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WELCOME MESSAGE

Dear Colleagues and Friends,

On behalf of the Organising and Educational Board of the 27th Meeting of the European Society of Veterinary Pathology and the European College of Veterinary Pathologists and of the Faculty of Veterinary Medicine of the University of Warmia and Mazury in Olsztyn, it is a great pleasure and honour to welcome all of you in Kraków, Poland.

Our Meeting will be held at the famous Jagiellonian University in Kraków, in a significant academic and cultural centre of Poland. We promise to do our best to make your stay in Kraków, the old Polish capital, the royal city full of charm of contemporaneity as well as the world-famous monuments, the most unforgettable scientific and social experience.

Let me introduce you to the 27th Meeting of the European Society of Veterinary Pathology and the European College of Veterinary Pathologists. Traditionally, ESVP Congresses are held every year. This Meeting in Kraków will be the first officially joint congress of the ESVP and the European College of Veterinary Pathologists. Moreover, it will take place together with meetings of the International Society of Veterinary Dermatopathology, C.L. Davis Foundation – European Division and the Polish Small Animal Veterinary Association, Oncology Section. Consequently, the 27th Meeting of ESVP and ECVP will offer an unique platform for exchange of information and experience as well as presentation and discussions of the latest achieve-

ments in our research. It should enhance international scientific cooperation and enable us to elaborate applications for daily practice. It will provide a comprehensive update of the state-of-the-art in all relevant disciplines contributing to pathomorphology, morphology and clinical pathology. Plenary lectures (6) and parallel 16 sessions with 44 oral presentations and 154 poster presentations will cover the subjects of histopathology, ultrastructural pathology, molecular pathology, toxicological pathology, experimental pathology, techniques in pathology and morphological sciences, clinical pathology and toxicological clinical pathology. The programme also includes a Mystery Slide Session on Dermatopathology and Workshop on Fish Pathology.

The organizers of the Meeting would like to thank all those who have contributed to the organization of these events in Kraków, particularly to the International Educational Board and boards of the European Society of Veterinary Pathology, European College of Veterinary Pathologists, International Society of Veterinary Dermatopathology, Polish Society of Pathologists and Polish Small Animal Veterinary Association. We are also grateful to all our sponsors and trade exhibitors for their help. We also thank all Delegates for coming to Kraków and for taking part in the creation of these events.

We believe that the 27th Meeting of ESVP and ECVP will have the same high standard as those of previous years and that they will be "good harvest of the research field" and also provide you with an opportunity to stimulate your own investigations.

We wish you a pleasant and fruitful stay in Kraków.

Prof. Dr hab. Józef Szarek
President of the Organising Committee

PLENARY LECTURES

EMERGING INFECTIOUS DISEASES AND INFECTIONS OF PIGS: WHICH IS THE ROLE OF THE PATHOLOGIST NOWADAYS?

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Introduction

Looking at the literature, there are many definitions of emerging infectious diseases. As an example, they have been defined as these whose incidence have increased in the past 20 years and threaten to increase in the near future. The natural history of new infections is rather simple from a generic point of view (fig. 1). However, even controlling the disease/infection is very difficult *per se*, to live with an enzootic scenario could be worse than to eradicate it and being threatened by the risk of re-infection. Such situation is especially important when dealing with livestock diseases/infections since the loss of production or competitiveness may cause significant economic disadvantages, not only for the producers but also for a country or region as a whole.

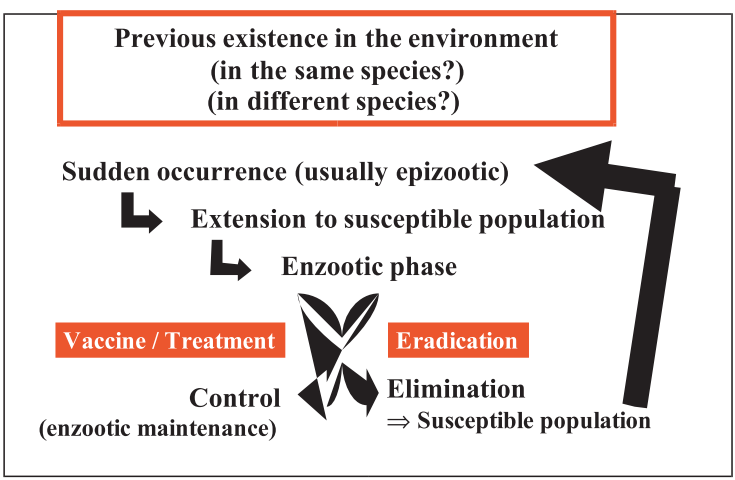

Fig. 1. Natural history of new infections.

Emerging infectious diseases of pigs

Within the terminology "emerging infectious diseases" there are potential sub-classifications (Fauci *et al.*, 2005): newly emerging diseases (those that have never been recognised before), re-emerging or resurging diseases (those that have been around for decades or centuries, but have come back in a different form or location) and deliberately emerging diseases (those that have been intentionally introduced). Talking about pig diseases, so far, it should be not expectable to experience diseases within the latest category. However, nowadays there are a number of very significant pig diseases that fall into the group of newly emerged diseases (such as porcine reproductive and respiratory syndrome (PRRS), which emerged by late 80s, or postweaning multisystemic wasting syndrome (PMWS), emerging by late 90s) or resurging ones such as classical and African swine fever and Glässer's disease).

Abovementioned diseases are of great concern for pig producers, but there are a number of non-well known infections that appear to be emerging in pigs as well. Some of them can be *Hepatitis E virus* (HEV) (which is a real zoonotic concern), *Torque teno virus* (TTV), *Porcine torovirus* (PToV), *Porcine enteric caliciviruses* (norovirus and sapovirus), *Porcine bocavirus* and probably others.

