hossein Mardjanmehr , Afshin Raoofi , Mir Adnan Mortazavi

Semnan University, Iran

Hepatocellular carcinoma has been identified in numerous species including dogs, cats, sheep, cows, pigs and the horse. To our knowledge there is no any report of Hepatocellular carcinoma in camel (*Camelus Dromedarius*) from Iran.

In an abattoir survey a liver of a 7-year-old male camel with a protruded and white color mass was observed.

The mass was 2 cm diameter and was located on the surface of the liver. Its consistency was looser than normal hepatic tissue.

Gllison capsule was covered the tumoural mass and appearance of cut surface was smooth and white to gray.

Histopathology revealed that tumour mass was surrounded by a capsule of connective tissue. Fatty change in adjacent lobules and congestion in sinusoids were observed. Massive necrosis and fibrosis were diagnosed in destroyed tissue of liver and remnant of affected lobules was seen as groups of hepatic cells or bands. Pleomorphism and individualization of neoplastic cells were observed. Neoplastic cells were swollen, spherical or ovoid predominantly. Cytoplasm of these cells was eosinophilic. Vesicular nucleouses were spherical or ovoid and located eccentrically in cytoplasm. Some of neoplastic cells were columnar. Cytoplasm of these cells was containing homogen eosinophilic material and had an ovoid or spherical and vesicular nucleus. Third group of neoplastic cells were spindle shape and had eosinophilic cytoplasm and spindle shape vesicular

nucleuses. These cells were scattered in the connective tissue of tumor stroma. Infiltration of mononuclear inflammatory cells in tumor mass was observed. Significant criterion of malignancy in this tumor was pleomorphism of vesicular nucleuses. According to microscopic examination of neoplastic cells and their growth pattern and similarity to hepatocytes this neoplastic mass was diagnosed as a hepatocellular carcinoma.

P40

A CASE OF CUTANEOUS AMYLOIDOSIS ASSOCIATED TO A FELINE POSTVACCINAL FIBROSARCOMA

Eider Salazar, Marta Pérez, Ester Biescas, Miguel Escalona, Armando Pañero, Julián Vázquez, Lluís Luján

University of Zaragoza, Spain

A case of nodular, cutaneous amyloidosis associated to a feline postvaccinal fibrosarcoma is described. An intact male, ten years old siamese cat was presented to a private veterinary clinic with a 5 cm dermal nodule located dorsocranial to the left scapula. The nodule had been detected by the owner 7 months earlier as a minimal growth. The nodule was firm and highly vascular. Histologically, an irregular, highly cellular mass was observed. Cells were spindle-shaped, with a moderate amount of cytoplasm. Nuclei were irregular but mostly elongated and they showed clumped chromatin. Nucleoli were prominent and there was a high mitotic index at HPF with presence of bizarre mitosis. At the periphery of the mass, numerous blood vessels were seen containing clumped tumoral cells, similar to the cells present within the tumor. The mass was infiltrated in all fields by variable amounts of eosinophilic material forming bands or islets. This material showed to be Congo Red positive and exhibited an apple-green birefringence under cross-polarized light, thus being classified as amyloid. No clinical evidence of metastasis or a systemic disease was observed at the time of surgery but at the present time the animal shows a marked cachectic process. Further studies in order to classify the type of amyloid are currently being carried out at the moment. To the best of our knowledge, this is the first description of amyloidosis associated to a feline postvaccinal fibrosarcoma.

MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL ASPECTS IN FELINE LYMPHOMAS

Emilia Ciobotaru, Teodoru Soare, Sanja Aleksić-Kovačević, Manuella Militaru, Georgeta Dinescu, Manolescu Nicolae

Faculty of Veterinary Medicine Bucharest, Romania

8 cases of cat malignant lymphoma were studied (3 cases alimentary lymphoma, 3 cases of multicentric lymphoma, 1 case of spleen lymphoma and 1 case of plasma cell tumor). Pathological investigations were used: necropsy, cytology, histology, immunohistochemistry. Fine needle aspiration, smears and imprints were May Grünwald Giemsa stained. Histological and immunochemistry investigations were performed on organs samples (lymph nodes, heart, spleen, kidney, pancreas, eye, and intestine) fixed in 10% formaldehyde solution and paraffin embedded. Routine histological sections were Masson trichromic stained. Immunohistochemistry was performed only in 5 cases and used avidin-biotin-peroxidase method and CD-3, CD-56, CD-57, CD-79 monoclonal antibodies.

Cytologically, 3/8 cases were centrocytic/centroblastic lymphoma, 2/8 cases immunoblastic lymphoma, 1/8 Hodgkin-like lymphoma, 1/8 large granular lymphoma and 1/8 plasma cell tumor.

2/5 lymphoma were CD-3+ (T-cell lymphoma, 1 as multicentric lymphoma and 1 as alimentary lymphoma), 1/5 was CD-3+ and CD-79+ (discrete reaction to the last antibody in spleen lymphoma), 1/5 was CD-79+ (B-cell lymphoma as multicentric lymphoma) and 1/5 CD-79+, CD-56-, CD-57- (large granular lymphoma).

Immunohistochemical investigation was made for the first time in Romania in feline lymphomas.

Gross, cytological and histological aspects of the investigated lymphomas are not relevant for the origin of tumoral cells. Large granular lymphoma (alimentary lymphoma) was CD-79+ (AMC anti-human) suggesting B-cell origin and CD-56-, CD-57-, excluding NK-cell origin.

P42

IMMUNOPHENOTYPIC CLASSIFICATION OF CANINE SOFT TISSUE TUMORS AND COMPARISON OF THE FINDINGS WITH THE PREVIOUS HISTOPATHOLOGIC DIAGNOSIS

Damla Haktanir Yatkin, Seçkin Serdar Arun

Istanbul University Faculty of Veterinary Medicine, Turkey

The objective of this study was to determine the immunophenotypic features

of soft tissue tumors obtained from dogs of varying ages and breeds, to assess relevance between these findings and conventional diagnosis and contribute to the diagnosis particularly in undifferentiated tumors. The study was carried out on 50 benign and malign soft tissue tumors, which were located in various anatomic sites. Previous conventional diagnosis consisted of hemangiopericytoma (n:1), dermatofibrosarcoma protuberance (n:4), fibromas (n:2), fibrosarcomas (n:4), hemangiomas (n:2), hemangiosarcomas (n:5), leiomyomas (n:8), leiomyosarcomas (n:3), malignant fibrous histiocytomas (n:4), neurofibromas (n:2), undifferentiated mesenchymal tumors (n:15). Formalin-fixed, paraffin-embedded specimens were labeled with streptavidin-biotin immunoperoxidase method, using a panel of commercially available antibodies raised against vimentin, muscle specific actin (HHF35), desmin, smooth muscle actin (SMA), s-100, keratin and F-VIII. All specimens stained positively for vimentin, negatively for keratin, indicating the mesenchymal origin of the neoplastic tissues. s-100 positive hemangiopericytoma, one case of dermatofibrosarcoma protuberance and one malignant fibrous histiocytoma and three cases of undifferentiated tumors were referred as peripheral nerve sheath tumors; three cases of SMA positive undifferentiated tumors as leiomyosarcomas; one leiomyosarcoma and one undifferentiated sarcomas as malignant fibrous histiocytoma; one case of neurofibroma and leiomyomas as fibroma and two cases of undifferentiated sarcomas were categorized as fibrosarcomas on the basis of immunohistochemical findings. Immunohistochemistry was considered to be useless in one case of neurofibroma and four cases of undifferentiated tumors. The specificity of HHF35 as a myogenic marker was found to be superior to desmin antibody. SMA and F-VIII were determined to be specific for smooth muscle and endothelial differentiation, respectively. One case of undifferentiated sarcomas was re-classified as haemangiosarcoma. Focal smooth muscle differentiation was shown in fibrous and fibrohistiocytic tumors. In conclusion our findings revealed usefulness of immunohistochemistry as an auxiliary technique accompanying histopathologic diagnosis, but ultrastructural and molecular studies should be carried out in the classification of dedifferentiated mesenchymal tumors.

P43

MASS-FORMING INTRAHEPATIC CHOLANGIOCARCINOMA IN A SHEEP

Azizollah Khodakaram-Tafti, Seyed Shahram Shekarforoush

School of Veterinary Medicine, Shiraz University, Iran

A mass-forming intrahepatic cholangiocarcinoma was diagnosed in a 4 years old sheep. This tumor was as a massive (large single) mass about 12 X 10 X

7 cm with relatively firm texture that involved entire right hepatic lobe. It was lobulated by fibrous bands and well circumscribed from adjacent normal tissue with irregular margins. The cut surface was gray-white with a few cystic areas containing yellow-brown fluid. Microscopically, the tumor composed of well differentiated cuboidal to columnar cells resemble to biliary epithelium. The neoplastic cells were organized into small, irregular, gland like structures embedded in fibrous stroma. Necrosis of deep parts of the tumor, infiltration of inflammatory cells scattered in the stroma and local invasion of the tumor cells into adjacent normal parenchyma were seen. Mitotic figures of neoplastic cells were remarkable but without any karyomegaly or giant cells. This primary hepatic tumor has been reported rarely in sheep.

P44

CANINE MAMMARY GLAND NEOPLASIA WITH ADNEXAL DIFFERENTIATION: A CASE REPORT

Diego Caliari, Laura Cavicchioli, Gabrita De Zan, Valentina Zappulli, Massimo Castagnaro

Department of Public Health, Veterinary Hygiene and Comparative Pathology, Italy

Two mammary gland nodules of approximately 1 cm in diameter were detected in a female yorkshire-breed dog and submitted for histology after excision. On the basis of the histological pattern and cytomorphology a tubular complex adenoma and a tubulopapillary simple carcinoma were diagnosed. In the first nodule, associated with the epithelial tubular component, multifocal areas of adnexal differentiation composed by multiple irregular and dysplastic hair follicles associated with small aggregates of polyhedral vacuolated cells consistent with sebaceous glands are detected. Marked multifocal squamous differentiation is also present. Immunohistochemistry for specific cytokeratins and myoepithelial markers was performed. A diffuse positivity for the presence of cytokeratin 8/18 is evidenced within neoplastic epithelial cells toward the lumen of tubular structures while a diffuse positivity for cytocheratin 5/6 is present within basal cells of adnexal and squamous epithelial cells. Both glandular luminal and adnexal epithelium are positive for pancytokeratins and negative for vimentin. Immunohistochemistry for cytocheratin 14 reveals a positivity for basal epithelial and myoepithelial cells of tubules and for basal cells of adnexal structures. Calponin and smoothmuscles actin are detected within myoepithelial cells around the tubules and within the hyperplastic areas of myoepithelial cells. Ki-67 expression revealed a low grade positivity. On the basis of the immunohistochemistry results the lesions is classified as tubular complex adenoma with adnexal differentiation. The second nodule consists of papillary and tubular structures lined by a single layered palizading epithelium.

In the centre of the nodule a focally extensive area of necrosis is observed. At the periphery there are occasional intravascular metastatic emboli. The epithelial neoplastic cells are positive for pancytokeratins, cytocheratins 8/18, cytocheratins 14 and mildly positive for cytocheratin 5/6. Numerous neoplastic cells are positive for vimentin and spindle cells at the periphery of neoplastic epithelium are negative for calponin.

P45

ATYPICAL SUBCUTANEOUS GIANT CELLS CARCINOMA IN THREE DOGS: HISTOLOGICAL, IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL FEATURES

Valentina Zappulli, Diego Caliari, Silvia Ferro, Laura Cavicchioli, Massimo Castagnaro

Department of Public Health, Veterinary Hygiene and Comparative Pathology, Italy

Three subcutaneous solitary nodules, from dorsal, cervical, and cheek regions, respectively, of three dogs, were incidentally detected, excised and submitted for histology. The nodules, ranging from 0.8 to 1.5 cm, were indolent and not-fixed to the surrounding tissues. For all three nodules histology reveals a well demarcated mass within the dermis and the hypodermis consisting of multiple irregular lobules associated with moderate stroma. Lobules are composed by pleomorphic cells arranged in sheets, in short bundles with mild palisading, and in a pseudoalveolar pattern; a central residual lumen is occasionally associated with mineralized PAS positive deposits. Polyhedral to spindle cells showed moderate to very large cytoplasm with marked irregular vacuolation, hyaline glassy material and/or granular PAS positive residues. Multifocally, discrete ballooning often multinucleated bizarre cells with abundant eosinophilic cytoplasm and occasional clear peripheral halo, is also present. Rarely, signet ring cells and variably sized areas of squamous-like differentiation are detected. Morphological differential diagnosis include atypical forms of amelanotic melanoma, neuroendocrine tumor, peripheral nerve sheath tumor, and anaplastic sarcoma/carcinoma. Histochemistry, immunohistochemistry and electron microscopy were performed. Neoplastic cells are negative to Grimelius silver impregnation and Fontana stainings. Immunohistochemistry reveals a coalescing to diffuse intensively positive staining for the presence of pancytokeratins; only scattered cells are positive for calponin (myoepithelial and smooth muscle cells), CK14 (basal epithelial and myoepithelial cells), CK8/18 (luminal glandular epithelial cells), CK5/6 (basal cells of stratified squamous epithelia). Frequently a concomitant expression of vimentin and occasionally S100 is detected in neoplastic cells, particularly in atypical giant cells. Neoplastic cells are generally negative for smooth muscle actin, glial fibrillar acidic protein,

neuron specific enolase, and chromogranin A. Electron microscopy allowed identification of numerous desmosomal structures associated with neoplastic cells. On the basis of these results, the lesions are classified as atypical carcinoma with pleomorphic multinucleated giant cells, possibly a new morphological type of sebaceous carcinoma.

P46

PHENOTYPICAL CHARACTERIZATION OF VASCULAR BLADDER TUMORS IN BOVINE ENZOOTIC HAEMATURIA IN SÃO MIGUEL ISLAND, AZORES

Tania Carvalho, Iolanda Fernandes, Diane Nayden, Carlos Pinto, Maria Peleteiro

Faculty of Veterinary Medicine, Technical University of Lisbon, Portugal

Bovine Enzootic Haematuria (BEH) is a syndrome of chronic onset, associated with the ingestion of bracken fern (*Pteridium aquilinum*). Bladder tumors represent the more serious manifestation of BEH and the presence of multiple different neoplasms in the same bladder is one of its most interesting characteristics. The incidence of BEH is particularly high on S. Miguel Island (SMI), Azores, with 21% of the dairy farms in this island presenting at least one animal with clinical signs and/or tumoral lesions. Moreover, approximately 10% of the adult cows presently slaughtered in SMI are rejected due to the presence of urinary bladder tumors.

Vascular tumours account for about 36% of all the neoplastic lesions found in BEH. Immunophenotyping was performed using a panel of antibodies directed to CD31, uroplakin III, cyclin D1 and p53, in a total of 24 haemangiomas, 16 haemangioendotheliomas and 20 haemangiosarcomas. Immunohistochemical staining was performed using a standard streptavidin-biotin technique.

All lesions were positive for CD31. No positivity for uroplakin III was seen within the neoplastic vascular cells. Nevertheless, an atypical staining pattern was frequently present within the transitional epithelia covering the neoplastic lesions, with loss of the normal staining of the apical aspect of superficial (umbrella) cells. Diffuse cytoplasmic staining of superficial, intermediate and basal cells was seen and also the presence of numerous lumina and microlumina exhibiting membranous immunostaining was noted.

Regarding cyclin D1 and p53, both were overexpressed in vascular bladder tumors, increasing with the grade of malignancy. Nevertheless, cyclin D1 overexpression revealed to be an early event in bladder carcinogenesis, a finding also reported by other authors in human pathology, in contrast with p53 overexpression which occurred particularly during the late stages of

tumorigenesis.

This work was supported by the Foundation for Science and Technology and Interdisciplinary Centre of Research in Animal Health (CIISA).

P47

PUTATIVE ROLE OF INHALED DUSTS IN THE DEVELOPMENT OF CANINE LUNG CARCINOMA

Giuliano Bettini, Maria Morini, Paolo Stefano Marcato

University of Bologna, Italy

Although some studies suggest an association between the exposition to urban pollutants and lung cancer in dogs, the relationship between the deposition of black dust matter in lung (also termed anthracosis) and primary pulmonary carcinoma has never been investigated.

In order to clarify this association we have scored the deposit of particulate matter (PM) in lung tissue of dogs with primary carcinoma and evaluated the correlation with the tumour histological type and grade.

34 canine lung tumours out of 64 selected from our records (formalin fixed, paraffin embedded, HE stain) were diagnosed as primary carcinoma on the basis of necropsy report, histological pattern and TTF-1 immunohistochemistry, and classified (WHO 1999) as 16 papillary adenocarcinoma, 1 acinar adenocarcinoma, 9 bronchioloalveolar carcinoma, 1 squamous carcinoma, 2 adenosquamous carcinoma, 4 large cell carcinoma, 1 bronchial gland carcinoma; the histological grading (McNiel et al, JAVMA 1997) prompted grade I in 7 cases, grade II in 20 cases and grade III in 7 cases.

Non-tumourous lung tissue in all cases of primary carcinomas displayed a considerable amount of black dusts within macrophages in the peribronchiolar and perivascular interstitium; PM score (Schoning et al, AJVR 1996) was 1 (minimal amount) in 4 cases, 2 (moderate) in 12 cases and 3 (massive) in 18 cases; in 13 cases there was also huge evidence of PM within tumour tissue. Nevertheless, there was no significant relationship (ANOVA) among PM score and the histological type or grade. The comparison of PM score in lungs with primary carcinoma with PM score in another series of 164 randomly selected canine lungs (Bettini et al, ProcESVP 2005) evidenced in the former group a significantly higher ($p=0,0009$) deposit of inhaled dusts.

These findings strongly suggest that canine pulmonary carcinomas more frequently develop in heavily anthracotic lungs, which may reflect the effect of inhaled carcinogens from urban pollutants.

GRANULOSA CELL TUMOR IN DOG (FIRST CASE REPORTED FROM IRAN)

Minosh Siavosh Haghighi, Abbas Tavasoly

University of Tehran, Iran

Introduction: Granulosa cell tumors are the most common ovarian neoplasms of cow and mare, and occur in older bitches with about equal frequency as ovarian papillary cystadenocarcinoma. They arise from the specialized stroma of the ovary and are regarded as sex cord-stromal tumor. They are usually unilateral, benign and may become extremely large.

Materials and Methods: The dog had been ovariectomized and the ovarian tumor mass was referred to the department of pathology. Tumor mass was fixed in 10% buffered formalin solution. Thin sections were stained with H&E.

Results: The gross appearance of this tumor was cystic, and the surface was smooth. The consistency of the tumor was soft and yellowish to orange in color. The size of the tumor mass was 5cm in diameter.

Microscopically neoplastic cells proliferated in a variety of patterns, including: predominantly follicular, and minimally sertoli cell-like patterns, as well as in diffuse sheets surrounding. Varying sized cystic spaces containing clear fluid. Neoplastic cells predominantly resemble normal granulosa cells in being small, polyhedral with foamy cytoplasm and round hyperchromatic nuclei with distinct nucleoli. A few Call-Exner bodies which consisted of small, central, round to oval space containing eosinophilic follicular fluid surrounded by a collar of radially arranged granulosa cells.

Conclusion : In dogs and cats granulosa cell tumors are often associated with clinical signs of hyperestrogenism, including continuous estrus, vulvar swelling, vaginal discharge, endometrial hyperplasia, and pyometra. A higher percentage of canine granulosa cell tumors are malignant and metastasize to regional lymph nodes and distant organs.

Call-Exner bodies, when present is diagnostic of granulosa cell tumor, however, they are not always present, particularly in extremely large tumors.

According to the microscopic pattern of this tumor, it was diagnosed as benign granulosa cell tumor.

P49

URINARY CORTICOSTERONE AS A STRESS BIOMARKER IN A PHARMACOLOGIC STRESS MODEL

Jean-Martin Lapointe, Patricia Snyder, William Reagan, Thomas Kawabata

Pfizer Global Research and Development, USA

Regulatory guidelines for toxicity studies of pharmaceuticals recommend using at least one dose near the maximum tolerated. At that level significant toxicities may occur, leading to systemic stress and secondary immune suppression which can be difficult to differentiate from a primary drug effect. Therefore there is a need for a biomarker of systemic stress easily applicable to toxicity studies. This study sought to evaluate urinary corticosterone as a stress biomarker in rats, using a pharmacologic stress model, the organophosphate fenitrothion, previously shown not to cause immune effects at pharmacologically active doses.

Rats were administered fenitrothion orally at 20 and 30 mg/kg daily for 2 or 8 days, with matched vehicle controls (n=6/group). Urine was collected for 24hr, before treatment and on day2 and day8. Urine was assayed for creatinine and corticosterone, separately for the first 6hr of collection and for the whole 24hr sample. Animals were euthanized on day3 or day9, and lymphoid tissue samples were weighed and examined histologically.

Treated rats showed significant neurologic clinical signs following treatment. Results showed time- and dose-dependent decreases in body weight, and spleen and thymus weight decreases supra-proportional to body weight on day9. Histologic changes were absent to mild at 20 mg/kg, but significant at 30 mg/kg, consisting mainly of lymphocytolysis at day3 and lymphoid depletion at day9. Urine corticosterone was increased on day2 and day8, in the 6hr samples but not the 24hr, at both dose levels. Urine corticosterone appears to be a sensitive biomarker of systemic stress when sampled concurrently with drug effects.

P50

AGE-DEPENDENT LEVEL OF ANTIOXIDANT DEFENCE SYSTEM AND LIPID METABOLISM STATE IN CALVES

N Kostromitinow, E Sumenkowa

Institute experimental veterinary, Russia

The findings obtained in the present study showed that the first days of life of the calves up to day 45 are accompanied by the naturally determined increase in the TBA (thiobarbituric acid)-active products and glutathione, which, in our opinion, is related to the transition period in feeding of the calves. The content of phospholipids and lipoproteins in blood of animals increases by day 30 and then stabilizes. The enzymatic activity of blood from day 5 to day 30 changes in a different manner. Thus, the catalase and ceruloplasmin values were noted to decrease by day 30 to be followed by an increase in and stabilization of the concentration of the enzymes by day 45-60. In contrast, the peroxidase value is steadily decreasing in the age-related dynamics. Although we registered the lowest index by day 30 of the calves' life.

LABORATORY AND MORPHOLOGICAL DIAGNOSIS OF PANCREATITIS IN DOGS

*Jozef Szarek, *Barbara Fojut-Palka, **Anna Winnicka*

*University of Warmia and Mazury in Olsztyn, Department of Forensic and Administration of Veterinary Medicine, Olsztyn, Poland; *Small Animal Clinic, Warsaw, Poland; **Warsaw Agricultural University, Chair of Clinical Sciences, Warsaw, Poland*

Introduction: There are still some difficulties in the diagnostic of pancreatitis and knowledge of its etiology and pathogenesis is not sufficient. The aim of the research was to elaborate principles of dog's pancreatitis diagnostics intravital on the base of clinical symptoms, conventional and newly introduced laboratory tests and also by morphological examinations.

Materials and Methods: The examination embraced 73 dogs. They were of different breeds, between 5 months and 17 years of age. Based on the history, clinical signs and results of laboratory blood analyses (complete blood count and serum analysis with pancreas, kidney and liver profile) and urinalysis and urine concentration of creatinine and also urine amylase and lipase level, in 25 dogs pancreatitis was suspected. Microscopic (HE) examination of the pancreas (20 sections in each case) and of the liver was carried out postmortem. The organs were also examined ultrastructurally (Opton TEM).

Results: Four dogs from the group of 25 animals suspected of pancreatitis died. Microscopic examination confirmed clinical diagnosis in each case, although there were also sections of the pancreas free from morphological lesions. Ultrastructurally in the parenchymatous cells of the examined organs changes in the rough endoplasmic reticulum and mitochondria and in endothelium of the blood vessels were most frequently noted.

Conclusion: The research has shown that the increased activity of amylase in serum and/or in urine can be accepted as the basic criterion in identifying pancreatitis, especially the chronic one in dogs.

Acknowledgement: Research financed from the sources for science in the years 2005 - 2008 as investigation project No 2 P06K 029 29.

THE CYTOLOGICAL DIAGNOSIS OF CANINE MAMMARY TUMOURS USING PAPANICOLAOU AND MAYGRÜN WALD-GIEMSA STAINS AND CORRELATION BETWEEN THE CYTOLOGICAL AND HISTOPATHOLOGICAL DIAGNOSIS

Funda Yildiz, Aydin Gurel

Istanbul University Faculty of Veterinary Medicine, Turkey

In this study, the cytological aspect of criteria of malignancy in canine mammary tumours were evaluated and a correlation was established between cytological and histopathological diagnosis by application of two different cytological staining methods: Papanicolaou and MayGrünwald-Giemsa.

The study was conducted on surgical mammary biopsy specimens, suspected of mammary tumour of 100 dogs, which were submitted to our laboratory. Cytological smears of the masses were prepared and stained with Papanicolaou and MayGrünwald-Giemsa. Tissue samples were fixed in 10% formaline, routinely processed and then stained with Hematoxylin&Eosin for histopathologic interpretation. Both were evaluated under light microscopy individually.

In our study, the major cytological criteria of malignancy in canine mammary tumours were listed as pleomorphism, large nuclei, nuclear membrane alterations, variable nucleolar size, shape and quantity, coarse chromatin and high cellularity. Cytological diagnosis was consistent with that of histopathology in 89% of all cases with Papanicolaou and in 85% with MayGrünwald-Giemsa, due to the malignant or benign features of the masses. The sensitivity, specificity and positive predictive value, negative predictive value and diagnostic accuracy of Papanicolaou and MayGrünwald-Giemsa were determined to be 97%, 96%; 70%, 57%; 91%, 88%; 11%, 19% and 91%, 87%, respectively.

Although no statistically significant difference ($p < 0,05$) was detected in terms of positive and negative predictive values, when both cytological staining methods were compared, statistically significant differences were obtained with regard to some criteria such as large nuclei, abnormal nuclear size, increase in nucleus to cytoplasm ratio and nuclear and cytoplasmic vacuolar patterns ($p < 0.001$) and coarse chromatin ($p < 0.05$). In terms of diagnostic accuracy, the criteria such as large nuclei, abnormal nuclei, variable nucleolar size and cytoplasmic vacuolisation were shown to be statistically significant ($p < 0.05$) as well as the increase in nucleus to cytoplasm ratio, nuclear vacuolar patterns ($p < 0.001$).

HYPOMYELINOGENESIS IN WEIMARANER DOGS: HISTOPATHOLOGICAL, IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDIES.

Carme Costa, Dani Sanchez, Mercedes Márquez, Raül Tortosa, Anna Domenech, Joan Mascort, Martí Pumarola

Universitat Autònoma de Barcelona, Spain

Hypomyelinogenesis is characterized by a defective myelin sheath formation in the central nervous system, although myelin present is biochemically normal. It has been reported in several animal species but it has only been well studied in humans, rat, mice and Spaniel dogs in which a genetic defect has been demonstrated. Moreover it has also been described in animals secondarily to infectious or toxic agents.

We report a case of Hypomyelinogenesis in two thirty-day-old Weimaraner dogs from the same litter. Clinically, they showed “intention tremors” and dysmetria since their birth.

At necropsy, no lesions were observed outside the nervous tissue. Histologically the central nervous system revealed a generalized white matter disorganization due to a diffuse lack of myelin, confirmed with Kluver-Barrera staining. Axonal abnormalities were ruled out using Bielchowsky argentic impregnation. Immunohistochemical staining for myelin proteins showed reduced PLP (proteolipid protein) positivity, but the amount of MBP (myelin basic protein) positivity was normal. The number of astrocytes immunoreactive against GFAP (glial fibrillary acidic protein) was increased. The immunoreactivity against HSP25 (heat shock protein 25) was also higher both in neurons and glial cells. Electron microscopic findings proved a defective myelin sheath formation together with apparently normal axons and the presence of oligodendrocytes.

Our results are in accordance with the described findings in humans (Pelizaeus-Merzbacher disease), jimpy mouse and Spaniel dogs. In all of them, the disease has been associated with a mutation in PLP gene.

P55

ROLE OF MACROPHAGES POPULATION ON GUT ASSOCIATED LYMPHOID TISSUES FROM MICE INOCULATED WITH SCRAPIE

J.L. Romero Trevejo, M Pedrera, P.J. Sánchez-Cordón, M.J. Bautista, J.C. Gómez-Villamandos

University of Córdoba, Spain

To study the role, numerical and qualitative changes of macrophages population on gut associated lymphoid tissues, 102 C57BL mice were orally inoculated

with the scrapie RML strain (CISA-INIA), while 30 animals with the same characteristics were maintained without inoculate and used as negative controls. Animals were slaughtered in batches of 6 from 15 to 400 days post-inoculation (dpi); samples from intestine and Peyer's patches were fixed in 10% buffered formalin and zinc solution and embedded in paraffin-wax. Haematoxylin-eosin and avidin-biotin peroxidase complex techniques were used for structural and immunohistochemical studies.

Animals showed typical symptoms of the disease from 270 dpi, consisting in stagger, arched back and erected tail and hair. However, no gross or microscopical lesions were found in the necropsies or in the structural study. The immunohistochemical study carried out using the Rb486 antibody showed the presence of PrPres in tingible body macrophages and in dendritic cells of Peyer's patches from 60 dpi, increasing the number of immunolabelled cells against this antibody until 180 dpi. To study the changes in macrophages population, Mac-3, anti-TNF α , anti-IL-1 α and anti-IL-6 antibodies were used, and positive cells were counted and tested for significance ($P < 0.05$) by Student's t-test.

This work will allow to profundice in the knowledge of the pathogenesis of prionic diseases, considering that it has become increasingly apparent that immune system cells could participate in clearance of the infectious agent at entry sites of infection, supply routes for agent spread and provide fertile ground for agent replication or accumulation.

P56

THE PATHOLOGICAL PHENOTYPE OF EXPERIMENTAL OVINE BSE IS MAINTAINED AFTER BLOOD TRANSFUSION

Silvia Sisó, Lorenzo González, Fiona Houston, Nora Hunter, Stuart Martin, Martin Jeffrey

VLA Lasswade, UK

Iatrogenic transmission by blood transfusion has been described from cases of human vCJD, experimental ovine BSE and natural sheep scrapie, demonstrating that blood in these prion diseases is infectious. However, comparative pathological features of the diseases in blood donors and recipients have not reported yet on the effect of blood exposure compared to the oral one. The present study describes the pathological phenotype of PrPd deposition in sheep recipients succumbing to clinical disease after blood transfusion from experimental ovine BSE or natural scrapie sheep donors.

Detailed immunohistochemical studies were carried out on brain and lymphoreticular tissues. Sheep challenged intravenously with BSE cattle brain homogenate were used as positive controls. Blood recipients were examined after they developed clinical signs or died from intercurrent disease, while

donors were culled at preclinical stages or left to develop clinical disease. We showed that blood can become infectious at early stages of sheep TSE infections and that the PrPd immunohistochemical phenotype of experimental sheep BSE was maintained after blood transfusion. Variability was observed within and between scrapie donors and recipients which might be attributable to the presence of more than one strain within the original source. Thus, the experimental sheep BSE model suggests that a change in pathological phenotype of vCJD would not be expected as a result of exposure through blood transfusion. We conclude that prion diseases of sheep can be of use to validate human blood diagnostic tests.

P57

SHREWS AS RESERVOIR HOSTS OF BORNA DISEASE VIRUS

Maria Puorger, Romana Herrsche, Monika Hilbe, Kati Zlinszky, Jürg Paul Müller, Jolanta Kolodziejek, Norbert Nowotny, Felix Ehrensperger

University of Zurich, Switzerland

Borna Disease (BD) is a severe, immunopathological disorder of the central nervous system (CNS), caused by infection with Borna Disease Virus (BDV), the prototype of a new virus family, Bornaviridae, within the order Mononegavirales. The main known naturally affected animal species in endemic areas in central Europe are horses and sheep, but many animals can be infected experimentally, like mice and rats. The precise pathogenesis and epidemiology of natural BDV infections are unknown; however, several unique epidemiologic features point towards the existence of BDV reservoir populations other than the final hosts. Here, we report on the detection of a natural host, the shrew (*Crocicudra leucodon*).

Eight moles, 3 shrews, and 87 mice of different species were trapped between 1999 and 2003 in a small village near Chur, Switzerland. The small mammals were euthanized and stored at -20°C for later examination. Their brains were divided into 2 equal parts, one half was fixed in 4% formaldehyde, cut transversally into several equal parts and embedded in paraffin for microscopic evaluation; the other half was stored in tubes at -20°C . IHC was performed as well. A recently established TaqMan real-time RT-PCR system (Applied Biosystems Rotkreuz, Switzerland) was used to detect and quantify BDV nucleic acid in all brain samples and selected heart samples from the mice, shrews, and moles.

Histologic examination showed no inflammation or degenerative processes in any of the 98 brains. Three of the 98 brains, however, were positive for BDV antigen by IHC. The 3 BDV-antigen-positive brains originated from the 3 shrews investigated, while all samples from moles and different species of mice proved negative. Identical results were obtained by TaqMan real-time RT-PCR; all 95

mouse and mole brains were negative, while the brain samples of all 3 shrews were positive. Sequence analysis of the shrew-derived isolate showed 99.9% identity to a BDV sequence from a horse from the same region.

P58

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF POST-MORTEM MUSCULAR PROTEOLYSIS IN SEA BREAM (SPARUS AURATA)

M^a José Caballero, Juan Carlos Escrig, Rafael Ginés, Pedro Castro, Gustavo A. Ramírez, Antonio Espinosa de los Monteros, Antonio Fernández

Veterinary School of Las Palmas of Gran Canaria, Canary Islands

The evolution of cytoskeleton muscle proteins along fish post-mortem is utilized as “in situ” marker of muscular proteolysis, together with that of proteinases calpains, which contribute to muscle deterioration. The objective of this study was to determine the immunoreaction patterns of these proteins along 14 days post-mortem in seabream. For this purpose, the presence of the cytoskeletal proteins desmin, actin and dystrophin and the endoproteases mu-calpain and m-calpain as well as its inhibitor, calpastatin, was monitored by ABC method.

Results showed that the morphological muscle structure was preserved intact up to the 7 day post-mortem (dpm), after that period a significant loss of connective tissue and cytoplasm detail fibres was observed. An intense immune reaction for desmin was found in the periphery of red and white muscle fibers. Anti-actin antibody showed a pattern in “bundles” with an homogeneous distribution throughout the cytoplasm which was more intense in red muscle fibres than in white ones. The immunoreaction to dystrophin was located in the cellular area adjacent to the sarcoplasmic membrane of the red fibres. Reaction to mu- and m-calpains and calpastatin all appeared as a microgranular deposition located next to the sarcolemma. Along post-mortem, positive immune reactivity to desmin was observed until the 7 dpm, whereas reaction to dystrophin disappeared earlier, at 2 dpm during the pre-rigor mortis period. Reduction of immune-reaction against actin was observed between the 7 and 10 dpm, although it was moderately conserved until the last sampling day. Mu, and m-calpains and calpastatin immunohistochemical reactions were intense during the first days (1-4 dpm) disappearing between the 7 and 10 dpm. The immunohistochemical techniques applied in the present study were found to be very useful to evaluate the post-mortem evolution of muscular proteolysis and denote the importance of muscle structural proteins and endoproteases as indicators of fish flesh quality.

A SLAUGHTERHOUSE SURVEILLANCE OF BOVINE SPONGIFORM ENCEPHALOPATHY IN BANGLADESH

E.H. Chowdhury, N. Marium, A. Islam and M.M. Rahman

Bangladesh Agricultural University, Bangladesh

Bovine spongiform encephalopathy (BSE) is a transmissible fatal neurodegenerative infectious disease first identified in the United Kingdom in 1987. This disease has a causal link with an old disease “Scrapie” which has been prevalent in sheep for over 200 years. The principal component of the infectious agent responsible for the disease appears to be an abnormal isoform of the host encoded prion protein (PrP), designated “PrP^{Sc}”. The emergence of variant Creutzfeldt Jakob Disease (vCJD) in humans in 1996 in UK and its causal links with BSE has highlighted the need for comprehensive study on its pathogenesis, diagnosis, prevention and eradication approaches. To control BSE within a country or to prevent the entry of BSE into a country, EU, USDA and few other countries have taken emergency measures in prevention, education, surveillance, and exportation of bovine origin materials (feeds, tallow, gelatin, etc) into these countries need BSE surveillance certificate. Bangladesh has no BSE surveillance so far. In this study, preliminary slaughterhouse surveillance is conducting in two districts, Dhaka and Mymensingh, in Bangladesh. Around 1000 brain samples from Cattle older than 30 months of age, slaughtered for human consumption in the district slaughterhouses, were collected for this study. The brainstems (obex), pyriform lobe, pieces of cerebellum and cerebrum were subjected for Histopathology, immunohistochemistry and ELISA using an anti-PrP monoclonal antibodies 6H4 (prionic AG, Switzerland) specifically reacting with epitopes on ruminant PrP^{Sc} and commercial immunoperoxidase and ELISA kit. Although some of the brainstems showed mild gliosis and inflammation, but none of the brainstems exhibited characteristic histopathologic lesions as found for BSE. No PrP^{Sc} was detected on these brainstems using immunohistochemistry and ELISA. From this study it apparently seems that BSE is not present in the native cattle in Bangladesh. Still samples are being collected from different regions and are analysing using above-mentioned methods.

P60

MALIGNANT PERIPHERAL NERVE SHEATH TUMOUR IN A DOG

Milijan Jovanovic, Vladimir Kukolj, Nenad Andric, Dimitra Psalla

Faculty of Veterinary Medicine, Belgrade, Serbia

Materials and Methods

The Siberian husky, male, 13 years old, started limping on left thoracic limb one year ago, and lameness increased during time. On MRI without contrast,

suspected diagnosis was neoplasia of brachial plexus peripheral nerves. Following euthanasia and postmortal examination tissue was taken for histological examination.

The tissue was routinely processed and stained with hematoxylin and eosin.

Immunocytochemical staining was done by use of a commercial avidin-biotin peroxidase complex kit. Antibodies for S-100 protein, glial fibrillary acidic protein (GFAP), cytokeratins AE1/AE3 and vimentin were tested. Diaminobenzidine (DAB) was used as the chromogen and Mayer's hematoxylin was used as the counterstain.

Results

Macroscopically, the tumour mass (6x8 cm) was located on the left axillar region closely connected with nerves of the brachial plexus, was nonencapsulated and infiltrated surrounding tissue. On section the tumour was solid with central core of haemorrhages and necrosis.

Histologically, neoplasm was infiltrative and poorly circumscribed. The cellularity and pattern of arrangement varied. In the more cellular areas, the spindle cells were arranged in fascicles, whorls, or sheets. The neoplastic cells had hyperchromatic oval to elipsoid nuclei with one or two prominent nucleoli, and fine, tapering, eosinophilic cytoplasm. The cell borders were indistinct. There were two to five mitotic figures per high-power field. Necrotic foci and hemorrhages comprised large areas of neoplastic tissue. In some nerves infiltrating neoplastic cells were seen in the endoneurium between apparently normal axons.

Immunohistochemically most spindle cells were positive for vimentin and negative for S-100, keratin and GFAP.

Conclusion

Based on the morphological and immunohistochemical features, the tumour was classified as a malignant peripheral nerve sheath tumour (MPNST) with dominant mesenchymal component.

P61

MORPHOLOGICAL ALTERATIONS IN OXYDATIVE MUSCLES ASSOCIATED WITH EQUINE ATYPICAL MYOPATHY

Dominique Cassart, Etienne Baise, Yan Cherel, Catherine Delguste, Nadine Antoine, Freddy Coignoul, Daniel Desmecht

University of Liege, Belgium

Thirty-two 0.5- to 7-year-old horses kept on pasture were referred for medical and necropsic evaluation of a sudden ataxia/myoglobinuria syndrome. Clinical examination and plasma CPK, LDH and AST levels were consistent with

extensive myopathy and, together with anamnestic data, with so-called “Equine Atypical Myopathy” (EAM), a disease of unknown etiology repeatedly reported in the literature since 1939. Necropsic examination revealed large areas of muscle necrosis, the extent and severity of which varied between cases and muscles, but which were clearly more constant and severe in respiratory and postural muscles and in the myocardium. Histology highlighted a multifocal and monophasic process compatible with Zenker degeneration/necrosis that mostly and segmentally affected type-1 fibres. Histochemical evaluation revealed a weak and disorganized pattern of NADH tetrazolium reductase staining, the absence of calcium salts precipitates and a dramatic accumulation of lipid droplets. Ultrastructural examination confirmed altered mitochondria and sarcoplasmic lipidosis. Taken together, the data suggest a primary alteration of mitochondria. The morphologic features reveal that EAM shares most of the characteristics of toxic myopathies. Interestingly, the pathogenesis of most of the latter includes mitochondrial poisoning.

P62

GENERATION AND CHARACTERIZATION OF A POLYCLONAL ANTIBODY FOR THE DETECTION OF THEILER'S MURINE ENCEPHALOMYELITIS VIRUS IN CELL CULTURE AND ANIMAL TISSUE

Maren Kummerfeld, Wolfgang Baumgärtner, Ludwig Haas, Susanne Alldinger, Andreas Beineke

University of Veterinary Medicine Hannover, Germany

Theiler's murine encephalomyelitis virus (TMEV) is a single stranded RNA virus of the family Picornaviridae. Intracerebral inoculation of susceptible mice with the BeAn-strain of TMEV causes a demyelinating leukoencephalitis in the late phase of the disease. Due to similarities of clinical and pathological findings, this chronic TMEV infection represents an important animal model for multiple sclerosis in humans. The aim of the present study was to generate a marker for TMEV by immunization of rabbits with purified virus. For the isolation of viral antigen BHK21 cells were infected with the BeAn-strain of TMEV and cultured for 24 hours. Cell-associated virus was harvested by repeated thawing and freezing of cultured cells. Subsequently, viral particles were purified by saccharose-gradient centrifugation. For initial immunization and subsequent boosting, three New Zealand White rabbits were injected subcutaneously with purified virus suspended in Ribí's complete and incomplete adjuvans, respectively. Occurrence of TMEV-specific polyclonal antibodies in post vaccination sera was tested on TMEV-infected L cells (murine fibroblast cell line) using immunofluorescence. Additionally, sera were tested on formalin-

fixed and paraffin-embedded TMEV-infected BHK21 cells pellets and spinal cord tissue of TMEV-infected mice using immunohistochemistry. Optimal concentrations for the visualization of TMEV were determined by serial dilution of post vaccination serum. Immunofluorescence revealed a specific signal in TMEV-infected L cells, while no immunoreactivity was found in non-infected control cells. Additionally, virus was exclusively detected in infected BHK21 cell pellets and associated with inflammatory lesion of the spinal cord of infected mice by immunohistochemistry. No cross-reacting antibodies were detected in pre-immune sera using these techniques. The present study describes the generation and characterization of antibodies for the detection of TMEV in cells cultures and mouse tissues. Now, these markers can be used for further studies upon the distribution and persistence of TMEV in the CNS and non-neuronal tissues of infected mice.

P63

TRANSMISSION OF CHRONIC WASTING DISEASE AGENT OF MULE DEER (CWDMD) TO SUFFOLK SHEEP BY INTRACEREBRAL ROUTE

Amir Hamir, Robert Kunkle, Randall Cutlip, Janice Miller, Elizabeth Williams, Juergen Richt. (*Deceased)*

National Animal Disease Centre, USA

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) that has been identified in captive and free-ranging cervids in the U.S. since 1967. To determine the transmissibility of CWD to sheep, 8 Suffolk lambs [4 QQ and 4 QR at codon 171 of prion protein (PRNP) gene] were inoculated intracerebrally with a pooled brain suspension from 28 mule deer naturally affected with CWD (CWDmd). Two other lambs (1 QQ and 1 QR at codon 171 of the PRNP gene) were kept as non-inoculated controls. Within 36 months post inoculation (MPI), 2 animals became sick and were euthanized. Only 1 sheep (euthanized at 35 MPI) showed clinical signs that were consistent with those described for scrapie. Microscopic lesions of spongiform encephalopathy (SE) were only seen in the sheep with the clinical signs of TSE and its tissues were positive for the abnormal prion protein (PrPres) by immunohistochemistry and Western blot. Between 36 and 60 MPI, 3 other sheep were euthanized because of conditions unrelated to TSE. The remaining 3 sheep remained non-clinical at the termination of the study (72 MPI) and were euthanized at that time. One of the 3 animals revealed SE and its tissues were positive for PrPres. Both sheep positive for PrPres were homozygous QQ at codon 171. Retrospective examination of the PRNP genotype of the 2 TSE-positive animals revealed that the sheep with clinical prion disease (euthanized at 35 MPI) was heterozygous (AV) and the sheep with the sub-clinical disease (euthanized at 72 MPI) was homozygous

(AA) at codon 136 of the PRNP. These findings demonstrate that transmission of the CWDmd agent to sheep via the intracerebral route is possible. Interestingly, the host genotype may play a significant part in successful transmission and incubation period of CWDmd.