The introduction of these emerging infections in the pig livestock is usually unknown, unless such introduction represents a significant overt disease and an economical hit for the swine industry. If studying some of the viral agents, one might foresee that a considered emerging viral infection in pigs would not probably represent a true new emerging virus, since it usually has remained unnoticed for a long period of time. This situation has occurred for *Porcine circovirus type 2* (PCV2), the essential etiological agent of PMWS, which was initially described in 1998 (Hamel *et al.*, 1998), but retrospective studies showed evidence of PCV2 infection as soon as 1962 in Europe (Jacobsen *et al.*, 2008). Moreover, its associated disease, PMWS, was also unnoticed for a number of years, since retrospective studies showed cases fulfilling disease case definition at least by 1985 (Jacobsen *et al.*, 2008). Similarly, swine HEV, described initially in 1997 (Meng *et al.*, 1997), has been shown in pigs as early as 1985 in Europe (Casas *et al.*, 2009). Another example would be swine TTV, which was initially detected by 1999 (Leary *et al.*, 1999), but retrospectively it has been shown to be present even in 1985. A common fact in all these retrospective works was that first evidence of viral infection coincided with the very first year of study and, therefore, it implied also the assessment of a widespread nature of these viral infections since then as well. The lack of very old pig serum or tissue samples impedes to date precisely the emergence of these viruses. PRRS virus (PRRSV), which caused an emerging pig disease by middle 80s in North-America and early 90s in Europe (Zimmerman *et al.*, 2006), would be an exception to

the previously mentioned viral agents. Evidence of PRRSV infection was recorded in 1979 in North-America (Carman *et al.*, 1995) and 1988 in Europe (Ohlinger, 1992), relatively close to its emergence as an overt disease. Therefore, to consider a number of diseases/infections as true emerging ones is probably to simplify the real situation. At the end, and this applies to all diseases/infections worldwide and to all species: "a given disease/infection may exist in a farm, region or country depending on the enthusiasm you are looking for it" (quoted by Dr. C. Pijoan, University of Minnesota, 1998).

The role of pathologists in emerging infectious swine diseases/infections

Sometimes it seems that if one is not working with molecular biology and high tech procedures such as real time quantitative PCR or microarray techniques, one is lost in the past. It is true that those new technologies have caused a revolution in the research and diagnostic world; however, like histopathology for example, they are just other techniques that must be interpreted and used according to the needs of each particular case. It is true that most pig diseases that show overt gross or microscopic lesions are of infectious origin; therefore, molecular biology techniques have helped a lot increasing sensitivity of pathogen detection as well as agent characterization.

Interestingly, scientists facing initially with significant emerging diseases of swine have been traditionally pathologists. PRRS and PMWS would be great examples of such situation. The first descriptions of PRRS as a new disease included basically the consistent finding of interstitial pneumonia (Zimmerman *et al.*, 2006). The first descriptions of PMWS as a novel entity included mainly lymphocyte depletion together with granulomatous inflammation of lymphoid tissues (Clark, 1997). Therefore, the role of the pathologist is still the cornerstone within the first line in detecting and characterising new emerging diseases (in pigs as well as in other species). The precise role of the pathologist in subclinical emerging infections of pigs (such as HEV, TTV and others) is, at present, marginal. However, this participation may still be significant, since it is important to assess subclinical pathological outcomes with a potential synergistic effect in the context of multifactorial diseases.

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RESEARCH UPDATE ON INFLUENZA AND EMERGING VIRAL DISEASES IN SWINE AT ARS' NATIONAL ANIMAL DISEASE CENTER

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LIVER PATHOLOGY – WHAT'S NEW AND WHAT'S STILL TRUE?

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A COMPARATIVE APPROACH TO METASTASIS BIOLOGY AND THERAPY

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The comparative oncology opportunity

The field of comparative oncology is uniquely positioned to take advantage of the completed canine genome to improve our understanding of cancer therapy and biology. Used most often when referring to the study of cancers seen in companion (pet) animals, comparative oncology describes a discipline that integrates the study of naturally occurring cancers in animals into studies of human cancer. Cancers in companion species are well suited to uniquely inform investigations of cancer biology and cancer drug development. The features of cancers in pet dogs that may uniquely contribute to our understanding of cancer pathogenesis, progression and therapy have been recently reviewed. Of most interest to the study of cancer biology is the biological complexity of cancers seen in pet animals. This is based in large part on the intra-tumoural (cell-to-cell) heterogeneity seen in these cancers. A natural consequence of this heterogeneity is the same deadly features of human cancers including acquired resistance to therapy, recurrence, and metastasis. Accordingly, cancers in pet animals capture the "essence" of the problem of cancer seen in human patients. The integration of these naturally occurring cancers into the study of cancer biology and therapy provides an opportunity to answer questions not answered in conventional model systems.

Cancer metastasis… Unmet needs

The development of metastasis is a universally grave development for cancer patients irrespective of specific cancer histology. Emblematic of this clinical problem is the biology associated with progression in the pediatric solid tumour, osteosarcoma. Indeed, for both human and canine cancer patients afflicted with osteosarcoma, the most common primary tumour of bone, metastasis to the lungs is the most common cause of death. Lung metastases develop in these patients despite highly effective treatment of the primary tumour. Improving our understanding of the biology of metastasis is needed to improve outcomes for these patients. It is important to emphasize that the study of metastasis must include two distinct biologic entities that are both referred to as metastasis. First metastasis is a *verb* that describes the process of cancer progression from a primary tumour site to a distant second-

ary site. Second metastasis also refers to the actual metastatic lesion that exists at the secondary site and is responsible for patient mortality. Progress towards understanding metastatic disease (both the *verb* and *noun*) and its inherent resistance to conventional treatments is limited by many factors. First, the process of metastasis is believed to begin very early in the course of disease progression and has occurred in most patients at the time of their initial presentation. This provides limited opportunities to study these events in human patients. Second, the genetic aberrations that are responsible for the development of metastasis are complex, heterogeneous and difficult to distinguish from the events responsible for the actual development of cancer. Finally, the access to well described patient samples is limited and often is available only after treatment with cytotoxic chemotherapy. For relatively rare human cancers such as osteosarcoma, these problems are amplified.

Comparative approach to osteosarcoma metastasis: identification and study of ezrin biology

To define genes and or proteins that contribute to the metastatic phenotype of metastasis in osteosarcoma, we have utilized a cross-species comparative approach that includes murine, canine, and human systems for gene identification and evaluation. This approach has leveraged the availability of murine, canine, and human genomes, to survey the expression of genes in normal and diseased tissues and then identify either patterns of gene expression of individual genes responsible for or associated with metastasis. As an example, we used this comparative approach to identify and then associate the cytoskeleton linker protein, ezrin, with metastasis. Ezrin is the best characterized of the ERM (Ezrin-Radixin-Moesin) family. ERM proteins exist in the cytoplasm in an inactive "closed conformation" though N-terminal to C-terminal associations within the protein or with other ERM members. The stability and consistency of our findings involving ezrin across species lines has strengthened our belief in this cross-species approach. Ezrin's linkage of the cell membrane to the actin cytoskeleton directly allows cells to interact with its micro-environment and functionally provides an "intracellular scaffolding" that facilitates signal transduction through a number of growth factor receptors and adhesion molecules. We have demonstrated that ezrin is necessary for metastasis in murine transplantable osteosarcoma and genetically engineered rhabdomyosarcoma models, that it is relevant in human-murine sarcoma xenograft models, and that its expression is associated with metastatic progression in pet dogs with naturally occurring osteosarcoma, finally we have found an association between ezrin expression and risk of relapse in pediatric osteosarcoma patients. The value of this comparative approach has been to define biologically relevant motifs that have sustained importance as we cross species lines.