P64

DIFFERENT SCRAPIE STRAINS DIRECT ALTERATIONS IN ABNORMAL PRP PROCESSING PATHWAYS

Martin Jeffrey, Gillian McGovern, Caroline Goodsir, Lorenzo González, Stuart Martin, Silvia Sisó

VLA Lasswade, United Kingdom

Using electron microscopy, we have studied the cellular changes and their relationship to PrPd accumulation in the brains of sheep infected with scrapie and compared these with the findings in conventional and transgenic mice infected with ME7, 263K, CWD and 87V. Scrapie-associated cellular changes included vacuolation, abnormal coated pits, membrane proliferation, increased lysosomes, and fibril formation. Fibrils were only seen in experimental rodent infections. Abnormally elongated and branched coated pits, sometimes connected directly to endosomes gave positive immunolabelling for both ubiquitin and PrPd. They were abundant in some sheep and rodent infections but were not found in others. They occurred on neuronal plasmalemma and along dendrites but not on axons, and were associated with increased cytoplasmic volume of endosomes and immature lysosomes. These changes suggest that a fraction of PrPd is not recycled but abnormally endocytosed. Membrane proliferation were found in astrocyte processes and in dendrites. These changes were directly and proportionally associated with PrPd localisation on cell membranes suggesting a causal relationship. Some PrPd was seen on cell processes adjacent to these areas suggesting PrPd release from infected cells to adjacent cell membranes. The proportions of each of these changes varied according to neuroanatomic site, cell type infected and source or strain. We suggest that different scrapie strains may be defined by distinct processing pathways of abnormal PrP. There are no consistent associations between PrPd accumulation and vacuolation or other non-specific degenerative features such as axon terminal degeneration or apoptosis.

P65

IMMUNOHISTOCHEMICAL EVALUATION OF THE PRESENCE OF PRPD IN THE INTESTINE, ILEOCAECAL LYMPH NODE AND CENTRAL NERVOUS SYSTEM OF CLINICAL AND SUBCLINICAL NATURAL OVINE SCRAPIE CASES

María del Carmen Ferreras, Miguel Fuertes, Carlos García-Pariente, Julio Benavides, Valentín Pérez, Juan Francisco García-Marín

Dpt. Patología Animal: Medicina Animal (Anatomía Patológica), Spain

Scrapie is a neurodegenerative disease of adult sheep characterized by the accumulation of the abnormal disease-specific prion protein (PrP^d). The aim of this study was to investigate the distribution of PrP^d in the gut associated lymphoid tissue -GALT-, enteric nervous system -ENS-, ileocaecal lymph node (ILN) and central nervous system (CNS) in clinical and subclinical Scrapie cases, and the relationship with the PrP genotype showed by the animals. Forty-six sheep from 3 flocks with confirmed cases of Scrapie have been studied. Animals were selected according to their age and genetic relationship with previous clinical cases, and the presence of clinical signs. PrP genotype was determined in 44 sheep. Immunohistochemical studies using 6H4 and L42 antibodies were performed in samples from the CNS, distal ileum, ileocaecal valve and ILN. PrP^d positivity in CNS was confirmed in 23 cases, all with clinical signs, and in 15 of them, these lesions coexisted with the presence of PrP^d in macrophages-like or dendritic-like cells in lymphoid follicles. Seven sheep showed positivity only in GALT and ILN. In 15 cases, both brain and digestive tissues were immunonegative. PrP^d was detected in the ENS in 6 animals, and in 5 of them also in the GALT. Positive cases were more frequent in 2-4 years-old individuals, although one sheep younger than one-year-old, daughter of a clinical case, showed intestinal positivity. PrP^d was found mainly in sheep with ARQ/ARQ (19) and ARQ/ARH genotypes (7). One sheep with ARR/ARR genotype presented immunopositivity in ILN and no signal was present in 3 ARQ/VRQ animals. Four sheep suffered both Scrapie and Visna virus infection. PrP accumulations can be detected in the ENS and the GALT both in clinical and subclinically affected sheep. Digestive PrP^d was detected in a Scrapie-resistant sheep carrying the PrPARR/ARR genotype, suggesting, as it has been pointed out, that genotype does not seem to affect the mechanisms of uptake of PrP^d.

P66

TELOMERASE REVERSE TRANSCRIPTASE (H-TERT) IMMUNOHISTOCHEMISTRY IN CANINE AND FELINE MENINGIOMAS

Luciana Mandrioli, Serena Panarese, Alessandro Cesari, Maria Teresa Mandara, Paolo Stefano Marcato, Giuliano Bettini

University of Bologna, Italy

Telomerase is a ribonucleoprotein complex that prevents the erosion of chromosomal extremities (telomeres) in eukaryotic cells.

The role of telomerase in the process of tumoral transformation is currently undergoing intense scrutiny, as its activation is considered a fundamental step in “immortalization” of neoplastic cells, and has been suggested to play a key role in the progression of several tumors, including human intracranial meningiomas.

This study aimed to evaluate in 25 cases of archived meningiomas (14 canine and 11 feline) the presence of telomerase, determined by immunohistochemistry (IHC) with an anti-h-TERT monoclonal antibody (clone 44F12, Novocastra), which detects the enzymatic catalytic subunit.

The positive reaction, identified by the evidence of scattered nuclear and/or nucleolar staining, was computed with an automated image analysis system (“Lucia 32G/Mutech”, Nikon) and expressed as percentage of positive tumoral cells.

According to WHO International Classification of Tumors of the Nervous System of Domestic Animals, meningiomas were grouped into the following histotypes: meningothelial (6 canine), transitional/mixed (1 canine, 6 feline), psammomatous (1 canine, 2 feline), fibrous/fibroblastic (1 canine, 1 feline), anaplastic/malignant (5 canine, 2 feline). In 15 out of 25 meningiomas (6 canine, 9 feline) h-TERT protein was localized in the nuclei, notably nucleoli and occasionally in the cytoplasm (two cases) of the cells, with mild to strong staining intensity. Even the percentage of positive cells was variable, ranging from 5 to 80%, regardless of the histological type.

Although the number of telomerase-negative meningiomas may depend upon overfixation, the heterogeneous h-TERT expression suggests the existence of alternative mechanisms to telomerase involved in the tumoral transformation and prompts the necessity to correlate h-TERT expression with proliferative activity and biologic behaviour.

P67

HISTOPATHOLOGICAL FINDINGS OF ABORTED FETUSES CAUSED BY NEOSPOROSIS IN DAIRY HERDS OF TEHRAN

FARIBORZ MOAYER , ARYA BADIEI

Islamic Azad University, Iran

Introduction: Neosporosis is one of the important causes of bovine abortion in Iran. In this survey Neospora Caninum was performed on 19 cases of aborted fetuses from dairy herds of Tehran submitted from summer 2005 to spring 2006 on the basis of histopathological findings.

Material and Methods: To detection cause of abortion, 75 aborted fetuses which occurred typically in mid-gestation with a mean age of 5.5 months (rang 3.5-7 months) were submitted for necropsy and microscopic examination. Tissue

samples obtained from the brain (cortex, midbrain, medulla and cerebellum), heart, lung, liver, spleen, kidney and skeletal muscle were fixed in neutral-buffered 10% formalin, routinely embedded in paraffin and stained with H&E.

Results: Gross findings from specimens during necropsy were non-specific and the fetuses were usually autolyzed with serosanguinous fluid accumulation in body cavities. Rarely there were subtle gross lesions, consisting of pale white foci in the skeletal muscles and the heart.

Histological lesions included widespread non-suppurative cellular infiltrates with occasional foci of necrosis in the brain and other organs. In this survey, the most characteristic lesions in the brain of fetuses were focal encephalitis with necrosis and non-suppurative inflammation. Myositis, non-suppurative myocarditis and portal hepatitis frequently with focal hepatic necrosis were mentioned.

In 12 aborted fetuses, protozoa of *Neospora* was seen in brain sections as a thick-walled tissue cysts.

Discussion: Fetal infections by the protozoa parasite, *Neospora* sp. is the newly recognized cause of abortion and congenital infection in cattle. This infection is the most common cause of abortion seen in many dairies throughout the world. *Neospora* can be transmitted congenitally (cow to calf) or horizontally (canid to cow). Congenital transmission occurs by passage of parasites from maternal blood to fetal blood across the placenta during pregnancy. In this study, cause of abortion in 19 out of 75 cases was detected on the bases of histopathological findings. Diagnosis of Neosporosis is based on microscopic examination of certain tissues of aborted fetuses. The brain is the most significant organ for diagnosing *Neospora* infection. Protozoa are not usually seen on routinely stained slides, but in some cases, thick-walled cysts may be present within CNS.

Abortion outbreaks can occur in chronically infected herds and are associated with severe depression of a cow's immunity by infection with other infectious agents (BVD virus), ingestion of toxins in moldy feed, or other stressors such as heat or overcrowding.

P68

EARLY DETECTION OF AXONOPATHIES IN CANINE DISTEMPER LEUKOENCEPHALITIS

Frauke Seehusen, Wolfgang Baumgärtner

University of Veterinary Medicine, Germany

Canine distemper virus infection (CDV) is commonly associated with demyelinating lesions in the central nervous systems of dogs. To further

investigate axonal pathology in this demyelinating disorder, the formalin-fixed, paraffin-embedded cerebella of 21 dogs, 17 of animals suffering from canine distemper leukoencephalitis and 4 healthy control dogs have been used in the present study.

For the histochemical investigations hematoxylin and eosin, luxol fast blue cresyl echt violet and modified Bielschowsky silver stain have been employed. In addition, antibodies specific for detection of axonopathies such as beta amyloid precursor protein (β -APP) and non-phosphorylated neurofilament (n-NF) as well as an antibody against the CDV nucleoprotein were used. Additionally, immunohistochemical double-labelling was carried out on selected slides.

Cerebellar areas of the healthy dogs served as a control group. The lightmicroscopic changes in the cerebella of the diseased dogs, suffering from demyelinating distemper encephalitis, were subdivided into a group of normal appearing white matter, early and late CDV-induced lesions.

The detection of β -APP, a marker for axonal damage, was found very early in lesions without signs of demyelination and showed the highest expression in subacute lesions. Damaged axons, which were positive for n-NF, were detected mainly in late lesions. By the modified Bielschowsky silver stain, detection of single spheroids have been observed.

Summarized, β -APP seemed to be the most sensitive protein for early detection of axonal damage. The provided data indicated that axonal damage occurred early in canine distemper demyelinating leukoencephalitis and can be detected before myelin loss appeared. It can be postulated that axonal damage plays an important role in the initial phase and during progression of canine distemper leukoencephalitis. Furthermore, the paradigm of canine distemper leukoencephalitis as a primary demyelinating disorder should be reconsidered

P69

PRIMITIVE SPINAL CRYPTOCOCCOMA IN AN IMMUNOCOMPETENT CAT

Sara Belluco, Jean-Laurent Thibaud, Thibaut Larcher, Monique Wyers, Stéphane Blot, Marie-Anne Colle

Veterinary school of Nantes, France

Cryptococcosis of lungs and meninges is a common finding in immunodeficient people and cats. It is often a disseminated infection associated to a minimal host inflammatory reaction. The Cryptococcoma described here is a discrete, primitive lesion localized in the thoracic spinal cord of an immunocompetent cat.

An 11-year-old male neutered cat was referred for a progressive tetra paresis. A clinical diagnosis of upper motoneuron syndrome localized in T3-L3 was made. An intramedullary mass was detected above T3 by MRI scans. Cerebrospinal fluid

tap was performed and revealed a mild mixed pleocytosis. The serology for FIV and FeLV, and PCR for Feline Coronavirus and *Toxoplasma gondii* infections were negative. No abnormalities were found on the myelogram. After two months the cat spontaneously died. At necropsy, a focal malacic area of 4 cm in length was found in the proximal thoracic spinal cord. The other organs were normal, including the respiratory system. The histopathological examination of the spinal cord revealed the presence of a cryptococcoma, characterized by a severe granulomatous inflammation which surrounded myriads of 7 μm basophilic yeasts with a clear halo. The yeast capsule was positive for PAS and Mucicarmin stain, indicative of a *Cryptococcus* spp. infection. Necrosis, digestion chambers, Gitter cells, spheroids and lymphocytic perivascular cuffs were observed. In conclusion, we report the case of cryptococcoma in a cat with a normal immunological status suggesting that immunodeficiency is not necessary for the development of infection. The contamination, occurred in a pigeon breeding farm, was probably due to the inhalation of a high concentration of yeast spores, which has overwhelmed the cat's immune defence.

P70

CORRELATIONS BETWEEN MAGNETIC RESONANCE IMAGING AND HISTOPATHOLOGY IN THE MDX (X-LINKED MUSCULAR DYSTROPHY) MURINE MODEL OF DUCHENNE MUSCULAR DYSTROPHY

Frédérique NGUYEN, Pierre-Antoine ELIAT, Mathieu PINOT, Florence FRANCONI, Laurent LEMAIRE, Jacques DE CERTAINES, Yan CHEREL
UMR 703 INRA - National Veterinary School of Nantes, France

Introduction. Magnetic Resonance Imaging (MRI) is used as a diagnostic tool for muscular dystrophies. Data are however lacking on the relationships between MRI features and histopathological aspects of skeletal muscle. Here we examine the correlations between MRI-Texture Analysis (MRI-TA), a quantitative assessment of MR images, and histomorphometry, in the murine model of dystrophin deficiency.

Material and methods. Eight mdx mice and eight control mice aged 1 year were used. MR images were acquired with a 7 Tesla system (high resolution images). Texture analysis was calculated using 3 methods (grey-level histogram, co-occurrence matrix, and runlength matrix). MRI-TA and histomorphometry were performed on 4 muscles of the calf (FDS: flexor digitorum superficialis, GLA: gastrocnemius caput laterale, GME: gastrocnemius caput medialis, and SOL: soleus). Myofibre size, and the extent of connective tissue, necrosis and regeneration were quantified.

Results. 10 MRI texture parameters discriminate mdx and control skeletal

muscles with an accuracy of 91%. In mdx mice, histomorphometry demonstrates myofibre atrophy, endomysial fibrosis, and an increased proportion of oxidative myofibers. Necrosis is grouped in 27-to-631-micrometer-large foci (1 to 44% of the total muscle area). Regeneration occurs in foci of 25 to 204 micrometers in diameter (0 to 17% of the total muscle area). In mdx mice, 26 MRI-TA parameters specifically correlate with the extent of necrosis and regeneration, including 8 with necrosis alone, and 2 with regeneration alone ($P < 0.05$ by linear discriminant analysis).

Conclusion. MRI-TA allows an in vivo discrimination between mdx and control mice, and correlates with histomorphometric parameters such as the extent of necrosis and regeneration. MRI-TA would be a promising tool for the non invasive monitoring of therapies addressed at muscular dystrophies.

P71

NEUROPATHOLOGICAL FINDINGS IN A STAFFORDSHIRE BULL TERRIER WITH L-2-HYDROXYGLUTARIC ACIDURIA

Emma Scurrell, Emma Davies, Elizabeth Baines, Giunio Bruto Cherubini, Simon Platt, Alun Williams

The Royal Veterinary College, UK

L-2-hydroxyglutaric aciduria (L-2-HGA) is a rare neurometabolic disorder described in humans, Staffordshire bull terriers and one West Highland Terrier. It causes a variety of progressive neurological signs accompanied by an elevation of the organic acid, L-2 hydroxyglutaric acid in plasma, urine and cerebrospinal fluid. An eleven month old, male neutered Staffordshire bull terrier presented with seizures, ataxia and behavioural changes. Magnetic resonance imaging (MRI) revealed symmetrical hyperintense lesions on T2 weighted images in the thalamus, pons and cerebellum, with no gadolinium uptake. The cortex appeared swollen. Considering the clinical signs and MRI findings together with the age and breed of the dog, L-2-HGA was suspected. The disease was confirmed by marked elevation of L-2-hydroxyglutaric acid in the urine. The dog was euthanased and submitted for post-mortem examination. No lesions were evident on macroscopic examination of the brain and spinal cord. The main microscopic lesion was spongiform change of the cerebral cortex, thalamus, cerebellum and pons, affecting mostly the gray matter, but also extending into the adjacent white matter. Spongiform change was characterised by the presence of numerous to confluent, clear vacuoles with well-demarcated margins, which measured up to 60 microns. In the gray matter, vacuoles were located adjacent to astrocytes, neurons and perivascularly. No significant findings were present in other organs/tissues. To the best of the author's knowledge, histopathological findings in dogs

with L-2-HGA have not been previously described in detail. Microscopic findings were similar to those reported from affected people, which included spongiform change and cystic cavitation affecting multiple brain areas. In the present case, MRI and pathological findings are suggestive of oedema. Currently however, the exact biochemical defect causing L-2-HGA and the mechanism of lesion development are uncertain.

P72

HISTOPATHOLOGICAL AND IMMUNOCHEMICAL STUDY OF SUSPECT ATYPICAL SCRAPIE CASES DETECTED IN THE SPANISH SURVEILLANCE PROGRAMME (2003-2005)

Cristina Acin, Eva Monleon, Marta Monzon, Rosa Bolea, Antonia Vargas, Juan Badiola

University of Zaragoza, Spain

During the last 5 years, scrapie surveillance has been encouraged with the aim of: detecting most of the scrapie-affected animals; eradicating the scrapie disease and improving the detection of BSE cases in sheep or goats. As a consequence of the increase in the number of analysis, the number of scrapie cases has also been incremented and atypical cases of scrapie have been described along Europe (E.g. Nor98 cases). The European Food Safety Authority has defined an atypical scrapie case as: samples mild positive to rapid Western Blot (high concentration of PK); clearly positive to modified Western Blot (low concentration of PK) and a low band of <12KDa and finally highly positive in the cerebellum and mildly positive in the brainstem by immunohistochemistry.

A retrospective study has been done analyzing suspect atypical scrapie cases diagnosed from 2003 to 2005 by the National Reference centre for TSEs of Zaragoza. Following the EFSA recommendations and the immunochemical protocols developed by Gonzalez et al, the envisaged techniques have been developed:

- Vacuolar lesional profile
- Reactivity of intracellular PrPd to N-terminal antibodies (epitope mapping) and phenotypic characterization by immunohistochemistry with the P4, 6H4, R145 antibodies.
- Modified Western Blot to determine de glycoform ratio and molecular weights.

In addition, the entire PRNP gene coding region of the selected animals has been sequenced in order to visualize the present SNPs.

The results obtained reveal the presence of atypical scrapie cases in our country. In addition, these cases could have been sub estimated because the surveillance applied was based on the analysis of the brain steam by rapid Western Blot.

EFFICIENT TRANSMISSION OF ARQ/ARQ SUFFOLK SCRAPIE TO BANK VOLES

Francesca Chianini, Michele Angelo Di Bari, Romolo Nonno, Scott Hamilton, Jeanie Finlayson, Samantha Eaton, Philip Steele, Susan Rodger, Mark Dagleish, Martin Jeffrey, Hugh Reid, Umberto Agrimi
Moredun Research Institute, UK

Scrapie is a transmissible spongiform encephalopathy (TSE) affecting sheep, goats and moufflon.

The influence of genetic susceptibility in sheep is well described and the occurrence of natural and experimental scrapie has been linked to polymorphisms at codons 136, 154 and 171 of the PrP gene. The effect of such polymorphisms is also dependent on breed and in Suffolk sheep the ARQ/ARQ genotype is considered the most susceptible whilst the ARR/ARR genotype is most resistant to natural disease.

The determination of levels of infectivity in tissues from scrapie affected sheep is fundamental to understand the pathogenesis, and the transmissibility of the disease. The most sensitive indicator of Suffolk scrapie infectivity is probably the parenteral challenge of ARQ/ARQ sheep. Such a system is however cumbersome and expensive. Detection of infection in laboratory mice is more rapid and economical, but is generally less sensitive, particularly in the case of primary isolation from Suffolk sheep. Lack of sensitivity could be overcome with the use of transgenic mice that express ovine PrP; however at the moment sufficiently characterised mice are not available.

Recently, the bank vole (*Clethrionomys glareolus*) has been shown to be highly sensitive to different sources of natural scrapie. After intracerebral inoculation, bank voles succumb to disease following a short incubation time (around 200 days post inoculation) and also show high attack rates.

With this study we established that voles are very susceptible to inoculation from ARQ/ARQ Suffolk scrapie, and that they succumb in 175 +/- 18 days after inoculation of 0.02 ml of brain homogenate.

SELECTIVE DEGENERATION OF THE CEREBELLAR CORTEX AND PROPRIOCEPTIVE NUCLEI IN A FIVE-YEAR-OLD CAT

Nicola M. A. Parry, Rolf Pfannl, Dominik Faissler

Tufts University, USA

Chronic neurodegenerative diseases in people comprise a wide spectrum of disorders with ataxia as the main symptom. These diseases are characterised by selective loss of specific neuronal populations over a period of years, and although their underlying aetiologies are mostly unclear, loss of neurones and neuronal contacts is an integral feature of disease pathology. This report documents striking selective degenerative changes in the cerebellar cortex and proprioceptive nuclei in a cat.

A five year old, castrated male, domestic shorthaired cat was euthanised following a history of slowly progressive cerebellar deficits from the time of adoption at the age of six months. Neurologic signs included intention head tremors with bilateral absence of menace reflex, ataxia, hypermetria and falling over. At postmortem examination the cerebellum and the rest of the brain were grossly normal. Microscopically in the cerebellum there was widespread loss of Purkinje cells with attenuation of the molecular and granular cell layers and pronounced Bergmann's gliosis. Degenerative changes were present in proprioceptive nuclei, with sparing of dorsal columns, and lesions consistent with Wallerian degeneration were seen in thalamic axons.

Numerous cerebellar disorders can affect cats of different age groups. Cerebellar dysfunction in young cats is most commonly attributed to foetal or neonatal infection by feline panleucopaenia virus, a parvovirus that interferes with cerebellar cortical development to produce cerebellar hypoplasia with non-progressive cerebellar disease. Progressive cerebellar dysfunction, however, can result from hereditary cerebellar degeneration, neuraxonal dystrophy and lysosomal storage diseases. The specific microscopic lesions and clinical signs in this case were consistent with a diagnosis of a progressive neurodegenerative

disorder. No information was known regarding the littermates or other family members of this cat.

In contrast to dogs in which numerous breed-related cerebellar degenerations are described, such conditions are very rare in cats, with only sporadic case reports documented.

P75

IMMUNOHISTOCHEMICAL CHARACTERISATION OF SKELETAL MUSCLE SAMPLES OF STRANDED CETACEANS

Eva Sierra, Manuel Arbelo, María José Caballero, Pedro Herráez, Antonio Espinosa de los Monteros and Antonio Fernández

Veterinary School, University of Las Palmas de Gran Canaria, Spain

Fiber types can be differentiated by analyzing the specific myosin heavy chain (MyHC) isoforms expressed by each fiber's phenotype. The two major skeletal muscle fiber types are type I (slow-twitch) and type II (fast-twitch). Skeletal muscle type II fibers can be further subdivided into types IIa (fast red) and IIb (fast white). In several mammalian species a IIx (IIc) myosin heavy chain isoform have been also described. Fiber-type composition varies extensively between muscles and in accordance with the functional requirements of the muscle. Endurance capacity is correlated with high percentages of type I and type IIa fibers whereas sprint capacity is correlated with high percentages of type II fibers. Samples from the dorsomedial area of the Longissimus dorsi skeletal muscle were analysed in order to compare fiber type composition among different cetacean species with different dives behaviour. Samples were collected following a standard necropsy procedure and fixed in 10% buffered formalin. Two monoclonal Anti-Myosin (Skeletal-Slow and Skeletal-fast) antibodies have been used for the localization of slow (Type I fibers) and fast (Type II) MyHC by using the immunohistochemical technique.

Previous studies based in the presence of intramyocellular lipids in cetacean skeletal muscles and these new results will be presented and discussed in relationship with their behaviour.

P76

PATOMORPHOLOGY OF THE LIVER IN EUROPEAN BISON (BISON BONASUS) FROM BIAŁOWIEŻA FOREST (POLAND)

Małgorzata Sobczak-Filipiak, Barbara Osinska, Wojciech Bielecki, Krzysztof Anusz, Jan Wiśniewski, Magdalena Matuszewska, Jerzy Dackiewicz

Warsaw Agricultural University, Poland

Between December 2004 and March 2005, 33 bison (18 females, 11 males)

aged from 2 months to 26 years, shot during culling, have been examined. The aim of this study was to determine pathomorphological changes in the liver.

During necropsy specimens of liver from 29 animals for histopathological analysis were collected. Samples were stained routinely (H&E) and by Masson's trichrome method. Animals were divided into groups, depends on age.

In the youngest group <1 year (11 animals) fatty degeneration of hepatocytes in 6 cases was seen. Inflammatory infiltrates in 6 cases were diffused in the liver parenchyma, in 3 - localized predominantly around billiary ducts in portal area; in 3 cases there were lymphatic nodules in the liver parenchyma. In 3 cases there was cholestasis, in 4 - haemostasis. In 10 cases proliferation of billiary ducts was seen.

In the next two groups >1-5 years (5) and >5-10 years (8) pathomorphological changes were similar: fatty degeneration of hepatocytes in almost all cases. Cirrhosis was seen in 3 cases; in 1 - F.hepatica in billiary duct. Proliferation of billiary ducts was seen in 2 cases (in 1 - with papillary growth of epithelium of the billiary duct). Moreover in 2 cases it was focal dilatation of sinusoids under capsula of the liver.

In the oldest group >10 years (5) fatty degeneration of hepatocytes was seen in 4 cases. Hepatocytes were different in size. Inflammatory cells in 4 cases were diffused in the liver parenchyma, in 4 - localized in portal area. In 5 cases there was cholestasis, in 2 - haemostasis. Cirrhosis was seen in 2 cases; in 1 case it was focal dilatation of sinusoids; in 2 cases - proliferation of billiary ducts.

Pathomorphological changes were observed in all examined animals.

P77

MAMMARY GLAND DEVELOPMENT AND LACTATION IN A JUVENILE FEMALE OF CUVIER'S BEAKED WHALE (*ZIPHIUS CAVIROSTRIS*) WITH AN OVARIAN GRANULOSA CELL TUMOUR

A. Fernández, A. Espinosa de los Monteros, A. Godinho, P. Herráez, M. Arbelo, P. Castro

Universidad de Las Palmas de Gran Canaria, Spain

Ovarian tumours are classified on the basis of the cell of origin as epithelial tumours, germ cell tumours and stromal tumours. In this latter group is included the granulosa cell tumour, the most common ovarian neoplasm in domestic animals. Granulosa cell tumours may secrete a variety of hormones, including progesterone, oestrogen, testosterone and inhibin. The production of hormones is frequently associated with abnormal behaviour and physiological events. In cetaceans, granulosa cell tumours have been described in only in four species of cetaceans (blue whale, fin whale, short-finned pilot whale and beluga). In all the cases, the animals were adult and two of them were pregnant.

In this communication, the gross and histopathological features of a granulosa cell tumour are described in a juvenile female of Cuvier's beaked whale (*Ziphius cavirostris*) found stranded in the coast of La Garrucha (Almería, Spain). Grossly, the right ovary was diffusely enlarged (6.5 x 2.5 cm) and on sagittal section included both solid and cystic areas. Microscopically, the ovary was replaced by neoplastic cells arranged in variable sized lobules, cysts, nests and cords separated by dense fibrous trabeculae. The cells were round to polygonal, with vacuolated cytoplasm and round nuclei. There was mild anisokariosis and nuclear pleomorphism. Some tumour cells were arranged in radial aggregates about a central deposit of eosinophilic material (Call-Exner bodies). Other lesions observed in the animal were a moderate development of the mammary gland with normal in appearance milk secretion and mild oedema in the external genitalia.

To the authors' knowledge, this is the first description of an ovarian granulosa cell tumour in a juvenile female of Cuvier's beaked whale. Furthermore, the animal showed an unexpected mammary gland development and lactation presumably occurred secondary to oestrogen and progesterone production by the tumour.

P78

SCALY LEG IN A BUDGERIGAR

Saeed Hesaraki, Abbas Javaheri, Naser Seirafy

Azad University of Tehran, Iran

Knemidocoptes Mutans causes the condition known as "Scaly Leg". The mites get on to the feet of the birds from the ground since the lesion usually develops from the toes upwards. The parasites pierce the skin underneath. The scales, causing an inflammation with exudates that hardens on the surface and displaces the scales. The diseases may lead to lameness and malformation of the feet.

A 2 years old budgerigar was referred to veterinary clinic of Tehran Azad University. It had scaly legs and was stepping on limping. The bird was weak and had a poor body condition. After euthanasia numerous *Knemidocoptes mutans* (sarcoptidea) were found from scaly leg. The mites were almost spherical in shape and short legged with strongly striated epidermis and the dorsal striations were not interrupted.

The gross lesions were characterized by scales and crust on the host's legs.

In histological study there were tunnels which bored into the epithelium causing proliferation of scales and crusts.

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF KISSELEV NODULES IN WILD BOARS

Chiara Palmieri, Marco Brunetti, Leonardo Della Salda

Teramo University, Italy

Kisselev nodules have been described in several parasitic infestations, even if their functional role and mechanisms of formation are not completely understood. The aim of the study was to investigate, for the first time, the T (CD3) and B lymphocytes (CD79acy), macrophages (lysozyme, alpha1antitrypsin) and follicular dendritic cells (CNA.42) distribution and vascular organization (vWF) of Kisselev nodules observed in wild boars. Tissue samples (lungs, kidneys, livers, tracheo-bronchial lymph nodes, spleens) were collected by 47 hunted wild boar, fixed in 10% buffered formalin, embedded in paraffin and stained with haematoxylin-eosin. Kisselev nodules were observed in lung (n=9), liver (n=1) and kidney (n=1), composed of lymphoid follicle-like structures with small amounts of interfollicular diffuse lymphoid tissue. The distribution of immunoreactive cells was similar to the cortex of lymph nodes: lymphoid follicles with germinal centers (composed mainly of CD79+ B cells, especially in the outer corona) predominate over interfollicular tissue (composed mainly of CD3+ T lymphocytes). These findings, in the cases associated with worms infections, suggest that the local humoral immune response is more important than the cellular response in parasitic diseases. The presence of follicular dendritic cells, involved in the antigen presentation and clonal expansion of B and T cells, confirms the high degree of organization of these lymphoid-like structures. Basing on the findings of similar structures in human chronic diseases (ectopic follicle, lymphoid neogenesis, tertiary lymphoid tissue), we can suggest, in the different cases, the following hypothesis about their genesis: (1) a direct derivation from BALT; (2) a primary involvement of monocytes/macrophages or follicular dendritic cells (major source of specific chemotactic factors for lymphoid cells, such as BCA-1); (3) the role played by the vascular system and the adhesion molecules expressed on the endothelial cells in the migration and recruitment of specific cells and the development of chronic inflammation.

LEAD INTOXICATION BY INGESTION OF LEAD SHOT IN RACING PIGEONS (COLUMBA LIVIA)

Stefan Roels, Paul Tavernier, Kristof Baert, Katleen Hermans, Frank Pasmans, Koen Chiers

Veterinary and Agrochemical Research Centre (CODA / CERVA), Belgium

Two 8 months old domestic pigeons were presented with a distended crop and regurgitation of liquids. They were thin and anaemic and displayed an unusual behaviour characterised by lethargy and a backwards stretching of the neck. Lateral to and fro rolling movements of the distended crop could be seen under the skin. Deep palpation of the crops induced regurgitation of foul smelling liquid crop contents. In one pigeon ptosis was obvious. The urate fraction of the excreta was watery in one pigeon and a soft consistency of the faeces was noticed in both.

Crop swabs for direct and cytological examination were negative for *Trichomonas gallinae* and *Candida* sp. A whole body radiograph of one pigeon confirmed the wide distention of the crop, which contained grit and food particles. In the gizzard one round radiopaque pellet could be distinguished from the grit particles. Haematological examination of a blood smear showed numerous erythroblasts, polychromatic erythrocytes and reticulocytes, indicating a severe regenerative anaemia. Atomic absorption spectrophotometry showed a blood lead concentration exceeding 7 ppm (7000 µg/l).

The pigeons were treated by I.M. injection of 40 mg/kg calcium disodium EDTA bid in intermittent 5 day courses. Mineral oil was administered orally instead of attempting surgery to eliminate the lead shot. The pigeon in which the high blood lead concentration was found, died after 5 days of treatment. A post-mortem radiograph and a necropsy revealed 11 lead pellets in the gizzard and in the intestine, whereas only the biggest one had been seen on the first radiograph. An impression smear of the kidney showed numerous intranuclear inclusion bodies, which were also demonstrated on HE and Ziehl Neelsen stained histological slides.

Possible effects of intoxication by ingestion of lead shot in wild bird populations in Flanders, as seen in domestic pigeons, need further examination.

P81

CROSS-REACTION OF HUMAN AND BOVINE CYTOKINES IN CETACEANS TISSUES

J. R. Jaber, J Pérez, M Arbelo, P Herráez, A Espinosa de los Monteros, A Godinho, M Mendez, A Fernández

Universidad de Las Palmas de Gran Canaria, Spain

Impairment of immune function is suggested to play a contributing role for the increasing incidence of infectious diseases in marine mammals. The integrity of the immune system is controlled by efficient cellular responses to cytokine stimulation. Consequently, the understanding of the role of different cytokines during inflammatory diseases could lead to better knowledge of the marine mammal immune system. This study evaluates the cross-reactivity of a panel of ten monoclonal antibodies to human and bovine cytokines in snap frozen tissue sections of lung, spleen, liver and mesenteric lymph nodes of three species of cetaceans: Atlantic spotted dolphins (*Stenella frontalis*), striped dolphins (*Stenella coeruleoalba*) and fin whale (*Balaenoptera physalus*), most of them stranded alive in the shores of the Canary Islands.

The IL-1alpha, IL-1beta, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-alpha, IFN-gamma and CD25 mAbs were used. In serial sections, anti-human CD3, IgG, and lysozyme were used to label T and B cells, and macrophages/monocytes respectively. The expression of cytokines varied in intensity and number of immunolabelled cells in the different organs examined. The anti-human IL-1alpha, IL-1beta, IL-2, IL-6, IL-8, IL-10, TNF-alpha, CD25 and anti-bovine IL-4, IFN-gamma mAbs yielded immunolabelling in cetacean lymph node tissue sections similar to that obtained in the species of origin and other species, a finding also reported in terrestrial mammals. Macrophages and lymphocytes were the most common cell population immunolabelled with the anti-cytokine mAbs. The results obtained in this study suggest that these antibodies cross react with cetacean cytokines and therefore they are suitable to evaluate cytokine expression in snap frozen tissue samples of the species of cetacean tested.

P82

ECCRINE CARCINOMA AND NEPHROSCLEROSIS IN AN ASIAN ELEPHANT (*ELEPHAS MAXIMUS*). CASE REPORT

Željko Grabarević, Andrea Gudan, Marko Hohšteter, Branka Artuković, Petar Džaja, Ingeborg Bata

University of Zagreb, Croatia

A case of eccrine carcinoma of the interdigital glands and nephrosclerosis followed with uremia in the 39-year-old female Asian elephant from Zagreb zoo is described. The tumor from the area between toenails of the right forefoot was surgically removed three years ago (2003.) and the histopathological diagnosis was eccrine carcinoma. During the last year the animal showed signs of kidney insufficiency which was followed with sudden death (May, 2006.) At postmortem severe nephrosclerosis of the both kidneys, lung edema, heart dilatation with hydropericardium, gastric healed and recent ulcers, liver fibrosis and atrophy of the lymphnodes and spleen was noted. Histopathologically, extirpated

tumor was compound eccrine carcinoma of the interdigital glands. Necrosis, papillary intraacinary proliferations, multilayer epithelium and myoepithelial cells proliferation were seen. Mitoses were rarely noted. Previous excision was succesfull because no sign of that tumor was noted on the necropsy. Diffuse glomerular- and tubular sclerosis with signs of purulent inflammation of the collecting ducts and micronephrolithiasis, severe lung edema, vacuolisation and lipofuscinosis of the myocardial cells, lymphocytic depletion in the spleen and lymphnodes, pancreatic atrophy with adenomatous hyperplasia of the islet-cells, interstitial hepatitis and gastric ulceration were found. In the dermal parts of the foot necrotic vasculitis with thrombosis was also noted. It should be notead also that BUN and creatinine were significantly elevated (blood urea nitrogen - 55.6 /2.165/ mmol/l, creatinine - 543 /141.44/ micromol/l).

P83

COMPARISON OF ANTIBODIES FOR CETACEAN CENTRAL NERVOUS SYSTEM (CNS) TISSUES

*Mariña Méndez, Eva Sierra, Manuel Arbelo, Antonio Fernández.
Veterinary School, University of Las Palmas de Gran Canaria, Spain*

The aim of the study was to identify diagnostic antibodies in cetacean Central Nervous System (CNS) tissues, and to homogenize the processes applied to cetacean samples, in order to get more information about those animals, looking for methods of improvement the identification /diagnosis of cetacean disorders.

CNS sections from a total of twelve cetacean stranded in Canary Islands coasts were chosen from archived formalin-fixed and paraffin-embedded samples tissues. The samples were taken from brain, olive nucleous, spinal cord and some central nerves.

To assess a reliable immunostaining for those tissues, different protocols were used for twelve primary antibodies: Vim (vimentin), Syn (synaptophysin), GFAP (Glial Fibrillary Acid Protein), HSP-70 (70kD-Heat Shock Protein), NSE (Neuron-Specific enolase), Beta-AP (Beta Amyloid Protein), MH (Myeloid/Histiocyte), S100 (S100 protein), FVIII (Factor VIII/ Von Willebrand), C-Jun (Jun protein) and C-Fos (Fos protein).

Peroxidase technique with aminoethylcarbazol or with diaminobenzidine as chromogens were used. All cases were also processed and stained routenately with hematoxilyn and eosin.

Each slide were assessed under light microscopic exam in order to obtain the most objective data as possible; they were evaluated in terms of sensitivity, specific and no specific background staining. An specific immunorreaction

was obtained with GFAP, HSP-70, NSE, MH, S100, FVIII, C-Jun and C-Fos antibodies in cetacean CNS tissues.

These antibodies are being investigated for their usefulness at the detection in Central nervous system tissue of different markers, and seem to be suitable for detection of possible disorders in cetaceans.

P84

DIAGNOSIS OF AA AMYLOIDOSIS IN LIVING CHEETAHS.

Yumi Une, Miyuki Fujino, Shoei Fu, Keiichi Higuchi

Azabu University, Japan

Introduction: AA Amyloidosis associated with chronic inflammation is the leading cause of death in cheetahs, which are a designated endangered species. Amyloid deposits have been identified in 95% of cheetahs dying in captivity. Accumulated stress and factors relating to the breeding environment, in particular transmission of amyloid fibril, are thought to bring about the onset of amyloidosis, but the cause remains to be clarified. To further pursue the pathogenesis and a possible treatment for this disease, we considered methods for diagnosing AA amyloidosis in living cheetahs.

Materials and Methods : 1) Detection of amyloid fibril from feces: Amyloid fibril was extracted from the liver of a cheetah which died of amyloidosis (C-68), using the water extraction method of Pras. The extracted fraction was partially broken down with 0.1N NaOH, then inoculated into rabbit to produce anti-cheetah AA amyloid antibody. Testing for the presence of amyloid fibril in the fresh feces of 18 living cheetahs and the intestinal contents of 2 dead cheetahs (C-67 and C-68) was carried out by Western blotting using this antibody. 2) Detection of SAA: SAA was isolated from the blood serum of cheetah C-68 and used to prepare SAA antibody. This was then used to test for the presence of SAA in the serum of 30 living cheetahs by Western blotting. The extent of amyloid deposition throughout all organs of the dead cheetahs C-67 and C-68 was also determined pathologically.

Results and Discussion: 1) Detection of amyloid fibril from feces: AA amyloid fibril was detected in the feces of 15 of 18 live cheetahs (83.3%) and the intestinal contents of dead cheetah C-68, but not of C-67. Pathological analysis showed the amyloid score for C-67 was intestine 0, kidney 2, liver 0, whereas for C-68 it was intestine 1, kidney 4, liver 3. These results suggest that digestive tract-related amyloidosis may be detectable by Western blotting analysis using this antibody from feces, and that this may be a useful tool for predicting the incidence of amyloidosis in living cheetahs with reasonably high probability, since 85.7% of the amyloid deposits in cheetahs dying from this disease are found in the gastrointestinal tract. 2) Detection of SAA: SAA was detected in

only 4 of 30 live cheetahs (13.3%), and in only 3 of the 15 cheetahs in which deposition of amyloid in the digestive tract had been strongly suggested by the presence of AA amyloid fibril in their feces. Increased levels of serum SAA are believed to accompany amyloid deposition and amyloidosis in other species. However in the cheetah, it may be that serum SAA does not increase under such conditions.

Conclusion: Our results suggest that feces testing for amyloid fibril, using anti-cheetah AA amyloid antibody, may be a useful non-invasive, highly specific method for diagnosing AA amyloidosis in living cheetahs with relatively high accuracy.

P85

HYPOTHYROIDISM, GOUT, AND AMYLOIDOSIS IN A CAPTIVE TURTLE - RED-EARED SLIDER (TRACHEMYS SCRIPTA ELEGANS)

Cătoi Cornel, Cătoi Adriana Florinela, Iacob Sorina

University of Agricultural Science and Veterinary Medicine, Cluj-Napoca, Romania

A body of Red-eared Slider turtle without any history data was received in department of Pathology. In gross examination tumefaction of the dermis and subcutis was obvious on the head, neck and legs. The necropsy showed enlarged, semitransparent thyroid gland, marked myxedema, enlarged, yellow, soft and fatty liver. The following changes were found in routine histology. The thyroid gland showed large follicular lumen, cubic and flat epithelium and homogenous, acidophilic colloid – colloidal goiter. The liver showed diffuse, uniform, fatty change with large and small droplets of lipids and necrosis. Some hepatic cells contain intracytoplasmic acidophilic body inclusions, apparently of viral origin. Slightly basophilic crystals surrounded or included in giant cells were observed in liver, diagnosed as gout tophi. Touton giant cells were also identified in liver. In the kidney, the perivascular spaces were dilated by accumulation of eosinophilic material, Congo red positive - amyloid. Amyloidosis was extensive an intensive, affecting glomeruli and tubule, and associated with atrophy, degeneration and necrosis of epithelium. In this case report the diagnosis of hypothyroidism is supported by colloidal goiter and myxedema. In this context, the fatty liver suggests hyperlipidemia and the presence of Touton giant cells in liver suggest persistent hypercholesterolemia. We have not informations about alimentation in this turtle but the nutritional origin of the goiter is more probable. The pathogenesis of the renal amyloidosis is not clear but the visceral gout observed in liver may be secondary to the failure of tubular secretion of uric acid. The intracytoplasmic acidophilic body inclusions, apparently of viral origin, were not investigated by additional techniques.

POXVIRUS INFECTION IN A GREAT TIT (PARUS MAJOR)

Andrea Gruber, Elvira Grabensteiner, Jolanta Kolodziejek, Norbert Nowotny, Gerhard Loupal

University of Veterinary Medicine, Veterinärplatz 1, Austria

Four great tits (*Parus major*) with nodular lesions suggestive of Pox could be observed in a garden in Austria. One bird out of these was caught and brought to University for examination. The bird showed multiple yellowish confluating pedunculated nodules, 3 to 15 mm in diameter, around the left eye, nearly covering the whole left side of the face reaching down to the breast. Due to its poor condition, the bird was euthanized and a necropsy was performed. Histological examination of the nodules revealed severe hyperplasia of the epidermis. The cells of stratum spinosum were swollen and most of them bore homogenous, eosinophilic, round to polygonal viral intracytoplasmic inclusions (Bollinger bodies). Ultrastructural examination revealed numerous oval virus particles with often dumb-bell shaped cores. Based on clinical, histopathological and ultrastructural findings, a poxvirus infection was diagnosed. This is the first report of avian pox in this species in Austria.