Evaluating antimetastatic therapies based on ezrin biology

The comparative approach is uniquely suite to rapidly translate findings from the laboratory to the clinic. With this interest in mind we have recently linked ezrin expres-

sion and the mTOR (mammalian target of rapamycin) pathway in the translation of proteins by metastatic cells. We have begun to validate these associations in murine, canine and human cancer cells and have conducted preclinical studies in mice that support the therapeutic role of inhibiting mTOR using rapamycin (and novel analogs). Indeed novel inhibitors of the mTOR pathway have entered early clinical trials for human osteosarcoma and sarcoma patients. There are many questions that remain unanswered regarding the optimal use of these agents in human patients. Indeed, many of the unanswered questions that exist in the development path of new drugs may be effectively answered by integrating studies that include pet dogs with cancer. To complete the translational effort based on ezrin and mTOR inhibition, we have now completed dose and regimen finding studies in pet dogs with osteosarcoma that will allow the evaluation of the inhibition of translation initiation (by rapamycin) in pet dogs with osteosarcoma. Ongoing studies will now define optimal treatment schedules for the use of rapamycin in children and dogs with osteosarcoma. Efforts to validate reagents and further characterize these models using more sophisticated techniques has been ongoing within several comparative oncology laboratories around the world. Contributing to this effort is Comparative Oncology Programme of the US National Cancer Institute's Center for Cancer Research and the not for profit, Canine Comparative Oncology and Genomics Consortium.

Based on the opportunities provided by the completion of the canine genome, the identical biological behaviour of canine and human osteosarcoma, and the increased prevalence and aggressiveness of this disease in dogs we recently used our cross-species comparative gene expression approach to uncover specific genes, gene families/functions, or pathways that were conserved across the dog and human and are commonly linked to metastasis. Collectively, our comparative approach has provided a novel, necessary and informative perspective for the study of cancer biology. The approach exemplifies the values of value of a veterinary and comparative perspective in the study of complex biomedical problems.

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CARDIAC DISEASE IN FARMED SALMONIDS

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With a production of more than 800 000 tonnes of farmed salmon, rainbow trout and cod, fish is by far the most important farm animals in Norway by comparison, cattle, sheep and swine production is 282 000 tonnes, approximately one third of the fish production. The present day production is dominated by Atlantic salmon (*Salmo salar*), followed by rainbow trout (*Oncorhynchus mykiss*) and cod (*Gadus morhua*). 95% of the biomass is produced in seawater cages. Salmonids (salmon and rainbow trout) will form the backbone of Norwegian fish farming for many years to come, as the farming of marine species such as cod and halibut (*Hippoglossus hippoglossus*) is more complicated and the market limited. Salmonids are fed energy-rich diets with up to 40% fat. An increasing proportion of modern feeds are of vegetable origin as marine resources are becoming fewer and more expensive.

From its beginning in the 1970'ies, the fish farming industry has suffered from numerous infectious and production-associated diseases. Historically, vibriosis (*Vibrio anguillarum*), cold-water vibriosis (*Vibrio salmonicida*) and furunculosis (*Aeromonas salmonicida*) have caused tremendous losses and extensive use of antibiotics. These bacterial diseases are now controlled through effective vaccination programs. The present disease situation is dominated by viral diseases such as infectious pancreatic necrosis (IPN), infectious salmon anaemia (ISA) and pancreas disease (PD). Additionally, production-related diseases with complex aetiologies associated to intensive feeding, breeding and general manipulation are becoming more common. Among such disease complexes are side-effects following vaccination, skeletal and soft-tissue malformations, cardiac abnormalities and enteritis. From a comparative point of view, the similarities to production-related disease in swine and poultry are many and striking. In this presentation, particular focus will be paid to cardiac diseases in farmed salmonids.

More than 30 000 species of fish are described and they occupy a wide range of different habitats requiring different solutions to physical and biological challenges. Salmonids together with scomberids (mackerel, tuna) and clupeids (herring) are among the athletes in the fish world. For example, Atlantic salmon migrate vast distances in the North Atlantic Ocean, chase their prey aggressively and negotiate long rivers and high

waterfalls on their migration to the spawning grounds. Their hearts (particularly the ventricle) are reflecting this lifestyle and the ventricle of a wild salmon has the shape of a sharp triangular pyramid with the apex pointing caudoventrally. The ventricle is composed of two distinct layers; an outer compact myocardium with a coronary blood supply and the inner spongy ventricle receiving only venous blood from the circulating blood pumped through the organ. This differentiation is also reflected in the pathological changes encountered in different diseases.

Cardiac diseases in salmonids may be categorized into those that are a part of a larger entity, e.g. systemic or localized bacterial and viral diseases, and idiopathic conditions. Among the former are furunculosis, PD and heart and skeletal muscle inflammation (HSMI). In this presentation, the main focus will be on idiopathic cardiac diseases such as cardiomyopathy syndrome (CMS), arteriosclerosis, ectopia cordis, aberrant shapes and several diffuse conditions such as endomyocarditis, epicarditis and degenerative lesions. This latter group appears to be an increasing problem and constitute an important background in understanding "unspecific mortality" often occurring during transportation to the slaughtering facility. Generally speaking, farmed salmon appear to be very fragile and tolerate grading, handling, transportation and medication poorly. This is partly alleviated by slaughtering the fish on site ("stun and bleed"), while the basic underlying problem of course remains unsolved.

Arteriosclerosis

Arteriosclerosis with resultant reduction of the blood-transporting capacity of the coronary artery has been known from sexually mature Pacific salmon since the 1960'ies and has been characterized as a "fact of life" as virtually every single individual is affected to varying degrees. The implications for the individual fish are probably limited. In farmed fish, this condition is of particular interest, as fast-growing individuals typically develop the most severe lesions and may therefore experience reduced threshold to stress such as low ambient oxygen level, grading and transport.

Ectopic heart

Ectopic heart resulting from absence of the transverse septum and situs inversus cordis both result in altered, suboptimal ventricular shape and misalignment of the normal blood flow through the bulbus arteriosus and ventral aorta. These conditions are primarily caused by too high temperature during the incubation of the eggs and the yolk-sac period.