P87

HISTOLOGICAL OBSERVATIONS OF BOVINE TUBERCULOSIS IN LUNG AND LYMPH NODE FROM UK DEER SPECIES

Linda Johnson, Ernesto Liebana, Julie Gough, Alejandro Nunez, Yvonne Spencer, Keith Jahans, Arun Wangoo, Richard Clifton-Hadley, Alastair Ward, Richard Delahay, Graham Smith, Alex Barlow

VLA-Weybridge, UK

The importance of wild deer as a wildlife reservoir for bovine tuberculosis infection in UK cattle is under investigation. A retrospective histopathology study of tuberculosis positive deer examined cases obtained from two surveys in the past 5 years. Samples were not random throughout the UK, but rather concentrated in the Southwest, the region most affected by bovine tuberculosis in cattle. Deer stalkers provided most tissue samples. A total of 120 deer, comprising 4 species, were studied and most samples were lymph node and lung tissue. Unlike tuberculosis granulomas in cattle, many of the granulomas in the deer tissue samples lacked complete fibrotic capsules. Fifty-nine cases (49%) had tissues with very high numbers of acid-fast bacilli, defined as more than 50 bacilli in a tissue counted in one plane of section. Fifty-four of these high bacilli deer cases (91%) had many hundreds of densely packed bacilli present in granulomatous inflammation. Species representation of these high bacilli cases were: Red- 65%, Roe- 36%, Fallow- 36%, unknown species- 64%

and 1 Muntjac (100%). These findings suggest tuberculosis lesions in deer may contain far greater numbers of bacilli than commonly reported in cattle and is consistent with some deer species being more likely to develop granulomas with abundant numbers of bacilli.

P88

RHABDOMYOLYSIS AND MYOGLOBINURIC ACUTE RENAL FAILURE (CAPTURE MYOPATHY) IN A STRIPED DOLPHIN (STENELLA COERULEOALBA)

A. Fernández, P. Herráez, E. Sierra, M. Arbelo, J.R. Jaber, A. Espinosa de los Monteros

University of Las Palmas de Gran Canaria, Spain

We report a delayed myoglobinuric capture myopathy in an adult striped dolphin found stranded alive on the coast of Fuerteventura (Canary Islands, Spain). The animal was kept in a small private swimming pool and transported to Gran Canaria dying 48 hours after the stranding, with no specific clinical signs. During necropsy, only cardiac lesions were detected and consisted with multifocal, whitish subendocardial areas of myocardium. Histologically, the main lesions were related to skeletal and cardiac muscle and kidneys, where acute rhabdomyolysis affecting both cardiac and skeletal muscle, and severe pigmentary nephrosis were observed. Immunohistochemically, degenerated muscle fibers showed complete depletion of myoglobin and an intracytoplasmatic immunoreaction for fibrinogen. Orange-red pigmented casts presented in the tubular lumen were strongly labelled by myoglobin. To our knowledge, no previous pathologic descriptions of capture myopathy with myoglobinuric tubular necrosis have been reported in stranded cetaceans. Stress, exertion, trauma and crush injury caused during the stranding, restrain and transportation were the main causes of rhabdomyolysis in this case. Capture Myopathy should be included in the differential diagnosis of acute death in active stranded cetaceans, emphasizing the importance of an exhaustive examination of skeletal muscle in order to find degenerative changes and the usefulness of the immunohistochemical demonstration of myoglobin and fibrinogen as markers of early ischemic muscle damage and myoglobinuric renal failure in dolphins.

P89

INTRACYTOPLASMIC EOSINOPHILIC GLOBULES IN HEPATOCYTES OF BY-CAUGHT HARBOUR PORPOISES (PHOCOENA PHOCOENA)

Ana Godinho, Paul Jepson, Robert Deaville, Mariña Méndez, Pedro Herráez, Antonio Espinosa

Veterinary School, University of Las Palmas de Gran Canaria, Spain

Intracytoplasmic inclusions have previously been described in cetacean hepatocytes. In those studies it was concluded that the globular inclusions were more frequent in animals that stranded alive with acute liver congestion. These globules are essentially composed of glycoproteins that include alpha-1-antitrypsin and fibrinogen (acute phase proteins). These lesions are not species-specific because they have been described in 15 cetacean species. In this investigation we studied 27 liver samples that were collected from fresh or slightly autolytic harbour porpoises (*Phocoena phocoena*) by-caught in gillnet fisheries in UK waters. Histologically, intracytoplasmic hyaline eosinophilic globules were found in 26 of 27 livers with the same eosinophilic characteristics as those reported in other species but with a low degree of liver congestion. Fibrinogen was demonstrated immunohistochemically in all 26 animals which had globules. The only negative liver without globules belonged to a neonate porpoise. These results will be presented together with a discussion of their possible pathogenic mechanism.

P90

SPLENIC FOLLICULAR VASCULITIS IN THE IBERIAN LYNX

Maria-Ángeles Jiménez, Belén Sánchez, Pilar García, Laura Peña

University of Madrid, Spain

The Iberian lynx is considered the most endangered felid species in the world. Its situation is critical due to habitat loss and fragmentation, being its only location nowadays a metapopulation in southwestern Spain. The remaining population of less than 150 animals has signs of inbreeding. Recent studies of our group have demonstrated a high prevalence of lymphoid depletion and membranous glomerulonephritis in these animals. Also, the presence of follicular hyalinosis in spleens has been detected. Our objective was to study this lesion by means of histology, immunohistochemistry and electron microscopy in order to determine its composition and pathogenesis. A retrospective study of the spleens of 20 Iberian lynxes necropsied during the years 1998-2005 was carried out. Samples were stained with H-E, PAS, Congo Red and Masson's trichromic and silver staining. Immunohistochemistry was done against IgA, IgM, IgG, type I, type III, type IV and type VIII collagens, fibronectin, laminin, and Von Willebrand factor. Follicular hyalinosis was detected in 19 of the 20 animals. The histopathology and immunohistochemistry revealed a vascular origin of the hyaline deposits due to the thickening of the vascular basement membrane and a progression of the lesion to sclerosis. IgA, IgM, IgG immunostaining was present in the deposits as well as other substances (type VIII collagen, type IV collagen, laminin, fibronectin, type I collagen and type III collagen). The ultrastructural study confirmed the sclerosis and the presence of electron dense

bodies in the basement membranes of centro-follicular arterioles compatible with immune complexes. These lesions are indicative of the existence of an immune mediated vasculitis in the Iberian lynx.

P91

OCULAR LESIONS OF TOXOPLASMOSIS IN A COMMON WOMBAT

Christina McCowan, Kate Bodley, Tony Ross

University of Melbourne, Australia

A juvenile common wombat was found wandering in daylight by the roadside, and taken to a wildlife sanctuary. The animal had bilateral cataracts and was blind, but in excellent body condition and otherwise seemed well. Nutritional cataract was suspected. Neither release nor rehousing were feasible, and euthanasia was performed. Tissues were collected at autopsy. Protozoal cysts consistent with *Toxoplasma gondii*, and confirmed to be such with immunohistochemistry, were found in several organs, but lesions were present only in the eyes, which showed severe bilateral inflammation and degeneration in the retina and choroid, the optic nerve and extraocular muscles. The lens was cataractous. The anterior segments of the eyes showed relatively mild inflammatory changes.

Australian marsupials are uniquely sensitive to infection with this organism, presumably as a result of evolutionary isolation from felids. Ocular lesions have been described in macropods, but this is the first report of ocular lesions in a wombat.

P92

EVIDENCE OF SEMINOMA PRECURSOR LESIONS (CIS) IN CONTROLATERAL TESTES OF DOG WITH SEMINOMA.

Elena Riccardi, Valeria Grieco, Valentina Ciampi, Mario Finazzi

Università degli studi di Milano, Italy

INTRODUCTION: Seminoma is frequent in human as in canine species. In the human WHO classification of tumours, 2 type of seminoma are described: classical seminomas (SE) and spermatocytic seminoma (SS). SE derives from undifferentiated gonocytes contained in testicular tubuli, arrested during embryonic development. These precursor lesions are called carcinoma in situ (CIS). CIS and SE cells are PAS positive and immunohistochemically positive for placental alkaline phosphatase (PLAP). On the contrary, SS arise from spermatocytes and are composed of PAS and PALP negative neoplastic cells. In human, CIS are detected in the testes with SE and in the controlateral ones but they seem not present in the patients with SS.

AIM OF THE STUDY AND MATERIAL METHODS: In order to asses the

presence of CIS in the dog, in this study testes with seminoma and the controlateral testes from 10 dogs were considered. All the samples, formalin fixed, were examined histologically with EE and PAS and immunohistochemically (ABC) for PLAP.

RESULTS: 5 samples, showing intratubular pattern or initial interstitial invasion, were both PAS and PALP positive and so considered SE. 5 samples, showing diffuse pattern, were both PAS and PALP negative and diagnosed as SS. CIS was found in 3/5 SE and in 3/5 SS. In the controlateral testes, CIS was found in 4/5 dogs with SE and in 4/5 dog with SS.

DISCUSSION: In human, CIS is considered a precursor lesion only for SE and its presence suggests an impaired gonadal development. In this study, CIS was found both in dogs with SE as with SS. Testes with CIS may be more prone to develop germ cells tumours other than SE, such as SS. In human, environmental chemicals are suggested as possible inducers of testicular dysgenesis and further study are required to assess their role also in canine species.

P93

INTRAGASTRIC INFECTION AS MODEL FOR THE STUDY OF THE CHLAMYDIAL ENZOOTIC ABORTION

Antonio J. Buendía, Nieves Ortega, Carlos M. Martínez, Joaquín Sánchez, Laura Del Rio, Jesus Salinas, Jose A. Navarro

Universidad de Murcia, Spain

Chlamydomphila abortus is able to colonize the placenta of small ruminants, and causing abortion during the last third of gestation. Experimental infection by different inoculation routes of pregnant ewes with *C. abortus* causes late term abortions regardless of the moment of infection. Experimental mouse models of parenteral inoculation have been developed to study the pathogenesis of the abortion, however those models does not explain other features associated to *C. abortus* infections. It will be interesting an alternative route of infection similar to those that occurred in the natural infection investigate the relationship between latency, pregnancy and abortion. To establish a mouse model of intragastric infection with *C. abortus*, pregnant mice were intragastric inoculated at day 7 of gestation. Mice were killed at day 9 postinfección, and after abortion or parturition. Samples from uterus, placenta and other organs were collected and immunostained with the ABC technique using an anti-chlamydial LPS antibody. A model of intraperitoneal infection served as control of abortion. When mice were killed at day 9 p.i. (day 16 of pregnancy), two of eight mice showed a great amount of chlamydial antigen with large inclusions associated to a suppurative placentitis, severe hepatitis and splenomegaly. The other mice in the group showed mild hepatitis and weaker immunoreaction. In the placenta

very mild lesions with weak immunoreaction was observed. When mice were let finish the gestation, three of ten infected mice aborted at day 19 to 20 of pregnancy. The litter size of the normal parturitions in the group was 10-11 mice. Mice killed after abortion or normal parturition showed immunoreaction against chlamydial antigen in uterus, liver and spleen. In conclusion, intragastric infection of pregnant mice caused systemic dissemination of *C. abortus*, which reached the placenta, causing late term abortions similar to those observed in the natural infection in small ruminants.

P94

EVALUATION OF PROSTATE BIOPSY COMPLICATION AFTER USING MEAN OF COOK NEEDLE BIOPSY METHOD.

Ali Rezaie, Arash Khaki, A Hajalilo

Department of Vet Pathology, Islamic Azad University, Tabriz Branch, Iran

Introduction: one of the study and evaluation of tissue and glands for diseases and cancers, whereby diagnosis is biopsy so it is important for physicians and surgeons to obtain best result after operations. For reaches this means we study prostate gland biopsy by using mean of cook needle in dog.

Material and methods: the study was performed by needle biopsy from region with sonography guide, clinical care sustained for 15 days, than for CBC&Acid phosphatase & urine sediment measurement the blood sample and urine samples were analyzed at the day 0(control)1,2,3,4,5,9,12 &15.for histopathological evaluation biopsy of prostate gland was done.

Results: In clinical care there wasn't any complication except oedema in the perineal region and in exploratory sonography only in one case the hyperplasia of gland were showed. There was no significant difference in CBC results and neither in serumic ACP. The increase of serumic levels of ACP were in the normal range of dogs (<13 IU/lit).urine sediment analysis revealed high volume of epithelial cells, cellular cast, granular and hyaline cast. In pathological findings only capsular and glandular hemorrhage and healing process were observed.

Conclusion: From the clinical, paraclinical,pathologic and statistical analysis of this results it seems that complication of needle biopsy is limited and there weren't any necrotic tissues and the architecture of gland were safe and this method is recommended for clinical diagnosis.

Key words: Dog,needle biopsy,prostate.

THE HISTOPATHOLOGICAL EFFECTS OF SUPEROVULATORY DRUGS ON THE MOUSE ENDOMETRIAL STROMA

Farzad Rajaei, Bazy Parviz, Zarea mohammad ali

Qazvin University of Medical Sciences, Iran

Introduction .

Usage of stimulatory drugs for harvesting more oocytes is very common during Assisted Reproductive Technique protocols. Studies have recently shown that the rate of successful implantation in stimulatory cycles is less than normal cycles due to detrimental effect of the superovulatory drugs on the endometrium. Since it has been shown that the endometrial basement membrane has a main role in implantation of the embryo, the possible effects of stimulatory drugs on the ultrastructures of endometrial basement membrane were investigated

Methods :

The endometrial samples were obtained from 30 normally cycling mouse and from 30 superovulated with PMSG(10 IU) and hCG(10 IU) at the time of implantation(120 h after hCG injection). The specimens were processed for electron microscopic studies. Qualitative and quantitative (Morphological and morphometric) studies were carried out on the micrographs. The data have been compared using statistical methods.

Results:

Morphological findings evaluated based on glycogen, vacuoles and nuclear euchromatin and morphometric findings have also shown that in case group, volume fraction of nucleus, RER, mitochondrium, glycogen to cell and euchromatin to nucleus had statistically no significant difference.

conclusions:

These results (based on present study findings) suggest that ovulation drugs have no effects on stroma of endometrial tissues at the time of implantation.

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

THE HISTOPATHOLOGICAL EFFECTS OF SUPEROVULATORY DRUGS GNRHA/PMSG/HCG ON THE GLANDULAR EPITHELIUM OF UTERINE ENDOMETRIAL TISSUE IN RAT

Parviz Bazzzy, Farzad Rajaei, Khaki Arash, Banafsheh Esmaealzadeh

Bushehr University of Medical Sciences, Iran

Introduction

Using of stimulatory drugs such as : GnRH α / PMSG / HCG for induction of superovulation and harvesting more oocytes is very common during Assisted Reproductive Techniques (ART) protocols. Studies and experiences have recently shown that the rate of successful implantation in stimulatory cycles is less than natural cycles. On the other hand, it is also shown that endometrium has a main role in implantation.

Methods: At the present study we used an experience animal (Rat) as a model. We have investigated the possible effects of above mentioned drugs on ultrastructures of endometrial tissues (Glandular epithelium). For this purpose, endometrial biopsies were obtained from female rats (N=30) which were under superovulation treatment and those rats which never given any drugs (N=30). The specimens were processed for electronmicroscopic studies. Qualitative and quantitative (morphologic and morphometric) studies were carried out on electron micrographs.

The data have been compared using statistically methods.

Results: Morphological findings evaluated based on three structures namely : nuclear channel system (NCS), giant mitochondria (GM) and glycogen vacuoles. Additionally, nuclear euchromatin. Morphometric findings has also shown that in case group, volume fraction of nucleus RER, mitochondrion, glycogen to cell and euchromatin to nucleus had statistically difference significance.

Discussion and conclusions: These results (based on present study findings) suggest that ovulation drugs have a negative effects on endometrial tissues at the time of implantation which may lead to low implantation rate.

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

IMMUNOHISTOCHEMICAL DIAGNOSIS OF INFECTIOUS ABORTIONS IN SMALL RUMINANTS

Carlos M. Martínez, Joaquín Sánchez, Antonio J. Buendía, Esmeralda Minguijón, Ana García-Pérez, Jose A. Navarro

Universidad de Murcia, Spain

Infectious agents are the most prevalent cause of abortion in small ruminants, but only a low percentage of the samples submitted to the laboratories can be diagnosed. Similar signs and gross or histopathological lesions are observed in mostly all the placental infections. Furthermore, co-infections are a common feature of these placental infections. Thus, an accurate differential diagnosis is necessary in this type of infections. A additional problem is the potential zoonotic risk associated. The infectious agents are able to colonize the cotyledonary trophoblast of placenta, an immunodeprived area that increased the multiplication, leading to the development of a necrotic placentitis. The availability of commercial antibodies against infectious agents causing abortion, let the etiologic diagnosis by immunohistochemical techniques in formalin-fixed, and subsequently paraffin-embedded cotyledons from aborted placentas. In The present study a wide panel of commercial antibodies was tested firstly on previously diagnosed samples using an ABC technique to assure the specificity of the immunoreaction against *Brucella melitensis*, *Chlamydia abortus*, *Coxiella burnetii*, *Listeria monocytogenes*, *Salmonella abortusovis* and *Toxoplasma gondii*. Once established the suitability of the antibodies, the panel was applied to twenty outbreaks of abortion (ten from ovine flocks and ten from caprine flocks). All the cases included in this study, showed immunoreaction against at least one antibody. Placentitis associated to the presence of the antigen, confirmed the implication of the correspondent infectious agent as causing of abortion. *C. abortus* antigen was detected in the cotyledons submitted from 19 of the 20 flocks, alone or together with other pathogens, as *S. abortusovis* or *C. burnetii* confirming the importance of the chlamydial aetiology in the ovine or caprine abortion. The results of the present study show the usefulness of the immunohistochemical technique as a safe and fast method for the diagnosis of infectious abortion in small ruminants.

P98

CASE OF OVARIAN CYSTS IN GUINEA PIG

Izabella Babińska, Anna Snarska, Sylwia Lew, Joanna Wojtacka

University Warmia and Mazury, Poland

Introduction: Pathological conditions of the reproductive system are often observed in guinea pigs (*Cavia porcellus*) and ovarian cysts are among them.

Clinical signs: At the age of 9 years, through 10 months rapidly growing abdomen was observed. This condition severely impeded movements 4 months before death. Fur coat was fragile, and mat, numerous alopecias were noted. There was non-curable trichophytosis on the nose. Oral mucosa was pale and aphthae and reddening were noted on the buccal side. Dental caries was also noted. Tumor of 4 x 2.5 x 2 cm was removed from left inguen earlier. Estrus stopped after the operation.

Anatomopathological and histopathological lesions: the ovaries had cystic structure. They were enlarged to the size of: right - 18 x 10 x 8 cm and left - 12 x 6 x 4 cm. The quantity of liquid was about 700 ml. Presence of liquid in body caves and in pericardial sac, hyperaemia of parenchymatous organs, heart enlargement and thickening of the walls of left ventricle were also noted. Thin wall cyst of about 1.5 cm filled with liquid was noted in right kidney. It was noted microscopically (HE staining) that the wall of the ovarian cyst is internally covered with single epithelium layer and it contains thin layer of connective tissue that did not have many cells and vessels. Parenchymatous degeneration, necrotic focuses, hyperaemia, extravasations, proliferation and oedema of the star cells and numerous binuclear hepatocytes were noted in the liver. Parenchymatous degeneration, droplet and hyaline necrosis of the cells of tubular epithelium and hyperaemia, extravasation and glomerulonephritis were noted in the kidneys. Haemosyderosis was noted in the spleen. Catarrhal gastritis and enteritis in the mucosa were also noted. There were parenchymatous degeneration and hyperaemia in the heart muscle. It was also stated that the tumor (3 x 2 x 1 cm) located close to mammary gland was adenocarcinoma.

P99

BIOMETRICAL AND PATHOLOGICAL STUDIES ON TESTES OF BULLS

Muhammad Hassan

Government of Sindh Pakistan

200 testes of cow bulls were collected from Mirpurkhas abattoir and were examined for biometrical values and pathological conditions. Out of 200 testes, 54 were collected from calves (below 01 Year of age), 97 from steers (up to 03 Years of age) and 49 testes from adults (age over 03 Years). Out of 200 testes 57 were found affected (11 calves, 29 steers and 17 adult cow bulls).

The mean length of left and right testes respectively was measured as 6.075 ± 0.214 cm and 5.763 ± 0.173 cm in calves, 8.602 ± 0.142 cm and 8.466 ± 0.143 cm in steers and 10.839 ± 0.097 cm and 10.701 ± 0.102 cm in adults. The mean breadth of left and right testes respectively was measured as 3.398 ± 0.173 cm and 3.269 ± 0.146 cm in calves, 4.863 ± 0.099 cm and 4.793 ± 0.870 cm in steers

and 6.206 ± 0.134 cm and 5.919 ± 0.116 cm in adults. The mean circumference of left and right testes respectively was measured as 8.483 ± 0.309 cm and 8.226 ± 0.213 cm in calves, 12.612 ± 0.264 cm and 12.493 ± 0.259 cm in steers and 15.463 ± 0.326 cm and 15.296 ± 0.300 cm in adult. The mean weight of left and right testes respectively was recorded as 16.841 ± 1.962 gm and 16.639 ± 0.1981 gm in calves, 62.689 ± 2.709 gm and 61.009 ± 2.369 gm in steers and 123.937 ± 5.123 gm and 121.813 ± 5.269 gm in adults.

Gross pathological lesions in affected testes varied and recorded; Orchitis 0.5 % in calves, 2.5 % in steers and 2.0 % in adults; Hypoplasia 0.5 % in calves, 1.5 % in steers and 1.0 % in adults; Cryptorchidism 1.5 % in calves, 2.5 % in steers and 1.5 % in adults; Adhesions 1.0 % in calves, 3.5 % in steers and 2.0 % in adults; Spermatocele only 1.0 % in calves and 1.0 % in steers; Degeneration 1.0 % in calves, 2.0 % in steers and 1.0 % in adults and Hydrocele 1.5 % in steers and 1.0 % in adults were seen in affected testes during study period.