Cardiomyopathy syndrome (CMS)

CMS is a chronic progressive disease leading to total destruction of the myocardial

trabeculae of the atrium and inner ventricle. CMS typically causes mortality in large fish close to slaughter and is therefore a major cause of economic loss. Histopathological lesions are characterized by widespread necrosis of spongy myocardium and massive infiltration of lymphocytic cells (?). Death results from cardiac collapse, severe congestion, intracardial coagulation and rupture of the atrium with resultant cardiac tamponade. Although transmission experiments have resulted in production of typical lesions, the causes remain unknown. It is an enigmatic fact that large and fat fish suffer the most heavy mortality.

Other cardiac diseases

Other cardiac diseases include gluconegenosis, ("Pompes disease"), myocardial hypoplasia, epicarditis and intracardial haemorrhage (Dissecting haemorrhage) and will be briefly discussed.

In summary cardiac diseases in salmonids are many and complex and represent an important cause of mortality and reduced tolerance to other diseases and stressors. Their causations are complex and challenging, but also of great interest from a comparative point of view.

VIRUS INFECTIONS OF FISH

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Introduction

Beside the "classical" aquacultured species in Europe as rainbow trout (*Oncorhynchus mykiss*) or common carp (*Cyprinus carpio*), new fish species like sheatfish (*Silurus glanis*), eel (*Anguilla anguilla*) or sturgeon (*Acipenser sp.*) introduced new disease agents and diseases into the world-wide aquaculture. Only the development of new diagnostical tools allowed the detection of these agents.

Diseases and their pathogens, notifiable to European Union (EU) and World Organization for Animal Health (OIE), are well known and mostly detectable by methods as cultivation in permanently growing fish cell cultures, antigen enzyme-linked immunosorbent assay (antigen ELISA), indirect immunofluorescence assay (iIFA) with polyclonal antisera or monoclonal antibodies (mabs), serum neutralization test (SNT) and, in some cases, polymerase chain reactions (PCR) or reverse transcription PCR (RT-PCR) as well as realtime PCR. Unfortunately, serological assays using serum antibodies directly from infected fish are not among the so called allowed diagnostical methods.

While most diagnostic laboratories have no problems to use validated and published methods for detection of notifiable infectious agents, new diseases occurred in newly aquacultured species. Instead of well validated diagnostical methods, mostly laboratory or in-house but accredited procedures are used for diagnosis.

Due to the enlargement of the EU, the cyprinid aquaculture production increased enormously since countries as Poland, Czech Republic or Hungary are main producers of these animals.

Salmonid diseases

Viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN)

VHS / IHN are notifiable diseases by EU and OIE and caused by VHSV and IHNV, respectively. While classical virological methods like histology and macroscopic patho-

logical anatomy (fig. 1) can give some information, more specific molecular and serological tools are often necessary.

Fig. 1. Macroscopically examination of VHSV infected rainbow trout. Bleedings and petechia in visceral fat, in muscle tissue (pathognomonic) and in the eyes.

Phot. D. Fichtner, FLI, Germany.

More often, both viruses can be isolated in permanent growing fish cell culture like RTG-2 (VHSV) or EPC (VHSV and IHNV) and the identified by iIFA using monoclonal antibodies (fig. 2).

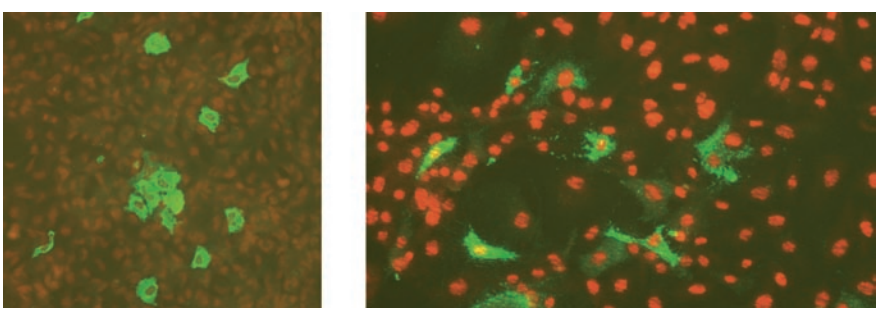

Fig. 2. On the left – iIFA with mabs against VHSV, IHNV in EPC cells and on the right – IHNV N protein for virus identification, VHSV in RTG-2 cells.

Phot. S.M. Bergmann, FLI, Germany.

In terms of IHNV some histological changes are visible. Mainly molecular assays as RT-PCR and sequence analysis of PCR products serve for genotyping of the different

VHSV and IHNV isolates, respectively. These methods are recently under validation for routine diagnostics inside the EU.

Infectious salmon anaemia (ISA)

One of the main threats for salmon (*Salmo salar*) production is the infection with ISA virus (ISAV). Norway, Faeroe Islands and more recently Chile diagnosed heavy lethal infection induced by European type of ISA. While RT-PCR (fig. 4) is an allowed diagnostic method, cell cultivation in "salmon head kidney cells (SHK-1)" (Dannevig *et al.*, 1997) and identification by iIFA using mabs are recommended by EU and OIE (fig. 3).

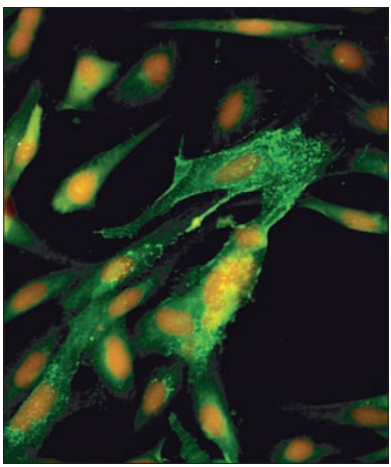

Fig. 3. Detection of ISAV by iIFA using mab, SHK-1 cells infected with ISAV isolate Glesvær (Norway) stained with mab ISA/3H6F8 (Falk *et al.*, 1997).

Phot. S.M. Bergmann, FLI, Germany.

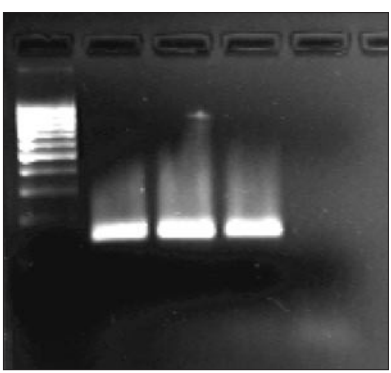

Fig. 4. RT-PCR acc. to Mjaaland *et al.* (1997), lane 1 DNA marker (peqlab), lane 2 isolate, Glesvær/2/90, lane 3 isolate Scotland 390/98, lane 4 positive control (Norway), lane 5 negative control.

Phot. S.M. Bergmann, FLI, Germany.

Sleeping disease (SD) of rainbow trout / pancreas disease (SPD) of salmon

Over that last 6 years a new disease occurred in rainbow trout in fresh water (SD) and in salmon in marine environment (SPD) caused by "atypical" *alphaviruses* named SD virus (SDV) and SPD virus (SPDV). The viruses could be identified by RT-PCR (Villoing *et al.*, 2000). Often samples obtained from SDV and / or SPDV infected fish were contaminated by Infectious Pancreatic Necrosis Virus (IPNV). IPNV was then identified either by cell cultivation in RTG-2 cell or by RT-PCR (Bergmann *et al.*, 2008, Taksdal *et al.*, 1997) (fig. 5).

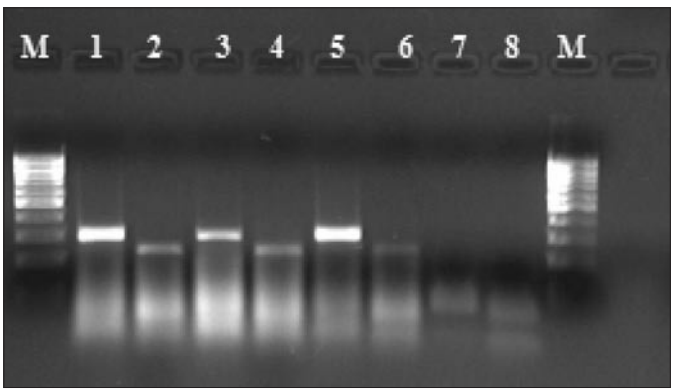

Fig. 5. M 100 bp marker (peqlab), lane 1 sample 1 SDV primers, lane 2 sample 1 IPNV primers, lane 3 sample 2 SDV primers, lane 4 sample 2 IPNV primers, lane 5 sample 3 SDV primers, lane 6 sample 3 IPNV primers, lane 7 neg. co SDV primers, lane 8 neg. co IPNV primers.
Phot. S.M. Bergmann, FLI, Germany.

To solve the problem of IPNV contamination, a new diagnostic procedure was implemented: RNA transfection by electroporation into RTG-2 cells (Bergmann *et al.*, 2008). While SDV / SPDV are positive stranded RNA viruses, their RNA is infectious itself, IPNV, as a negative stranded RNA virus, is not replication in cell cultures when this technique is used. After transfection or in case of a successful isolation, newly designed mabs against both viruses could be used as well.

Cyprinid diseases

Koi herpesvirus diseases (KHVD)

Over the last years, a new disease, named KHVD, was introduced and spread into the cyprinid aquaculture world-wide. This herpesvirus started to kill enormous amount

of common carp as well as koi. While cell cultivation in koi fin cells (KF-1, Hedrick *et al.*, 2000), molecular assays like PCR or realtime PCR were implemented as "golden standards" for disease diagnostics. Infected carps did show external clinical signs like gill necrosis, enlarged skin and gill mucous production and enophthalmus (fig. 6).

Fig. 6. External signs of KHVD in common carp: on the left – bleedings in the skin, mucous, on the right – gill necrosis.
Phot. Teifke, FLI, Germany, Bräuer, TSK Saxony, Germany.

For confirmation of these results only sequence analysis of PCR products, electron microscopy and IFA or *in-situ* hybridization (ISH) on tissue sections are available. Serological methods as antibody ELISA or SNT are recently under validation for implementation into allowed diagnostic methods.

Angullid diseases

An additional problem is the diagnosis of new agents in newly used species for aquaculture, e.g. eel or elvers. Over the last years picorna-, birna-, herpes-, rhabdo- and reoviruses have been found in sick elvers but also in adult eels. It is completely unknown, but assumed, if glass eels caught in the wild already carry these pathogens and it is not known which pathogen is influencing the production process. One main virus, reducing eel production world-wide, is the *herpesvirus anguillae* (HVA). While HVA can be easily isolated in eel-kidney cells (EK-1, Davidse *et al.*, 1999) (fig. 7), molecular tools as PCR or nested PCR (fig. 8) are often faster more accurate.

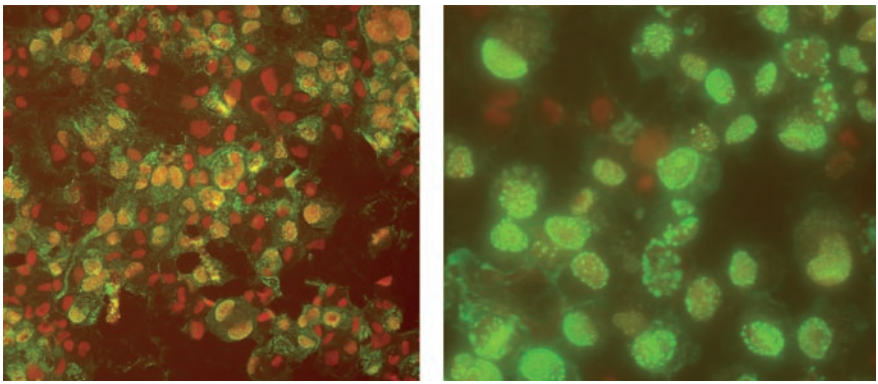

Fig. 7. Identification of HVA in EK-1 cells by iIFA, on the left - Polyclonal HVA antiserum from rabbit F 37 produced by D. Fichtner (1:10 000), on the right – α-HVA mab 16A8 produced by M. Dauber (1:5). Phot. S.M. Bergmann, FLI, Germany.

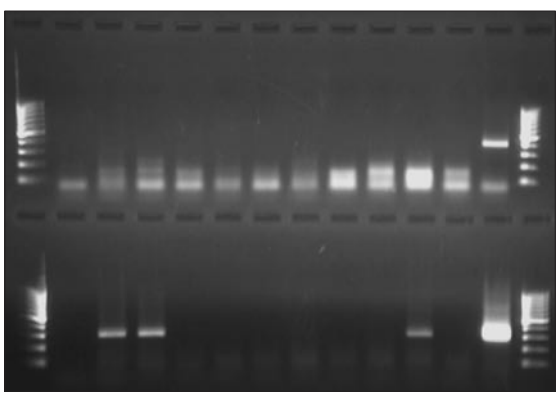

Fig. 8. Detection of HVA in samples from adult wild eels from Baltic Sea by nested PCR M DNA marker (peqlab), lane 1 negative control, lanes 2–11 wild eel samples (kidney, spleen, heart), lane 12 positive control PCR (RIJSEWIJK *et al.*, 2005) nested PCR (S.M. Bergmann and Schrudde, unpublished). Phot.: S.M. Bergmann, FLI, Germany.