P100

EXPRESSION OF TRANSFORMING GROWTH FACTOR B (TGFB) AND INSULIN GROWTH FACTOR-I (IGF-I) IN THE OVINE GENITAL TRACT DURING THE PREIMPLANTATION PERIOD AFTER TWO DIFFERENT ESTRUS SYNCHRONIZATION TECHNIQUES

M^aPaz Vilar, Pilar García, M^a Ángeles Sánchez, Belén Sánchez, Rosa Ana García, Juana M^a Flores

Complutense University of Madrid, Spain

The aim of this study was to determine the pattern of expression for Transforming Growth Factor b (TGFB) and Insulin Growth Factor-I (IGF-I) proteins in the oviduct and uterus during ovine preimplantation (days 4 and 7 after mating). We used 40 adult Manchega sheep. In 20 of the animals (Group PA) estrus was synchronised using cloprostenol, while the remaining ewes (Group P) were synchronised with progestagens. Immunohistochemical determination of TGFB and IGF-I was performed on dewaxed sections of oviduct and uterus using the streptoavidin-biotin peroxidase method. Different compartments of the oviduct (epithelium, stroma and muscle of the isthmus) and uterus (luminal epithelium, superficial glands, superficial stroma, deep glands, deep stroma and myometrium) were evaluated. Both groups presented the greatest expression of TGFB in the myometrium on days 4 and 7. Group PA presented higher uterine and oviductal expression of TGFB on day 7 than on day 4 in every compartment studied, being the difference of expression in the luminal epithelium of the uterus statistically significant ($p < 0.05$). On the other hand, Group P presented higher expression of TGFB on day 4 in almost every compartment of the oviduct and

uterus studied, being the expression in the epithelium of the isthmus significant ($p = 0.05$). IGF-I was expressed only in the uterine stroma in both days in Group P and Group PA, being negative all other compartments. A decrease in the expression of IGF-I was observed in Group P, being lower on day 7, although it was not statistically significant. The different pattern of expression of these growth factors in both groups suggests their involvement in the endometrial changes and in supporting the progression of early embryos throughout the preimplantation period studied.

This work was supported by the Spanish Comision Interministerial of Science and Technology (CYCYT) AGL 2001-196.

P101

TARGETED INACTIVATION OF INTEGRIN-LINKED KINASE IN PODOCYTES RESULTS IN PROGRESSIVE GLOMERULOSCLEROSIS IN MICE

Rüdiger Wanke, Nadja Herbach, Chiraz El-Aouni, Simone Blattner, Maria Pia Rastaldi and Matthias Kretzler

University of Munich, Germany

Background: Podocyte function is essential for the maintenance of the glomerular filtration barrier. Cytoskeletal disruptions and alterations in podocyte cell-cell and cell-matrix contacts are key events in progressive glomerular failure. Integrin-linked kinase (ILK) has been implicated in podocyte cell-matrix interactions and is induced in proteinuria. In this study, the role of ILK in podocytes was investigated by podocyte-specific deletion of ILK.

Methods: Mice expressing the Cre recombinase under the control of the podocin promoter in a podocyte specific manner (podocin-Cre) were crossed with ILK-floxed mice. Urinary protein analyses, light and electron microscopy were applied to characterize kidney alterations of mice harbouring a podocyte-specific deletion of ILK (podoILK^{-/-} mice). Cre-negative littermates served as controls.

Results: PCR analysis of DNA prepared from kidney cortex demonstrated Cre-mediated excision of the ILK locus. The lack of ILK protein in podocytes was shown using immunohistochemistry. Selective albuminuria was first evident in three-week-old podoILK^{-/-} mice and the true harmonic mean thickness of the glomerular basement membrane (gbm) was significantly increased vs. controls. At four weeks of age, glomerular lesions included mesangial expansion with increased matrix deposition and distortion of glomerular capillaries. At eight weeks of age, podoILK^{-/-} mice showed non-selective proteinuria, focal and segmental glomerulosclerosis as well as tubulo-interstitial lesions. Podocyte

changes of eight-week-old podoILK^{-/-} mice included hypertrophy, microvillous transformation and protrusions of the epithelial surface, severe vacuolization, as well as widespread foot process effacement and focal detachment from the gbm. The gbm exhibited diffuse and irregular thickening and showed electron-lucent areas, and was sometimes collapsed and tortuous. At 16 weeks of age, the animals exhibited end-stage renal disease.

Conclusion: Using the Cre-LoxP system, the podocyte-specific ILK deletion was generated and resulted in progressive glomerulosclerosis. This study indicates a direct role of ILK in gbm structure and podocyte function.

P102

IN VITRO TRANSMISSION OF BOID INCLUSION BODY DISEASE (BIBD)

Udo Hetzel, Ian Ashpole, Roz Wright, Juan Alberto Morales, Lance Jepson
University of Liverpool, UK

Inclusion Body Disease (IBD), affecting snakes within the family boidae is a highly infectious and lethal disease, the suspected aetiology of which is a viral infection. The morphological hallmarks of IBD are eosinophilic intracytoplasmic inclusions within cells in blood, nervous system, respiratory, gastro-intestinal and uro-genital tract.

Outbreaks of the disease have exclusively been reported in captive boid snakes from zoological gardens and private collections in Europe, the US and Costa Rica. Neonates, juveniles and adults can be affected and horizontal transmission via direct contact or mites as vertical transmission are considered likely. At present, the ante-mortem diagnosis relies on blood smears or liver biopsies.

To develop more reliable diagnostic tests, permanent tissue cultures were established from juvenile, *Boa constrictors* with and without IBD. In vitro transmission studies were performed, using light and transmission electron microscopy to identify the inclusions.

Transmission was successful with supernatant from IBD tissue cultures filtered through 400, 200, and 100 nm. The first inclusions were seen on day 3 p.i.; by day 6, inclusions of variable diameters (1-20 µm) developed. Filtration through 50 nm, 400 and 100 kD did not lead to infection, indicating that the infectious agent has a size between 50-100 nm.

Whole blood from a long-term IBD positive *Boa constrictor*, yielded positive results after the 3rd passage in tissue culture, with only few small intracytoplasmic inclusions developing. In none of the cultures, viral particles were detected in supernatants or cells.

Boid tissue cultures are a useful tool for studies on the aetiology of IBD and for diagnostic purposes, in particular in questionable cases. The differences observed

in the development of inclusions, using positive tissue culture supernatant or blood from an IBD positive animal could indicate adaptation to the individual host system or a variable pathogenicity of the infectious agent.

P103

USE OF DERMACID IN TREATMENT OF DERMATOMYCOSES IN SMALL DOMESTIC ANIMALS

Elena Sumenkowa

Institute Experimental Veterinary, Russia

A new preparation called Dermacid that we have developed and are presenting herein is a highly efficient means possessing fungicidal properties. It is not toxic for warm-blooded animals, possessing no cumulative properties and is a highly efficient means for treatment of dermatomycoses in cats and dogs.

P104

RENAL INTERSTITIAL FIBROSIS IN NEONATAL RATS INDUCED BY CISPLATIN

Jyoji Yamate, Yuuko Machida, Mitsuru Kuwamura, Takao Kotani

Osaka Prefecture University, Japan

Cisplatin (CDDP)-induced renal lesions in rats prove a useful model for analysis of the pathogenesis of post-tubular injury-renal interstitial fibrosis. This study investigated the histopathological changes in 10-day-old neonatal rats induced by a single injection of CDDP (4.5 mg/kg). Compared with age-matched controls, on post-injection (PI) days 1 to 6, the number of apoptotic cells, demonstrable with TUNEL method, was significantly increased in CDDP-treated neonates, and there was no marked epithelial necrosis nor fibrotic lesions. Fibrotic lesions began to be developed solitarily around some nephrons with dilated ducts in the corticomedullary junction on PI day 10 and the lesions became more prominent until PI day 20. Alpha-smooth muscle actin-positive myofibroblastic cells were seen exclusively in the fibrotic lesions. Additionally, the numbers of macrophages reacting with ED1 (specific for exudate macrophages), ED2 (for resident macrophages), and OX6 (recognizing MHC class II antigens expressed in antigen-presenting macrophages/dendritic cells) were significantly increased around the affected renal tubules. A greater immunoreaction for TGF-beta1 was seen mostly in the renal epithelial cells of CDDP-treated neonates. These findings indicated that macrophage populations and myofibroblastic cells as well as TGF-beta1 may be responsible for the production of neonatal renal interstitial fibrosis. Compared with CDDP-injected adult rats that develop extensive interstitial fibrosis (Yamate et al., J Comp Pathol, 1995), the formation of

fibrotic lesions was delayed, and the lesions were limited to the area around the affected nephrons; this could be attributable to differences in renal morphology between neonates and mature kidney of adult rats.

P105

APPLICATION OF BCG ANTIBODY FOR RAPID IMMUNOHISTOCHEMICAL DETECTION OF BACTERIA, FUNGI AND PROTOZOA IN FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUE SAMPLES

Levente Szeredi

Central Veterinary Institute, Hungary

Identification of various infectious agents in histological specimens requires special staining methods. The parallel use of different histochemical staining methods is time consuming and expensive. There are some reports about the successful application of BCG antibody in the immunohistochemical detection of various bacteria and fungi in skin biopsies. The aim of this study was to detect a broad range of bacteria, fungi and protozoa with immunohistochemical technique using rabbit, polyclonal BCG antibody.

Forty-four tissue samples of animal origin were fixed in formalin and embedded in paraffin: 35 tissue samples contained different bacteria species, 4 different species of fungi and 5 different species of protozoa. Three serial sections were prepared in each case. One was pre-treated with 0.1% pronase at 37°C for 10 minutes, the second was heated in a microwave oven at 750 W for 20 min in citrate buffer (pH 6.0) and to the last one BCG antibody was added directly without any pre-treatment.

Pre-treatment with microwaves was the most effective in case of almost every pathogens resulting in a minimal background staining. Strong or moderate positivity was observed in 23 bacterial, all fungal and 2 protozoa species, while weak reactions occurred in 2 bacterial species. Only 3 protozoal and 10 bacterial species, including *Leptospira* and *Mycoplasma*, showed no reaction in this test.

The above-described method can be used for the rapid detection of various bacteria, fungi and protozoa species in tissue samples. The test is especially helpful in cases where only formalin-fixed samples are available for laboratory examination. The simultaneous use of different histochemical staining methods can be replaced with this one single method in case of a broad range of microorganisms.

IDENTIFICATION OF INTERSTITIAL INFLAMMATORY CELLS IN THE KIDNEY OF OSBORNE-MENDEL RATS

Kinji Shiota, Tomoko Asano, Ryota Kashiwabara, Yosuke Sakurada
Azabu University, Japan

Introduction: Progressive glomerulonephropathy with severe proteinuria develops in Osborne-Mendel (OM) rats. The tubulo-interstitial lesions develop following glomerular lesion with age, and these might significantly contribute to the deterioration of renal function. This study was performed to identify the interstitial inflammatory cells for further studies on the mechanisms of tubulo-interstitial damage in OM rats.

Materials and Methods: The kidneys of 129 OM rats aged 3 to 35 weeks were examined. Paraffin sections were immunostained by antibodies against CD3, CD8, CD45R or ED-1. Serial sections of the kidneys were made and immunostained by each antibody. The first sections of serial ones were stained with hematoxylin-eosin (HE). Fifteen high-power fields of the cortex were randomly selected in the HE-stained sections and positive cells for each immuno-marker were counted at the same fields in the serial sections. As a preliminary study, ICAM-1 mRNA in the cortex was quantitatively evaluated by real-time PCR.

Results: Interstitial inflammation was initiated by T-lymphocyte infiltration at 5 weeks of age, and the number of T lymphocytes increased with age until 25-week-old in male rats. A half of the infiltrating T lymphocytes was CD8-positive. CD45R-positive B-lymphocytes were scattered in the cortex at young age, and the number of these gradually increased after 13 weeks of age. The number of T and B lymphocytes became same after 20 weeks of age. The macrophages appeared in the interstitium around 13 weeks of age, but were far less than lymphocytes. In female rats, the proportion of each infiltrating cell was similar to that in male rats, but these cells were significantly less than those in male. ICAM-1 mRNA was increased with age.

Conclusion: These results suggest that T lymphocytes might be important for initiation and development of tubulo-interstitial lesions and the role of macrophages might be minor in OM rats.

STABILITY OF DNA STANDARDS DILUTED IN 50% GLYCEROL AFTER MULTIPLE “FREEZING AND THAWING” CYCLES

Christiane Herden, Dirk Schaudien and Wolfgang Baumgärtner

Institute of Pathology, Germany

Standard curves are important tools in real-time quantitative PCR to precisely analyze gene expression patterns under physiological and pathological conditions. Handling of DNA standards often implies multiple cycles of freezing and thawing which might affect DNA stability and integrity. This in turn might influence the reliability and reproducibility of quantitative measurements in real-time PCR assays. In this study, three DNA standards such as murine TNF, murine IFN γ and rat kainat-1 receptor were diluted in 50% glycerol or water after one, four and sixteen cycles of freezing and thawing and amplified copy numbers after real-time PCR were compared. The standards diluted in water showed a reduction to 83%, 55% and 50% after four, to 24%, 5% and 4% after sixteen cycles for kainat-1 receptor, TNF and IFN γ standards, respectively when compared to a single cycle of freezing and thawing. Interestingly, all cDNA samples diluted in 50% glycerol were amplified in comparable copy numbers even after sixteen cycles of freezing and thawing. In conclusion, glycerol storage of DNA standards contributes to an accurate and reproducible quantification of cDNA species in real-time PCR analysis.

P108

ANOMALIES OF THE URINARY BLADDER IN CAMELS (CAMELUS DROMEDARIUS) IN AN IRANIAN ABATTOIR SURVEY

Seyed Hossein Mardjanmehr, Afshin Raoofi, Saied Bokai, Ganji Reza Asgharneghad

University of Tehran, Iran

Introduction: Urachal remnants are the infrequent malformation of the urinary bladder in domestic animals. Urachal remnants are divided into: urachal sinuses and urachal cysts. After the birth occasionally, urachal obliteration is partial, and rests of epithelium remain intact to develop into sinuses at the first segment of urachus near the bladder, or develop into cysts at any other point between the umbilicus and the apex of the bladder. The sinuses may have varying length. The cysts may become quite large but usually are small and multiple and are attached to the midline of the bladder. Urachal remnants have not any clinical manifestation and were diagnosed at necropsy or when the animal was slaughtered. These congenital defects of the urinary bladder have been described in all species but they have not been studied in Iran. Therefore this study was

POSTERS

performed to investigate the occurrence of urinary bladder anomalies in Iranian slaughter camels.

Materials and Methods: The study based on camels admitted to Kahrizak abattoir in Tehran province during a three months period between May 2003 and July 2003. Initially the urinary system of each camel was examined by naked eyes for presence of any gross lesions, immediately after slaughter. Then the urinary bladder was separated from the rest of urinary system, and incised longitudinally to assess the lumen of the bladder and examined the mucosa. The detected lesions were also examined by histopathology. They were fixed in 10% neutral buffered formalin solution, processed routinely and stained with haematoxylin and eosin. The individual data including age and sex of the camels were recorded. The age of examined camels determined by their dentition. For the statistical evaluation of the results chi-square test and two-tailed Fisher test were used.

Results: A total of 407 urinary bladder from selected camels were examined. 246 of the camels were males and 161 were females. They were grouped into the <6, 6, 7, 8, 9, and >9 years of age. Urachal remnants found in 19 (3.68%) inspected urinary bladder. From these congenital anomalies 4 (1%) cases were diagnosed as urachal sinuses, and all affected camels were females. Urachal cyst observed in 15 (3.69%) camels consisting of 14 males and only one female. Urachal sinuses appeared as a blind funnel form channel opened into the centrum of the bladder with 6 to 10 cm length. The lumen of these structures communicates with the urinary bladder and lined by transitional epithelium same as bladder mucosa. Urachal cysts appeared as a large, single, spherical cyst containing clear fluid and with thin wall, varied in size from 1 to 5 cm in diameter. These cysts firmly attached to the midline of the bladder or adhere to the free border of the lateral ligament. Histopathological examination of the urachal cysts showed that the wall of cyst lined by a layer of transitional epithelium. The rest of wall contain connective tissue and a thin smooth muscles layer which lined by a single mesothelial cells in the external surface.

Conclusions: From the results of this study and related studies in Iran, it is concluded that the urachal remnants are seen more often in camels than in other domestic animals. The statistical analysis showed that there were significant ($p < 0.05$) difference between the urachal remnants and sex, and significant ($p < 0.05$) difference between the urachal cysts in different age groups. No significant ($p > 0.05$) difference was established between the urachal sinuses in different age groups. Although, urachal remnants in the bladder wall are considered to be incidental findings, it may give origin to neoplasms. In addition urine stasis can occur in the urachal sinuses, predisposing the camels to cystitis or urinary calculi. It seems that neonatal omphalitis cause failure of complete involution of the urachus and can result in urachal remnants.

EXPERIMENTAL INOCULATION WITH CLASSICAL SWINE FEVER VIRUS (ISOLATE SPAIN 1/2001) IN PIGS: A CLINICAL, PATHOLOGICAL AND LABORATORY DIAGNOSTICS STUDY.

Francisco J. Salguero, Paloma Fernández-Pacheco, Jovita Fernández, Miguel A. Sánchez-Martín, Víctor Sánchez, María Fernández-de-Marco, Marisa Arias CISA-INIA, Spain

Classical swine Fever (CSF) is a haemorrhagic viral disease of swine that has been well known for decades and epizootics still occur. The clinical diagnosis continues to cause problems for veterinary practitioners due to extensive differential diagnosis together with the differences in the clinical signs and lesions depending on the CSF virus (CSFV) strain/isolate affecting the animals. In Spain, recent epizootics have occurred in 1997-98 and 2001/02 caused by CSFV isolates belonging to genotype subgroups 2.1 and 2.3, respectively. In this work we inoculated 3 animals (group #1) with a high dose (105 TCID₅₀) of CSFV isolate Spain 1/2001, sharing a BSL-3 box with 3 non-inoculated contact animals (group #2) simulating the conditions of the infection occurring in farms. Blood samples were taken and clinical signs were monitored daily. The onset of hyperthermia and clinical signs such as diarrhoea and dyspnoea was at 3-5 dpi in inoculated animals and at 7-10 dpi in contact animals. Animals were painlessly slaughtered at 18-20 dpi because of ethical reasons. Animals from group #1 showed petechial and ecchymotic haemorrhages in the lungs, intestine, urinary bladder and kidney, haemorrhagic lymphadenitis and cyanosis. Interestingly, only one animal from group #2 showed a few peripheral infarcts in the spleen and a few pinpoint haemorrhages in pleura. Viraemia was detected by RT-PCR from 3 dpi in animals from group #1 and from 9 dpi in animals from group #2. Viraemia was always accompanied by a lymphocyte depletion affecting B and T lymphocyte subpopulations as observed by flow cytometry analysis. Antibodies against CSFV were not detected by commercial ELISA kits nor by NPLA during the experiment in any animal, indicating the difficulty to detect CSFV in the recent epizootics occurred within Europe.

P110

ADRENAL EPITHELIAL-LINED CYSTS IN THE FERRETS

Isao Narama, Yutaka Nakahara, Naoko Ano, Kohji Nomura, Kiyokazu Ozaki Setsunan University, Japan

Solitary or multiple cysts and tubular structures lined by monolayered epithelium were rarely detected in the surgically resected adrenal glands of the

pet ferrets. Almost all adrenal glands were resected because of onset of clinical signs such as alopecia, enlargement of prostatic gland or swelling of vulva. The aim of this study is to clarify the origin and pathological nature of the epithelial-lined cysts and tubular structures.

Total 338 surgically resected adrenals and unilateral adrenals from 2 necropsy cases were examined histopathologically. Epithelial-lined cysts or tubules were detected eleven out of 440 adrenal glands (0.03%). Seven adrenals with cysts or tubules were obtained from total 52 right adrenals (13.5%), and remaining 4 cases were from 388 left ones (0.010%). All ferrets were gonadectomized at early age.

Many cysts were multilocular and were dilated filling with the eosinophilic mucoproteinaceous materials and varying amounts of cell debris. Monolayered flattened or cuboidal epithelium lined the all of tubular structures and cysts. Some cysts were surrounded by small amount of connective tissue and metaplastic bone was seen in a part of surrounding interstitium in two cases. In other two cases, cluster of hepatocytes was detected near the cysts. Right adrenal gland closely adhere the hepatic lobe and small pieces of adrenocortical tissue were embedded in the hepatic tissue in one necropsy case. Bile ducts adjacent to the embedded adrenal tissue proliferated and formed epithelial-lined cysts similar to those in adrenal glands. Epithelial-lined cysts were formed in the right adrenal gland of this case. Results of immunocytochemical staining for cytokeratin 7,14 and 19, AE1/AE2 in the epithelial cells of the cysts and tubular structure in adrenals were identical to those of bile duct.

These results suggested that adrenal epithelial-lined cyst in the ferrets are derived from bile duct in ectopic hepatic tissue.

P111

TIME-COURSE STUDY OF HISTOPATHOLOGICAL MARKERS FOR DRUG-INDUCED HEMOLYTIC ANEMIA IN RATS

Mihoko Ono, Kenji Nakano, Kyoko Ito, Nobuyuki Izumisawa, Satoko Izumisawa, Hajime Tabata, Kinji Shirota

Astellas Pharma Inc., Japan

jective] Anemia is one of problematic findings for development of drugs. When mild anemia without bone marrow suppression is induced by drugs, hemolytic anemia (HA) may be suspected, however, pathological diagnosis of HA is difficult because of the difficulty to perform clinicopathological examination at an optimal time. Using animal models of drug-induced HA with different mechanisms, the spleen was histopathologically examined to investigate the useful markers of hemolytic anemia. [Materials and Methods] Acetylphenylhydrazine (APHZ) inducing unstable hemoglobin HA with Heinz body, and ethylene

glycol monobutyl ether (EGB) inducing intravascular hemolysis due to red cell membrane damage, were given 10 mg/kg/day (SC) and 100 mg/kg/day (PO), respectively, to F344 rats for 1 or 2 days. Each dose expected to induce mild anemia was selected. Autopsy was performed at 6 and 24 hours after a single dose and at 24 and 48 hours after 2 daily doses. The expression of the following proteins in the spleen was examined by immunohistochemistry: heme oxygenase-1 (HO-1) that is an essential enzyme in heme catabolism, Bach-1 that regulates HO-1 expression, and ferritin heavy chain (Fh) that is an iron stored protein. The level of plasma haptoglobin was also evaluated. [Results & Conclusion] There were pathological differences between two animal models. In APHZ-induced anemia, all findings were progressive over time due to Heinz body formation. The expression of HO-1 and Fh in the spleen, and plasma haptoglobin may be useful markers of HA with Heinz body formation. In EGB-induced anemia, characteristic findings such as decreased plasma haptoglobin and increased expression of HO-1 and Fh due to direct destruction of red blood cells were observed at an early stage but rapidly disappeared. Therefore, the diagnosis is particularly difficult when loss of red blood cells is mild.