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ORAL PRESENTATIONS

Session 3 A – Tumour Pathology 1/O

COMPARISON OF THREE DIFFERENT GRADING SYSTEMS IN CANINE PERIVASCULAR WALL TUMOURS

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Introduction
Canine soft tissue tumours comprise the group of perivascular wall tumours (PWT) that are considered locally invasive with poor metastatic potential. A grading system with predictive behavior has never been applied to PWTs.

Materials and methods
Three different grading systems (GS) A, B, and C were applied to 35 PWTs with follow up data. GS-A evaluated cell differentiation, percentage of necrosis and mitotic index (MI). GS-B included also tumour growth and GS-C evaluated proliferative index by MIB1 labeling, replacing the MI.

Results
The percentage of recurrence in GS-A was 8% for grade I, 37% for grade II, and 33% for grade III; recurrence in GS-B was 0% for grade I, 43% for grade II, and 25% for grade III; and recurrence in GS-C was 20% for grade I, 26% for grade II and 100% (one case) for grade 3. The majority of cases arising on the neck or head recurred and the majority of cases in other locations did not recur.

Conclusions
The behaviour of PWTs correlates with anatomic location but not with the type of growth. The GS-C grading system gives the best prediction of recurrence. The cut-off values of MIB1-PI should be refined by statistical analysis.

IMMUNOHISTOCHEMICAL EXPRESSION OF VEGF, FLK1 AND CTGF IN CANINE PERIVASCULAR WALL TUMOURS

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Introduction

Soft tissue sarcomas (STS) represent 15% of canine cutaneous and subcutaneous tumours, including perivascular wall tumours (PWTs). Vascular endothelial growth factor (VEGF) and connective tissue growth factor (CTGF) in human STS have pathogenetic and prognostic significance.

Materials and methods

Immunohistochemical evaluation of VEGF, its receptor Flk1, CTGF and proliferation index (PI) were evaluated and compared with histologic grade.

Results

The majority of tumours were grade I (13) or II (13). In 23 cases 70–100% of the cells expressed VEGF, and in 14 cases expression was more intense in 5% to 20% of the cells. In 21/23 cases, more than 70% of the cells expressed Flk1. Two of 23 cases were Flk1 negative and had the lowest PI. In 17 cases more than 70% of cells and in 2 cases 5–20% of cells expressed CTGF while 8 cases were negative.

Conclusions

VEGF and Flk1 expression are unrelated with grade or PI, although among PWTs with high VEGF expression, negativity to Flk1 is associated with the lowest PI, suggesting an autocrine and paracrine VEGF-Flk1 pathway. PWTs contain a subpopulation of cells producing higher levels of VEGF possibly acting on the adjacent neoplastic cells. A wider caseload is required to confirm these hypotheses by statistical analysis.

CANINE GASTROINTESTINAL STROMAL TUMOURS: MORPHOLOGIC AND IMMUNOLOGIC STUDY

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Introduction

Gastro-intestinal Stromal Tumours (GISTs) are digestive mesenchymal tumours and are seldom diagnosed in veterinary medicine. In human beings, GISTs are now recognized as a specific tumoural entity derived from interstitial cells of Cajal, the cellular pacemakers of the digestive tract. Diagnosis of GISTs is based on histological and immunohistochemical characteristics, as most tumoural cells express KIT, the tyrosine kinase receptor encoded by the *Kit* gene. GISTs are particularly resistant to radiotherapy and chemotherapy and KIT tyrosine kinase inhibitors have revolutionized treatment.

Materials and methods

In a review of the Idexx-Alfort database, from January 1st 2003 to September 30th 2008, 100 canine cases of malignant digestive mesenchymal tumours were collected. Immunohistochemical labelling was performed using antibodies against KIT, smooth muscle actin, desmin, vimentin and pS-100. KIT-expressing tumours were subclassified according to their histological pattern.

Results

60 cases of digestive canine tumours were identified as GISTs based on KIT expression in tumoural cells. The great majority of these tumours showed a fusiform histological pattern similar to the most frequent pattern found in human GISTs.

Conclusion

We identified a large series of canine GISTs. Better recognition of these tumours in dogs will facilitate a new therapeutic approach.

EXPRESSION OF CLAUDIN-1 IN A CANINE PERINEURIOMA

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Introduction

Perineuriomas are rare benign peripheral nerve sheath neoplasms composed exclusively of perineural cells. These cells differ from almost all other mesenchymal cells based on their ability to form tight junctions.

Materials and methods

A 4-year-old male Leonberger was presented with a 2 year history of left foreleg lameness. Surgical exposure revealed a thickened distal median nerve. The lesion was excised and microscopically examined.

Results

Histological examination of HE-sections revealed an irregular enlarged, hypercellular nerve fascicle. The neoplastic tissue consisted of spindle-shaped cells arranged in pseudo-onion bulb-like whorls around axons. Cells were embedded in a loose myxoid matrix. Neoplastic cells were immunoreactive for laminin and claudin-1, but not for S-100. Electron microscopy showed some cells with a discontinuous basal membrane and some containing pinocytotic vesicles, confirming their perineural nature.

Conclusions

As in humans, claudin-1 can be used as a marker for perineuriomas in dogs. More canine perineuriomas should be examined to confirm the consistency of claudin-1 expression.

GLOBAL GENE EXPRESSION OF METASTATIC CANINE MAMMARY TUMOURS

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Introduction

The molecular mechanisms of canine mammary tumour carcinogenesis and metastasis are unclear in most aspects. To identify biochemical pathways associated with metastastic potential of canine mammary tumours the global gene expression of metastatic tumours was compared with that of normal mammary gland.

Material and Methods

Macro dissected samples of snap frozen canine mammary tumours with lymph node metastases and normal mammary gland of the same dogs (n = 12) were used to isolate total RNA. RNA integrity was controlled and cRNA samples were hybridized onto the Affymetrix© Canine Genome 2.0 array. Differences in gene expression were analyzed using the Partek GS and the Database for Annotation, Visualization, and Integrated Discovery (DAVID).

Results

529 not annotated mRNA/EST-based transcripts and 1326 annotated canine genes were differentially expressed ($p < 0.05$, fold change >2 or <-2) in adenocarcinomas versus normal mammary gland. Genes belonged to several functional groups including cell cycle control, DNA repair, p53 signaling and the TGF-beta superfamily.

Conclusion

Global gene expression analysis using canine specific cDNA microarrays is a promising approach to identify genes involved in canine mammary tumour carcinogenesis.

HEMANGIOPERICYTOMA IN DOG: AN ENDANGERED SPECIES? ULTRASTRUCTURAL STUDY OF CANINE CUTANEOUS PERIVASCULAR TUMOURS

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Introduction

Perivascular wall tumours (PWTs) reported in dogs are hemangiopericytoma (HEP), angioleiomyoma/sarcoma and glomus tumour. HEP remains a controversial entity due to the lack of specific morphological and immunohistochemical features. The aim of this study was to characterize cases of undifferentiated canine cutaneous PWTs by transmission electron microscopy (TEM).

Materials and methods

Samples from skin masses were fixed in 2.5% gluteraldehyde, post-fixed in OsO_4 and embedded in epoxy resin. Semi-thin and ultrathin sections were stained with toluidine blue and counterstained with uranyl acetate and lead citrate, respectively.

Results

The evaluation of "key" cellular features (cytoplasmic processes, external lamina, pinocytic vesicles, subplasmalemmal densities, myofilaments, fibronexus junctions) allowed the identification of the following categories: hemangiopericytoma-like undifferentiated PWT (6/12), hemangiopericytoma (3/12), myopericytoma (1/12), undifferentiated fibroblastic-like PWT (1/12), undifferentiated angiomyofibroblastoma (1/12). One myopericytoma diagnosed by TEM had a benign behaviour, while two hemangiopericytomas recurred.

Discussion

The main problem in the immunohistochemical diagnosis and classification of canine PWTs derives from the loss of specific differentiation markers by neoplastic cells and from the phenotypic plasticity of vascular mural cells (pericytes and perivascular fibroblasts) reflecting their origin from vascular wall resident mesenchymal (stem) cells (perivascular niche). This problem may be solved by TEM analysis in association with an ultrastructural diagnostic algorithm.

BOVINE PAPILLOMAVIRUS E7 ONCOPROTEIN EXPRESSION AND ITS COLOCALIZATION WITH E5 IN NATURALLY OCCURRING CARCINOMAS OF THE URINARY BLADDER IN CATTLE

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Introduction
Urinary bladder tumours in cattle are caused by chronic ingestion of bracken fern and infection with bovine papillomavirus (BPV). The objective of the present study was to assess, in a subset of E5 positive urothelial bladder carcinomas harboring BPV-2 DNA, the expression of BPV E7 oncoprotein and its co-localization with the viral E5 oncoprotein.

Materials and methods
Fifteen samples were analysed by immunohistochemistry for detection of E7 and three BPV E7 positive samples were investigated by double-labelling immunofluorescence for E5 and E7 co-localization.

Results
All the BPV-2 positive carcinomas expressed the E7 viral oncoprotein. The E7 immunoreaction was mainly detected in the cytoplasm and in the nuclei, although membrane staining was also observed in urothelial cells. Additionally, E7 was also found to be co-expressed with E5 in carcinoma lesions.

Conclusion
This is the first report of BPV E7 detection and its coexpression with E5 in carcinomas of the urinary bladder in cattle. These results suggest a possible role for the E7 oncogene in urothelial carcinogenesis.

ORAL PRESENTATIONS

Session 4 B – Pig Pathology

DETECTION OF MSG1 AND HSPA1 SURFACE ANTIGENS OF MYCOPLASMA SUIS BY IMMUNOELECTRONMICROSCOPY

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Introduction

The antigenic structures of the haemotrophic *Mycoplasma suis*, an epicellular parasite of erythrocytes, are largely unknown due to its unculturability. During a complex study, the genes and proteins of MSG1 and HspA1 were identified and characterized in detail. The present investigation aimed to verify the localisation of these proteins by immunoelectron-microscopical techniques using self-produced antibodies against rMSG1 and rHspA1.

Materials and methods

Erythrocyte-pellets of experimentally infected pigs (n = 3) and non-infected pigs (n = 3) were fixed in paraformaldehyde and embedded in LR White (-20°C and +65°C, respectively). For post embedding method, 1:10 MSG1 or 1:10 HspA1 antibodies were incubated and 10 nm gold labelled anti-rabbit IgG (1:50) were applied. Grids were stained with 1% uranyl acetate and 0.1% lead citrate.

Results

The membrane and surface localisation of MSG1 and HspA1 in *Mycoplasma suis* was verified only in -20°C embedded material.

Conclusions

Immunoelectronmicroscopy is not useful for routine diagnosis of *Mycoplasma suis,* but was an illustrative complementary tool in this study.

EVALUATION OF THE APOPTOTIC PHENOMENON AND EXPRESSION OF PROINFLAMMATORY CYTOKINES IN THE TONSIL OF PIGS INFECTED WITH A EUROPEAN PRRSV FIELD ISOLATE

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Introduction
A characteristic feature of Porcine Reproductive and Respiratory Syndrome (PRRS) is the presence of apoptosis in macrophages and lymphoid cells. However, the mechanism of induction of apoptosis has not been elucidated. The aim of this study was to investigate possible correlation between the expression of PRRSV antigen, proinflammatory cytokines and the apoptotic phenomenon in the tonsil of pigs inoculated with a PRRSV field isolate.

Materials and methods
Twenty eight specific pathogen free pigs were randomly distributed in batches of four and inoculated intramuscularly with PRRSV field isolate 2982 and killed at 3, 7, 10, 14, 17, 21 and 24 days post-inoculation (dpi). As controls, four other pigs were inoculated with 1ml of sterile medium and killed at the end of the study.

Results
The highest expression of viral antigen was observed at 7 dpi mainly in macrophages, and to a lesser extent in dendritic cells, and coincided with increased expression of TNF-α by macrophages and lymphocytes. Expression of IL-6 and IL-1α was observed at 3 dpi. Apoptotic phenomena, evaluated by Caspase 3 expression and TUNEL technique, were principally seen in lymphocytes at 24 dpi.

Conclusion
These results indicate that, in our study, the apoptotic phenomenon does not show a significant correlation with the expression of proinflammatory cytokines. Other indirect mechanisms appear to be involved.

GP5 AND M PROTEINS OF PRRSV COULD BE RELATED TO INFLAMMATORY RESPONSES

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Introduction

The immunopathogenesis of Porcine Reproductive and Respiratory Syndrome (PRRS) is poorly understood. In the present study, the role of different viral proteins was examined.