P112

ARTERIO-URETERAL FISTULA IN A FOAL

I. Van Dosselaer, G. van Loon, L. Lefère, R. Ducatelle, K. Chiers
University of Ghent, Belgium

Arterio-ureteral fistulation is rare in human patients and animals and is considered life threatening. Often the diagnosis is not considered, which may lead to diagnostic delay. The main symptom is haematuria which can be intermittent. Hypotension and shock can occur during periods of heavy bleeding.

When a five month old foal was referred to the University of Ghent because of severe haematuria, trauma was suspected. On general examination the foal was weak and showed pale mucous membranes, tachycardia and an enforced respiration. Blood analysis showed anaemia, hypoproteinemia, uraemia and leucocytosis. On transcutaneous ultrasound of the right kidney, a moderately echogenic and fluidly content, compatible with the presence of blood, was found under the renal pelvis and in the dilated proximal part of the ureter. An echogenic area was found under the renal capsule and was thought to be blood, urine or oedema. A large amount of blood was found in the bladder. The foal received a blood transfusion and antibiotics for several days. Ten days after presentation, the foal returned home in good general condition. Blood examination and ultrasound findings had completely normalised.

Six weeks later the foal returned because of haematuria and symptoms of colic. Packed cell volume was low but serum urea was within normal limits.

POSTERS

Echographic examination revealed a dilated ureter with presence of a blood clot and a large blood clot in the bladder. A similar therapy was instituted but the foal died due to a fatal bleeding.

At necropsy, an anastomosis between the abdominal aorta and right ureter was found. Histological examination of several sections of the anastomosis revealed an organized thrombus with recanalisation. *Strongylus vulgaris* larvae were not found.

In conclusion, this foal had a chronic aorta-iliac thrombosis. Recanalisation of this thrombus lead to fistulation with the ureter, resulting in fatal haematuria.

P113

EFFECTS OF INSULIN TREATMENT ON DIABETIC NEPHROPATHY IN ALLOXAN-INDUCED AND SPONTANEOUSLY DIABETIC RATS.

Chinami Kikkawa, Kiyokazu Ozaki, Tomoya Sano, Tetsuro Matsuura, and Isao Narama

Setsunan University, Japan

Aims: Male WBN/Kob rats spontaneously show diabetic condition from about 40 weeks of age, and develop diabetic complications such as peripheral neuropathy and retinopathy, but nephropathy is mild. Glomerular mesangial volume was much increased and thickening of glomerular basement membrane deteriorated in this strain of rat with accelerated diabetic condition and prolonged period by single injection of alloxan. We examined the effects of insulin treatment to diabetic kidney lesions morphometrically.

Materials and Methods: Total 25 male WBN/Kob rats were sacrificed about 95 weeks of age. Eight rats received single dosing with 40 mg/kg of alloxan at 15 to 20 weeks of age and were affected with diabetic condition for about 75 weeks (A group). Seven rats were treated in a similar way but maintained blood glucose control with insulin implants (AI group). Remaining 10 rats were untreated and affected with spontaneously occurred diabetes for about 40 weeks (Control).

Results: The blood glucose level of AI group fluctuated variously from hyperglycemia to normal level comparing constant high level of A and control group. The levels of BUN and inorganic phosphorus of AI and control groups were significantly lower than those of A group. The severity of Armani-Ebstein lesion and increased glomerular mesangial volume were A > control > AI group in order. The thickening of the glomerular basement membrane was milder in AI group comparing to A group, but was comparable to control group.

Conclusions

Insulin treatment prevents the deterioration of diabetic renal lesions such as

increased glomerular mesangial volume and Armani-Ebstein lesion as well as elevation of BUN level. However, an inhibitory effect of insulin treatment on the thickening of the glomerular basement membrane was not clear. These results may suggest that thickening of glomerular basement membrane reflects the effect of chronic progressive nephropathy rather than diabetic nephropathy.

P114

DIAGNOSIS OF CLINICAL AND PRECLINICAL SHEEP SCRAPIE IN RECTAL BIOPSY SPECIMENS

Lorenzo González, Mark P. Dagleish, Sue J. Bellworthy, Steve A.C. Hawkins, Stuart Martin, Silvia Sisó, Roy Jackman, Linda Terry, Robert Horton, Glenda Dexter, Philip Steele, Hugh W. Reid, Martin Jeffrey

Veterinary Laboratories Agency (VLA-Lasswade), UK

The current approach for sheep scrapie surveillance is based on laboratory examinations for disease-associated prion protein (PrP^d) on samples of central nervous system (CNS). We have conducted a large survey of immunohistochemical (IHC) detection of PrP^d in CNS, lymphoreticular system (LRS) tissue and rectal mucosa samples of scrapie-exposed sheep with and without signs of clinical disease. Scrapie confirmed cases included 244 with clinical disease, of which 241 were positive in the rectal mucosa (97.1%) and 121 apparently healthy sheep, 104 of which were RAMALT positive (86%). Preclinical detection of PrP^d was similarly efficient in the other LRS tissues examined (86.4-91.5%) and less so in the CNS (77.7%). The stage of infection, therefore, affected the probability of a positive result in the rectal mucosa, while the breed, PrP genotype, age and sex had little or no independent effect. Two ELISA assays were initially assessed on rectal mucosa samples and one of them was selected for further optimisation; once completed, the sensitivity of this rapid test reached 94% of that of IHC.

A total of 328 biopsy specimens of rectal mucosa were examined by IHC, while another 46 samples from sheep with previous IHC positive results were examined by ELISA. Positive IHC results were obtained in both naturally and experimentally infected sheep, with similar efficiency regardless of the route of inoculation. The efficiency of rectal mucosa biopsies for the detection of abnormal PrP was totally comparable to that of the palatine tonsil, and found that ELISA testing of biopsy samples provided a high diagnostic sensitivity.

We conclude that rectal mucosa samples taken at post-mortem could be considered as an alternative to CNS samples in active and passive surveillance programmes, and that rectal mucosa biopsies are a promising approach for the preclinical diagnosis of scrapie in the live animal.

PCNA EXPRESSION IN BOVINE EXPERIMENTALLY TREATED WITH 17 BETA ESTRADIOL

Luca Aresu, Faffaella De Maria, Selina Iussich, Gabriele Zancanaro, Francesca Spada and Bartolomeo Biolatti

Università di Torino, Italy

17b-estradiol is one of the most effective sex steroids used illegally as an anabolic in animal production, inducing hyperplasia and metaplasia of the genital accessory gland epithelium. These lesions are considered indicative as primary screening of illegal treatment with growth promoters. PCNA (Proliferating cell nuclear antigen) is one of the most used makers of cellular proliferation in human medicine as prognostic factor in tumors. The aim of this study was to evaluate the expression of PCNA in prostate, urethra and bulbourethral gland in bovine experimentally treated with 17b-estradiol. Two groups of three male veal calves each, 130 days old, were included in the project. Group B was treated with 10 mg/animal of 17- b-estradiol diluted in benzilic alcohol. Treatments of calves were carried out by four i.m. injections every 15 days for two months: the animals were slaughtered 15 days after the last dose. Group A was used as a control. Formalin fixed, paraffin sections of selected tissues were used for histological study and stained for PCNA. Results showed a PCNA labelling-index increased in prostate and bulbourethral gland of animals from Group B; while low PCNA immunoreactivity was demonstrated in normal animals (Group A). Immunohistochemical staining for PCNA was confined to the nucleus and showed a diffuse or granular pattern in the epithelial cells. Histological findings showed a diffuse hyperplasia and squamous metaplasia of the urethral and bulbourethral epithelium associated with the PCNA positive area. In conclusion, from this study it shows that animals treated with 17b-estradiol increased the level of PCNA in the epithelial cells of urethra and bulbourethral gland. Immunohistochemical nuclear labelling with anti-PCNA on routinely processed tissue is a simple technique for the assessment of proliferation and could be considered an important bio-marker in association with Ki-67 and AgNor to detect possible illegal treatment in calves.

IMMUNOHISTOLOGICAL DETECTION OF MHC II ANTIGENS IN FELINE SKIN SAMPLES

Maïke Huisinga, Kernt Köhler, Manfred Reinacher

Institut für Veterinär-Pathologie der Justus-Liebig-Universität Giessen, Germany

MHC II proteins present peptides derived from extracellular proteins to immune cells in order to induce an immune reaction. Therefore we suspected that MHC II expression increases in inflammatory skin changes. To verify this hypothesis we examined 50 skin samples from cats with dermatitis in consideration of perivascular dermatitis, nodular eosinophilic and pyogranulomatous dermatitis and biopsies with folliculitis of different aetiology. The control group consisted of skin biopsies of 10 necropsied cats without obvious skin lesions.

The detection of MHC II antigens was performed with a feline MHC II specific monoclonal antibody.

The immunohistological assessment of the MHC II expression revealed MHC II proteins in all samples on macrophages and dendritic cells whereas lymphocytes and plasma cells did not show expression in each case. In relation to this result, the whole MHC II expression correlated positive with the number of infiltrating inflammatory cells. Conspicuously, biopsies from all cats with folliculitis (11 cases), 5 biopsies (out of 10) from cats with eosinophilic granuloma and 1 biopsy from a cat with a nodular pyogranulomatous dermatitis (out of 8) showed MHC II expression on follicular keratinocytes. Additionally, in 2 cases with pyogranulomatous dermatitis we observed MHC II molecules on epidermal keratinocytes. The endothelial cells exhibited MHC II in 40 out of 50 cases.

Results indicate that the MHC II expression of non immunologic cells – especially of follicular keratinocytes – is dependent on infiltrating inflammatory cells. This finding coincides with results of similar examinations in canine skin samples. Why some cases of eosinophilic granuloma and one case of nodular pyogranulomatous dermatitis showed MHC II expression on follicular keratinocytes has to be clarified in future studies.

RETROBULBAR MYOSITIS ASSOCIATED TO LYME DISEASE IN A DOG

J. Gómez-Laguna, A.I. Raya, J.C. Afonso, L.E. Cortade, J. Martín de las Mulas, L. Carrasco

University of Córdoba, Spain

In this report, we made the first description in a dog of ophthalmologic manifestations associated to infection by *Borrelia burgdorferi*. An 8-years-old, female, crossbreed dog, was admitted to a small animal clinic due to fever and a severe inflammation at the right side of the face, involving the right eye. After an antiinflammatory and antibiotic therapy without improvement, a progressive exophthalmos in the right eye was noticed, reason for which were postulated a retrobulbar tumor or abscess as the cause of the exophthalmos. TAC showed diffuse homogeneous thickening of the extraocular muscles in the retrobulbar area, without evidence of an abscess. The bony walls of the orbit appeared intact.

The right eye and tissues of the orbit were enucleated and submitted for histopathological study, that showed a piogranulomatous inflammation, with perivascular lymphoplasmacytic aggregates. There was no evidence of tumoral cells in any of the samples.

One month later, the animal came back to the clinic showing a suppurative inflammation in the right side of the face, a suppurative conjunctivitis and keratitis in the left eye. The dog had fever, inappetence and enlargement of lymph nodes. Serologically, the animal was positive to *B. burgdorferi* infection by ELISA test. After a treatment with doxycycline, the animal has no fever, and the suppurative inflammation has disappeared.

Because of the clinical symptoms, that were indicative of an infectious disease, the positive serology and the response to doxycycline, we have concluded that *B. burgdorferi* was the cause of the orbital myositis observed in this dog. In recent years, orbital myositis has been described in human patients with Lyme disease.

P118

OUTBREAK OF OSTEOCHONDROSIS DUE TO HAZARDOUS CALCIUM DEFICIENCY

Antti Sukura DVM, PhD, Teppo Heinola DVM, Elias Jukola DVM, PhD, Päivi Näkki MSc

Faculty of Veterinary Medicine, University of Helsinki, Finland

In Finnish feedlot farm a hazard on mineral supplementantion yield to severely

distorted Ca/P ratio. Fattening bulls received by mistake only 60-70 % of the recommended calcium intake, while simultaneously receiving twice the amount of phosphorus. Confusion with the feeding of minerals last over seven months before it was revealed after lameness and leg problems became obvious to the farmer. At the farm fattening bulls were divided into three groups depending on the time of their arrival to the farm. This enabled the effect of mineral imbalance to be analyzed at different growth phases. Animals were divided to three groups: group 1 was exposed to mineral imbalance from age of 9 to 15 months, group 2 from the age of 4.6 to 11.6, and group 3 was feed with balanced diet. After slaughtering, the bones of both front and hind limbs were macroscopically evaluated. Over 80% of the animals with calcium deficiency in the diet had at least one severe osteochondrotic lesion.

The scapula was the bone most often affected (75% of the studied bones). All lesions of osteochondrosis (OC) in scapulas were located in the glenoidal cavity, on its weight-bearing surface. Predilection sites of OC lesions in the humerus were the medial condyle of the distal humerus (40% of bones affected) and the head of the humerus (27% of bones affected). In the femur OC lesions were most often found in the trochlea ossis femoris (60% of affected bones). OC lesions were commonly bilateral.

The weight gain per day varied between groups. The exposed groups had similar weight gains, but the control group (3) had a significantly ($P < 0.001$) higher gain. Animals in group 3 produced 20% better income than those in group 2 and over 30% better income than those in group 1.

P119

COMPARATIVE STUDY(HISTO-PATHOLOGICAL)OF ADRENAL GLANDS LESIONS BETWEEN CATTLE AND BUFFALOES IN AWAZ-IRAN

Mansour Sayari, Payman Bagherzadeh, Shahrokh Bohadori

School of Veterinary Medicine of Shiraz University, Iran

Adrenal glands being a member of the endocrine system are important in consequences of the functioning of the body. Bacterial and parasitic agents frequently localize in adrenal glands. Focal inflammatory usually are suppurative, arising in the course of bacterial septicemias. The investigation was carried out to study the histo-pathological conditions of adrenal glands of cow and buffaloes slaughtered of municipal slaughter house (Ahwaz). A total of 230 pairs of adrenal glands of buffaloes and 250 pairs of adrenal glands of cattles, 100 pairs from each of two animals suspected to be pathologic on gross observation were collected. After recording the measurement and gross lesions appropriate tissue were fixed in 10% formal saline, tissue were processed through paraffin

embedding method and sections cut at 5-7 μ were stained with haematoxylin and eosin. Special stain involving gimsa stain and gram's stain were also used wherever found necessary. Tissue sections were examined microscopically and various pathological conditions diagnosed. Comparison of the lesions in cattle and buffaloes (invagination 7%,12%), (accessory 4% ,10%) ,(nodular hyperplasia of cortex 21%, 18%), (degenerative and inflammatory 18% , 21%),(telangiectasis 1%,4%) (,adenocarcinoma of cortex 1%, 0%) (hemangioma 1%, 0%),(fibrosis of capsule 10%, 13%) .The many of the lesions were similar in these two species . Accessory cortical nodules are common in the adrenal glands of animales and found in capsule cortex and medulla .Nodular hyperplasia also is common in adrenal cortex..Degenerative and inflammatory lesions were same .Adenocacinoma and hemangioma cavernous diagnosed only in cattle.They can develop in any location in the body,but hemangioma in the adrenal glands is reported rare .

P120

NEUROENDOCRINE CELLS EVALUATION IN THE URETHRA OF STEROID HORMONES TREATED VEAL CALVES: PRELIMINARY RESULTS

Lisa Poppi, Marta Orazi, Anna Terreran, Roberta Merlanti, Massimo Castagnaro

Università di Padova, Legnaro, Italy

Neuroendocrine cells (NE cells) of human prostate have been related to proliferative disorders from benign hyperplasia to prostatic cancer. In the bovine prostate, multifocal areas of severe hyperplasia and squamous metaplasia of the urethral and glandular epithelium are found in estrogens treated animals and, to a lesser extent, in animals treated with other sexual hormones or hormones cocktails. A consistent number of NE cells is present in the bovine male urogenital organs: they are more numerous in the prostate complex and especially in the urethra. In this work, a correlation between hormonal treatment and variation on NE cells in treated veal calves have been immunohistochemically analyzed using mouse anti-human serotonin (5-HT) mAb on formalin fixed and paraffin embedded samples of prostate obtained from three homogeneous experimental groups of 9 veal calves (treated groups EB = 17 β oestradiol + boldenone, ET = 17 β oestradiol + testosterone; C = control group). Assessment of the number of cells positive for mAb 5-HT was performed by screening the urethral extension present in the specimens under light microscope examination (200x magnification). The location of NE cells near the observed lesions (areas of urethral hyperplasia or squamous metaplasia) were also considered. The results show that treatments affect the presence of NE cells in the urethral epithelium: labelled cells are more abundant in treated animals, particularly in EB (mean

3.30 cells/field) than in ET (3.04 cells/field) group, as compared to controls (1.80 cells/field). Data agree with distribution of hormonally induced lesions observed in HH staining. Furthermore, positive cells seem to be more densely distributed near hyperplastic lesions as regards morphologically normal and histochemically negative areas. These preliminary results suggest that also NE cells are sensitive to hormonal treatment. Further investigation may provide more detailed informations about their precise role in the development of prostatic lesions after hormonal treatment.

P121

SCREENING PITUITARY MICROSCOPIC LESIONS IN DOG

Barbara Osińska, Maria Katkiewicz

Warsaw Agricultural University, Poland

Introduction: The aim of this work was the pathomorphological evaluation of the adeno- and neurohypophysis and to find out the correlation between the relative mass and pituitary's pathological changes. There are few data concerning the problem especially of the neurohypophysis.

Material and methods: Fifty dogs, 5 ages' groups from 4 months to 22 years old, of both sexes were examined post mortem using pathomorphological methods as follow: H-E, PAS reaction, silver impregnation method according to Grimelius and immunocytochemical methods with monoclonal antibody anti-ACTH. For mathematic analysis Microsoft Exel 977 were used.

Results: The results of the research revealed pathological changes as follow:

- the adenohypophysis: hyperplasia 16% (expression ACTH 4%), adenoma 40% (expression ACTH 26%), cysts

48% (PAS-positive), hyperplasia connective tissue 2%, haemorrhage 2%, congestion 44%

- the neurohypophysis: adenoma 10% (expression ACTH 4%), cysts 12% (PAS-positive 10%, focal necrosis

10%, focal infiltration mononuclear cells 2%, infiltration neoplastic cells (ependymoma, myeloma) 8%,

thrombus/embolia 4% / 2%, haemorrhage 4%, congestion 38%.

The relative mass of pituitary presented individual differences and occasionally correlated with pathological changes.

Conclusion: Results of this work indicated, that pituitary's pathomorphological changes were relatively frequent, especially in neurohypophysis changes like neoplasm were observed. The pathomorphological changes observed in dogs' pituitary suggest disturbances of endocrine glands functions. There was not close correlation in the degree of pathological changes and pituitary's relative mass.

DETECTION OF IMMUNE SYSTEM CELLS IN PARAFFIN WAX EMBEDDED CHICKEN TISSUES

Alejandro Núñez, Daniel Hicks, Roberto La Ragione, Steve Cartman*, Arun Wangoo, Martin Woodward* and Bill Cooley†*

Department of Pathology, Food and Environmental Safety and TSE Molecular Biology†*

Veterinary Laboratories Agency-Weybridge, Addlestone, UK

Detection of immune system cells in avian tissues is hampered by the sensitivity of some cell surfaces epitopes to traditional fixation with aldehyde based fixatives. The aim of this study was to compare the suitability of buffered formalin (BF) fixative and zinc salts based fixative (ZSF) for preservation and immunolabelling of known specific fixation sensitive cell markers in chicken tissues.

Tissue samples from white leghorn SPF chickens, 41 to 73 days old were collected. The samples included spleen, bursa, cecal tonsil, lung and liver and were fixed in BF and ZSF and processed to paraffin blocks. Immunohistochemistry was performed using monoclonal antibodies against chicken B cells, CD3 T cells, CD8 T cells, macrophage/monocytes and Major Histocompatibility Complex type II. Our results showed specific immunolabelling for all the markers in tissues fixed in ZSF, whereas only detection of B cells was achieved in samples fixed in BF, despite the use of different antigen retrieval procedures. Double immunolabelling for T and B cell using fluorochrome labelled secondary antibodies was carried out on ZSF sections by confocal microscopy and the results showed colocalization of these two cell populations. The study highlights the use of ZSF fixatives in studying immune cell markers.

P123

ANALYSIS OF A REPRESENTATIVE SET OF APOPTOSIS-RELATED MOLECULES IN THE DOG

Benjamin Schade, Andrea B. Rickenbacher, Julia Wimmershoff, Stefan Keller, Eliane J. Müller, Maja M. Suter, Franco Guscelli

University of Zurich, Switzerland

Comparison of whole genome sequence data revealed that canines, as a species, are more closely related to humans than mice. Spontaneous diseases in dogs such as cancer, hence, would serve as suitable models for human diseases. The recent second release of the canine whole genome sequence allows rapid identification of putative sequences of canine orthologs of human genes and facilitates cloning of their coding sequences. The aim of this study was to clone the coding region

of canine genes encoding predicted proteins of the intrinsic apoptotic pathway and to perform an initial sequence comparison with the corresponding human and murine orthologs.

We have cloned and sequenced the first representative set of canine apoptosis-related molecules from the dog, including members of the Bcl-2 family (Bcl-2, Bcl-XL, Bcl-w, Mcl1, Bax, Bak, Bad, Noxa), the caspases (Caspase-8, Caspase-9, and Caspase-3), the Inhibitors of Apoptosis Proteins (XIAP, cIAP-1, cIAP-2, and Survivin), their mitochondrial inhibitors (Smac/DIABLO and Omi/HtrA2) and p53. Eleven of these sequences are novel for the dog. We have compared all nucleotide sequences, the deduced genomic organization spanning the coding sequences, and the deduced aminoacid sequences with the human and murine counterparts.

As expected, the overall sequence homology at the nucleotide and aminoacid level (and within specific subdomains) was higher between canine and human orthologs than murine. Interestingly, we nevertheless identified a small number of likely significant aminoacid differences in functional elements of dog and/or mouse proteins. The most significant of these changes was the absence, in canine Omi/HtrA2, of an IAP binding motif that is otherwise conserved from humans to *D. melanogaster*.

In conclusion, this type of analysis appears to be a beneficial strategy for identification of potential functional variations between canine and human genes, prior to using the dog as a model system for studying human diseases.

P124

PATHOLOGY OF EXPERIMENTAL SARCOPTES SCABIEI INFESTATION IN GREEK SHEEP

Dimitrios Tontis, Dimitrios Doukas, Elias Papadopoulos, Jens Mattsson, Maria Ioannou.

University of Thessaly Veterinary School, Greece

Introduction:

Sarcoptic mange is probably the most common ectoparasitic disease of sheep in Greece. In general, *Sarcoptes* mite infestation of man and animals has been known for over a century, yet dermatopathology of the disease is not fully described in sheep.

Material and methods:

From a flock of 20 greek breed Karagouniko naïve lambs, 3 months old, 16 lambs were experimentally infested with *Sarcoptes scabiei* var *ovis*. The animals were divided into two groups A and B (of 8 lambs each) and 4 animals were used as controls. Animals of group A were infested on the face, whereas of group B on the ventral skin.

Skin biopsies were taken under local anesthesia from developing lesions on d4, d7, d11, d18, d25, d33, d40, d46, d53, d60, d67, d75, d82, d90, d97, d105 and d130 respectively. Biopsies from body ventral area were taken only until d46. Routine staining methods (HE, Giemsa and Toluidine blue) and immunohistochemistry (CD3) were performed. Skin scrapings were examined after biopsies were taken, for the presence of all mite stages.

Blood samples for serological study were collected 20d prior start and on d11, d26, d38, d52, d66, d80, d94, d130. The ELISA was performed using standard methods employing an extract as an antigen from *Sarcoptes scabiei* var *vulpes*, in order to describe the serum antibody response to infestation.

Results:

Macroscopic lesions on the face were seen beginning at the 3rd day post infestation, such as erythema, pustules, crusts, excoriation and alopecia. Although the lesions began on face, finally were isolated only around nostrils and the margins of ear pinnae. Only one animal had periorbital involvement and another lamb had bacterial contamination. A remarkable itching was noticed approximately 4 weeks after infestation. In group B, the ventral skin of animals did not develop the above typical lesions, except of erythema and pustule formation, while lesions were self-limited and self-cured (d46).

Increased levels of circulating antibodies IgG were found in animals of group A, evident 4 to 5 weeks following the application of mites, with maximum level at d130, indicating provocation of a humoral immune response. Antibody levels in animals of group B were not significantly different compared to control group.

Histopathological lesions of the skin were described in details and scored for each animal at every period. These include orthokeratotic and parakeratotic hyperkeratosis, crusting, intracellular and intercellular oedema, marked spongiosis, hypergranulosis, subcorneal and intraepidermal pustule formation with few acanthocytes, intracorneal and intraspinal presence of parasites, although mites were difficult to find at early stages of infestation. Epidermis also showed hyperplasia, with marked acanthosis or pronounced rete ridge formation in many biopsies. Marked infiltration of epidermis with eosinophils was the earliest inflammatory cell response, associated with numerous PMNs and followed by exocytosis of lymphocytes (CD3 positive) especially at the sites of mites. Hyperplasia of the apocrine glands with periglandular inflammation and hidradenitis was prominent. Other findings, yet not seen in each animal, were increased number of apoptotic keratinocytes, angiectasia and hyperemia in superficial dermis, eosinophilic folliculitis and fibrosis. The inflammatory pattern was mainly dermoepidermal, diffuse and/or deep perivascular, and less focal periadnexal or nodular. The predominant cells in the infiltrates were

initially eosinophils, whereas T-lymphocytes (CD3+) were at the last stages of infestation. Plasma cells, histiocytes and mast cells participated at different degrees during the development of lesions.

Conclusion:

The predilection sites for the parasite seem to be skin around nostrils and ear pinnae and not whole areas of the face. The preference of mites for sparsely haired skin does not result in a predominance of ventral truncal distribution as seen in other animal species. These maybe due to the thickness of the epidermis and especially the stratum corneum, the odor or moister of the host's skin. Moreover, the development of lesions is dependent to the immune response of the infested skin.

P125

ASSESSING THE PROTECTIVE EFFECT OF DIFFERENT MAEDI-VISNA DNA VACCINATION STRATEGIES

Marta Pérez, Ester Biescas, Ignacio de Blas, Ramsés Reina, Ximena de Andrés, Damián de Andrés, Beatriz Amorena, Juan Badiola, Lluís Luján
University of Zaragoza, Spain

The objective of this work was to evaluate different strategies of DNA vaccination against Maedi Visna virus (MVV). Experimental groups of MVV-free sheep were immunised with recombinant vectors carrying the MVV envelope (env) and core (gag) genes. Vectors were delivered either to skin biolistically (using the gene gun device) or to mucosa by naso-pharyngeal aerosolization and intra-tracheal injection, using polycation (polyetilenimine, PEI) – DNA complexes. The effect of inclusion of recombinant plasmids carrying the interferon gamma (IFN-gamma) gene at the primer and booster recombinant plasmid immunisations was also assessed. Animals received a second booster immunisation with modified vaccinia virus Ankara recombinant for env and gag MVV genes, and were challenged intratracheally with the homologous MVV strain EV1. Animals were slaughtered three months after challenge and samples were collected from lungs and mediastinal lymph nodes to evaluate lesions and determine the presence of virus. Samples were analysed by anatomopathology, immunohistochemistry and MVV 'in situ' hybridisation techniques. Immune response was also evaluated by additional techniques, but in this work only pathologic studies are presented.

Results indicate that biolistic immunisation with gag genes significantly prevents lung lesion development. Incorporation of the IFN-gamma gene in the inoculum does not increase protection significantly. The remaining immunisation strategies do not seem to induce a significant protection.

COMPARISON OF ZIEHL-NEELEN AND IMMUNOHISTOCHEMISTRY ACCURACY FOR THE DIAGNOSIS OF BOVINE TUBERCULOSIS IN THE FRAME OF ERADICATION PROGRAMMES.

Katia Varello, Marzia Pezzolato, Valentina Audino, Daniela Mascarino, Maria Caramelli and Elena Bozzetta

Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, Italy

Rapid diagnosis of tuberculosis in cattle reacting positive to ante-mortem assays is pivotal in areas subjected to eradication programmes to confirm the presence of the infection in tuberculosis-free herds. However, mycobacterial culture and molecular investigation, besides being time consuming, don't allow to identify non-specific causes of positive in-vivo reactions.

Aim of this study was to investigate the accuracy of immunohistochemical technique using polyclonal anti-BCG antibody compared with Ziehl-Neelsen stain when lesions are caused by low infective doses and detected at early stages of the disease, and mainly consist in primary complexes.

We analyzed lesions belonging to 59 bovine lymph nodes collected in Piedmont in the frame of eradication campaign. The lesions were previously classified on histopathology as positive (caseation or mineralization with epithelioid and Langhans giant cells) and inconclusive (granulomatous lymphadenitis with macrophages and epithelioid cells). Serial sections obtained from paraffin embedded block were subjected to Ziehl-Neelsen (ZN) and Immunohistochemistry (IHC). Results were compared to mycobacterial culture and *M. bovis* identification as reference test.

IHC sensitivity was 67,86% (IC 55,62%-80,10%) with a predictive value of 100,00 (IC 100,00%-100,00%); the specificity resulted 100,00%, while the detection of acid-fast bacilli by ZN staining was less sensitive 48,21 % (IC 35,13%-61,30%), but high specific too (100%, IC).

Our study indicates that IHC is more sensitive than ZN method, detecting not only intact organisms but mycobacterial antigens, fragments and living or dead organisms with defective cell wall too. However this technique alone is not enough sensitive: in fact the inconclusive lesions weren't identified as positive. On the other hand it is specific and easy to perform, the granulomas caused by *Rhodococcus equi* resulting negative. Following these results the authors suggest that IHC could be used in eradication plans for early identification of mycobacterial infections.

HISTOPATHOLOGICAL FINDINGS, ANTIGEN DISTRIBUTION AND INFLAMMATORY CELL TYPES IN SYNOVIAL MEMBRANES OF CALVES WITH EXPERIMENTALLY INDUCED MYCOPLASMA BOVIS ARTHRITIS

Marion Hewicker-Trautwein, Rama Devi Vemuri, Kathrin Hermeyer, François Poumarat, Renate Rosengarten

University of Veterinary Medicine, Germany

In calves with *Mycoplasma (M.) bovis*-induced pneumonia the organism often spreads to the joints and induces severe arthritis. The mechanisms of pathogenicity of *M. bovis* arthritis are still largely unknown. The aim of this study was to characterise the distribution of antigens and phenotypes of inflammatory cells in synovial membranes from calves with experimentally induced *M. bovis* arthritis. Two calves were inoculated into one carpal joint with *M. bovis* strain 1067. Two calves were inoculated intravenously with the same strain at days 0 and 29 before they were challenged at day 49 into one carpal joint. Two non-inoculated calves served as controls. Necropsy was carried out at days 12, 14, 57 and 61 post infection (p.i.). From each animal, synovial fluid and tissue samples were collected from 12 different joints. Paraffin sections of synovial membranes were examined by applying the ABC method and antibodies to *M. bovis* antigens, T- and B-lymphocytes, macrophages and inducible nitric oxide synthase (iNOS). Histologically, all infected calves showed necrotic areas in the joint capsule of the inoculated joint. In 2 calves, lesions were also present in non-inoculated joints. There were only few T- and B-cells, but numerous neutrophils and macrophages surrounding necrotic tissue areas. Macrophages showed marked expression of iNOS. *M. bovis* was isolated from all inoculated joints and, in addition, from up to seven non-inoculated joints of 3/4 calves. *M. bovis* antigen was only present in necrotic tissue areas. *M. bovis* organisms and antigens together with inflammatory lesions, in spite of previous immunisation, were still detectable at 57 and 60 days p.i.. In conclusion, our results demonstrate that activated macrophages are the predominant cell type in *M. bovis*-induced joint lesions. The presence of numerous iNOS-expressing macrophages associated with necrotic tissue areas may indicate that they are involved in the pathogenesis of these lesions.

PORCINE DERMATITIS NEPHROPATHY SYNDROME (PDNS): DISCUSSION OF PATHOGENESIS FOCUSED ON THE CHARACTERISTIC LESIONS

Monir Majzoub, Mathias Ritzmann, Walter Hermanns

University of Munich, Germany

Data are presented from ten pigs with pathomorphological findings associated with Porcine Dermatitis Nephropathy Syndrome. Following characteristic lesions of 77 examined pigs with PDNS were found: systemic vasculitis, acute necrotizing glomerulonephritis, often accompanied by sclerosis of other glomeruli, leading to haemorrhages and multifocal necroses of the skin. An accumulation of electron dens material in the glomerular tufts was detected by electron microscopy.

The aim of the study is to contribute to an understanding of the pathogenesis of PDNS and the causative role of PCV-2. Our evaluations, using specific molecular techniques (PCR and in-situ-hybridization technique), are based on the detection of virus material in the lesions described above.

P129

XENOTRANSPLANTATION: POTENTIAL PRESENCE OF VIRAL ZONOSIS IN PIG ORGANS

Maria Teresa Capucchio, Frine Eleonora Scaglione, Deborah Catalano, Gianluca Scrivano, Loris Alborali, Maura Ferrari, Riccardo Villa, Simonetta Appino, Franco Guarda, Loretta Masoero

University of Torino, Italy

Xenotransplantation is the possibile solution for the lacking of human tissues. Multiple problems exist including immunological reactions, metabolic differences between animals and humans and potential risks of the transmission of infections. Pigs are the most considered species for xenotransplantation. Numerous potential viruses have been identified including Rotavirus, Hepatitis E virus (HEV), Porcine Circovirus type 2 (PCV2), Porcine Herpesvirus and Porcine Endogenous Retrovirus (PERV). The aim of this study is to look for the presence of these viruses in different organs of n° 99 pigs healthy slaughtered in Piemonte region. Samples of small intestine, kidney, heart and inguinal lymph nodes were collected, fixed in 10% neutral buffered formalin for histological investigations or stored at -80°C for virological researches. Rotascreen Dipstick (Microgen Bioproducts), an immunochromatographic test from fecal specimens was used to identify the presence of Rotavirus. Electron microscopy was used to detect HEV-like morphological particles on faeces. The

presence of Herpesvirus was evaluated by virus isolation on PK15 cell cultures and determination of the cytopathic effect. PCR and Real-time PCR, employing primers specific for the pol region and for the env region were performed to evaluate the PERV cell infection and to identify the specific retroviral classes, respectively. Immunohistochemistry using Mab 1A5 permit the detections of PCV2 on lymph nodes. No significative lesions were observed on histological sections. All samples were negative for the presence of Rotavirus, HEV, Herpesvirus and PCV2. PERV infection was observed in all swine specimens. All three classes of PERVs were identified: A,B and C. Almost all animals were A and B positive, whereas the 26% of pig organs were positive for PERV type C too. This study represents a further confirm of the presence of PERV in pig organs potentially usefull for xenotransplantation.

This work was supported by funds of Piemonte Region 2004.

P130

CANINE DIGESTIVE MESENCHYMAL TUMORS: HISTOLOGICAL AND IMMUNOHYSTOCHEMICAL STUDIES.

Alberto Benito-Peña, Carolina Mancho-Alonso, Mercedes García-Sancho Téllez, Ángel Sainz-Rodríguez, Fernando Rodríguez-Franco, Antonio Rodríguez-Bertos

Pathological Service of Veterinary Teaching Hospital. UCM. Madrid, Spain

In human being a new group of digestive neoplasms, called gastrointestinal stromal tumors (GISTs), have received special attention in the last decades. Up to date, many stromal tumors were diagnosed like a muscular neoplasms, but now it is known they constitute a new entity characterized by immunoreaction against c-kit protein (CD 117). These neoplasms are assumed to have likely two different origin: undifferentiated mesenchymal cells or interstitial cells of Cajal. These type of tumors are considered the most frequent digestive mesenchymal neoplasms, even more than smooth muscle derivated tumors.

In veterinary medicine, stromal tumors have been reported in dogs, horses and monkeys. It is necessary to realize differential diagnostic with other neoplasms came from gastrointestinal wall, which include muscular and nervous tumors. The routinary examination with histochemical techniques is not enough to differentiate the fenotypical appearance, been necessary the use of immunocytochemical techniques to characterize these kind of tumors.

The aim is realize a retrospective study of canine digestive tumors and their immunohistochemical characterization. We have checking the digestive neoplasm of the last ten years from the Pathological Service of Veterinary Teaching Hospital (UCM. Madrid). The results were 22 canine mesenchymal tumors: 8 leyomiomas (2 from stomach and 6 intestinal wall), 11 leyomiosarcomas (1

esophageal, 4 gastric and 6 intestinal origin) and 3 neoplasms without accurate diagnoses (2 gastric and 1 intestinal wall).

To immunohistochemical filiation we use panel marker: monoclonal antibodies against desmine, vimentine and smooth muscle actine; polyclonal antibodies c-kit and S-100. In addition, to end the proliferation cell index we employ a monoclonal anti-Ki 67 to determine the nuclear nonhistone protein. Finally, it is very important to know the tumoral prognosis, for this reason we study p21 protein in these tumors, since recent reports have marked its expression is correlated with a aggressive behaviour of the gastrointestinal neoplasm.

P131

FIBRILLARY NON CONGOPHILIC GLOMERULAR DEPOSITS IN A CAT: HISTOLOGICAL AND ULTRASTRUCTURAL FEATURES

Frine Eleonora Scaglione, Maria Teresa Capucchio, Paola Cavana, Enzo Cornaglia, Andrea Bovero, Anna Maria Farca, Davide Ripanti, Deborah Catalano

Department of Animal Pathology, University of Torino, Italy

Glomerular fibrillary deposits are an uncommon feature described in human and in horses characterized by extracellular randomly scattered microfibrils (Congo red negative) located within renal glomeruli. A five year old European cat was referred to the Teaching Hospital of the Faculty of Veterinary Medicine of Turin. The cat was suffering of depression, polyuria and polydipsia Anorexia, vomiting, weight loss, weakness and recurrent dysentery were also reported. Upon physical examination, the cat was lethargic, thin, cachectic, dehydrated with ascitic fluid in abdomen and subcutaneous edema. Serum biochemistry showed a mild increase in creatinine (2 mg/dl, range 0.8-1,8 mg/dl-), high urea concentration (110 mg/dl, range 43-65 mg/dl), low total protein (4.3 g/dl, range 6-7.5 g/dl) and hypoalbuminemia (0.65 g/dl, range 2.2-3.5 g/dl) with 0.18 albumin-to-globulin-ratio (range 0.6-1.2). Urinalysis revealed specific gravity 1.030 (range 1.35-1.60), high proteinuria (396.4 mg/dl) with 1.2 protein-to-creatinine ratio (range < 0.6). Sediment abnormalities included granulous cylinders. Because of the poor prognosis, at the owner's request, the animal was euthanized and a complete necropsy was done. Histological examination of the kidneys revealed a diffused membranous glomerulonephritis with multifocal thickening of the Bowman's capsule. Interstitial inflammation and fibrosis as well as hyaline casts were observed too. Congo red stained sections were totally negative. A severe, diffuse chronic non suppurative enteritis were also seen. Electron microscopy revealed diffused podocyte foot process effacement, thickening of GBM with widespread fibrillary (18-26nm) deposits in GBM, subepithelial and subendothelial regions and in mesangium. The nature of

POSTERS

these deposits is unknown. In man a fibrillary glomerulonephritis is considered an immuno-mediated pathology frequently associated to the deposition of immunoglobulins and C3. Further studies are needed to evaluate if this case could be represent a different manifestation of the same pathology.

This work was supported by funds of Piemonte Region 2004.

Social Programme

Social Events

Wednesday 30 August

1900 Welcome Reception - Summerhall, Royal (Dick) School of Veterinary Studies

Friday 1 September

1930 Reception, Conference Dinner and Entertainment - Dynamic Earth

Welcome Reception

The Conference will open with a Welcome Reception at 1900 hours on Wednesday 30 August. The reception is being held in Summerhall, the Veterinary School at Summerhall Square. Summerhall is situated at the corner of the Meadows and within walking distance of Pollock Halls and David Hume Tower (see maps).

Summerhall has been home to Edinburgh vet students since William Dick moved his Veterinary School, established in 1823, to the site in 1917. The School became part of The University of Edinburgh in 1951 as the Royal (Dick) School of Veterinary Studies.

Conference Dinner

The Conference Dinner will take place in Dynamic Earth on Friday 1 September. Dynamic Earth is situated in the heart of Edinburgh at the foot of Arthur's Seat, adjacent to the Palace of Holyroodhouse and the site of the new Scottish Parliament. Dynamic Earth is a 15 minute walk from David Hume Tower and Pollock Halls. A map showing Dynamic Earth can be found at the end of these Proceedings.

The evening will begin at 1930 hours with pre-dinner drinks in the Earthscapes galleries, which recreate different regions of the world including rainforest and polar regions.

Dinner will be served in the Stratosphere after which there will be the opportunity to enjoy some traditional Scottish music and dancing. The dress code is casual but if you have tartan we encourage you to wear it!

Entry to Dynamic Earth is by ticket only. A ticket to the Conference Dinner is included in all registration fees except the day rate. Additional tickets can be purchased at the registration desk. Please contact the Conference Secretariat.

Accompanying Persons

While we have not arranged an accompanying persons programme there are a huge range of activities to enjoy in and around Edinburgh. There will be information on things to do in and around Edinburgh beside the registration desk.

Festival Time

Edinburgh in the summer is the Festival City and definitely the place to be with six festivals taking place. They are all independently organised, but collectively they form one of the biggest and most intoxicating celebrations of the arts in the world. A whole array of events takes place including film, theatre, books, children's events, talks, music, dance, comedy, military bands and many more. So there is, quite literally, something for everyone. Further information on all the festival events taking place can be found at www.edinburgh-festivals.com/festivals

Refreshments

Tea and Coffee

Tea and coffee during both morning and afternoon breaks will be served in the Refectory located in the basement of David Hume Tower, next to the exhibitors area. Please refer to the programme for timings.

Lunch

Lunch will be served in the Refectory at Teviot Student Union. Teviot Student Union, which is shown on the University map, is a 2 minute walk from David Hume Tower. Please refer to the programme for timings.

BSVP Meeting

The inaugural meeting of the British Society of Veterinary Pathology will take place on Thursday 31 August between 1230 and 1300 hours in David Hume Tower Lecture Theatre A.

ECVP AGM

The AGM of the European Committee of Veterinary Pathology will take place on Thursday 31 August between 1730 and 1930 hours in David Hume Tower Lecture Theatre C.

ESVP Committee Meeting

The Committee Meeting of the European Society of Veterinary Pathology will take place on Friday 1 September between 1300 and 1400 hours in The Office of Lifelong Learning, no 9 Buccleuch Place. This is directly across the street from the conference venue.

ESVP AGM

The AGM of the European Society of Veterinary Pathology will take place on Friday 1 September between 1700 and 1800 hours in David Hume Tower Lecture Theatre B.

Mystery Slides

The Mystery Slides can be viewed between 0930 and 1730 on Friday 1 September in David Hume Tower Room 611. The Mystery Slide Session will take place on Saturday 2 September between 1100 and 1230 hours in David Hume Tower Lecture Theatre A. The organiser for this session is Anja Kipar.

ISVD Workshop

The ISVD Workshop will take place on Saturday 2 September between 0900 and 1730 hours in the William Robertson Building, beside David Hume Tower. The organisers for this workshop are Professor Maja Suter and Dr Monika Welle - see separate website www.isvd.org/.

UNIVERSITY OF EDINBURGH - CENTRAL AREA



Key:

28: David Hume Tower (Conference Venue)

20: Teviot Student Union (Venue for Lunches)

CITY OF EDINBURGH (INSERT DYNAMIC EARTH)