Material and Methods

Sixteen piglets were vaccinated (days 0, +21 and +42) with plasmid DNA as follows: empty plasmid (A, n = 4); ORF7 (B, n = 4); ORFs 5 + 6 (C, n = 4); ORFs 4 + 5 + 6 + 7 (D, n = 4). At day +63, the piglets were challenged intranasally with 2 ml of PRRSV (1×10^6 $TCID_{50}$/ml PRRSV Spanish isolate L450) and monitored for the next 21 days. The piglets were then euthanized and necropsied and samples of lung and lymphoid organs were collected. Throughout the study period, the humoral response was examined by ELISA (Herdchek 2XR, Idexx Laboratories), cell-mediated immunity (CMI) was monitored by means of the IFN-g ELISPOT and viremia was assessed by a nested RT-PCR.

Results and Conclusions

After challenge, fever (> 40°C) was higher ($p < 0.05$) in piglets in groups C and D and piglets receiving ORFs 5 + 6 had more severe lung lesions. Seroconversion occurred earlier in piglets in groups B and D and vaccinated animals showed earlier development of CMI. Taken together, our data suggest that previous immunity against GP5 and M proteins could lead to exacerbated clinical signs and lesions mediated by proinflammatory responses. This should be considered in the development of new vaccines.

MODULATION OF THE PULMONARY IMMUNE RESPONSE BY CYTOKINE EXPRESSION IN PRRS

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) replicates mainly in porcine alveolar macrophages (PAMs) but the mechanism whereby the immune response is triggered in the lung is not well understood. This study focused on determining changes in cytokines in the lungs of PRRSV-infected pigs.

Material and Methods

Twenty-eight piglets were distributed in groups of four and killed at 3, 7, 10, 14, 17, 21 and 24 days post-inoculation (dpi). Another group of four piglets was used as controls. Lung samples were fixed in 10% buffered neutral formalin and in Bouin solution for histopathological and immunohistochemical studies (PRRSV, IL-10, IL-12 p40, TNFα, IFNα, IFNγ antigens), respectively.

Results

Cytokines were expressed mainly by septal macrophages, whereas PRRSV antigen was observed mainly in the cytoplasm of PAMs. Correlation was observed between the expression of PRRSV and IL-10, IL-10 and IFNα, IL-12 p40 and IFNγ, and TNFα and IFNγ. However, no correlation was found between IFNα and IFNγ.

Conclusions

These results indicate that PRRSV modulates the immune response by the expression of IL-10, which might induce lower levels of other cytokines implicated in viral clearance. Stimulation of expression of IFNγ by IL-12 p40 and TNFα, but not by IFNα is also indicated.

PROINFLAMMATORY CYTOKINE EXPRESSION IN PRRSV INFECTION: PARACRINE SYNTHESIS AND CORRELATION WITH LUNG LESION EXTENT

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Introduction

Expression of cytokines is reportedly poor in pigs following infection with the porcine reproductive and respiratory syndrome virus (PRRSV). The aim of this study was to compare the serum concentrations with respect to tissue expression of proinflammatory cytokines and the extent of pulmonary lesions in PRRSV infection.

Materials and methods

Twenty-eight piglets were randomly distributed in groups of four and killed at 3, 7, 10, 14, 17, 21 and 24 days post-inoculation (dpi). Four identical pigs were used as control group and killed at the end of the study. Serum levels of cytokines were determined by means of commercial ELISA kits for IL-1β, IL-6, and TNF-α. Lung samples were fixed in 10% buffered neutral formalin for the histopathological study and in Bouin solution for the immunohistochemical study, the latter using commercial antibodies for PRRSV, IL-1α, IL-6 and TNFα antigens.

Results

Serum concentrations of proinflammatory cytokines showed a mild increase throughout the length of the study. Nevertheless the immunohistochemical expression of cytokine antigens in the lung was accentuated mainly in areas of interstitial pneumonia.

Conclusions

The low serum levels of cytokines together with the increased expression of these cytokines in the lung parenchyma point to a paracrine expression of proinflammatory cytokines during PRRSV infection.

REDUCED EXPRESSION OF INTESTINAL P-GLYCOPROTEIN FOLLOWING INGESTION OF DEOXYNIVALENOL (DON) CONTAMINATAD FEED IN PIGS

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Introduction

P-glycoprotein (P-gp) mediates active transmembrane transport of a variety of substrates. We investigated whether Fusarium mycotoxin deoxynivalenol (DON) alters the expression of P-gp in the intestinal tract of pigs.

Materials and methods

Nine pigs (5.90 ± 0.169 kg BW) were randomized into three groups receiving respectively blank feed, feed contaminated with 1 μg/g (ppm) DON and feed contaminated with 3 ppm DON, *ad lib* for 10 days. After euthanizing, samples of small intestine and colon were fixed in formalin for P-gp immunostaining using the antibody C219.

Results

Staining with antibody C219 showed reduced expression of P-gp at the brush border of the small intestinal villi in animals receiving 1 μg DON per gram of feed. A dose of 3 μg/g DON resulted in complete disappearance of P-gp.

Conclusion

Oral bioavailability of many xenobiotics is normally limited by P-gp mediated intestinal secretion. Loss of P-gp expression following DON ingestion may lead to a higher uptake of noxious substances present in the intestinal lumen such as xenobiotics. This may partly explain the reduced zootechnical performance of pigs exposed to DON.

DEVELOPMENT OF IMMUNOHISTOCHEMISTRY FOR DETECTION OF LAWSONIA INTRACELLULARIS IN FORMALIN-FIXED TISSUES FROM PIGS

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Introduction

The aim of the study was to develop an immunohistochemical method for detection of *Lawsonia intracellularis* (*L. intracellularis*) and to compare this method with PCR.

Materials and methods

Samples of intestines and feces from 10 pigs with clinical signs of proliferative enteropathy were analyzed using PCR and immunohistochemistry. Demonstration of *L. intracellularis* in tissue was obtained using monoclonal antibodies (EuroClone) and positive controls kindly provided by dr. T.K. Jensen (Danish Institute for Food and Veterinary Research, Copenhagen, Denmark). Sections of intestines from clinically healthy pigs served as negative controls. All sections were additionally stained with hematoxylin-eosin (HE).

Results

DNA of *L. intracellularis* was found in seven samples of intestines and in the corresponding feces. Positive signal for *L. intracellularis* was detected by immunohistochemistry in all positive controls and in two samples from diseased pigs. The remaining five samples were negative by immunohistochemistry, but showed typical microscopic lesions.

Conclusions

The results show that *L. intracellularis* antigen can be detected in formalin-fixed, paraffin-embedded tissues of pigs using monoclonal antibodies. The high specificity and low sensitivity of the immunohistochemical test can be attributed to DNA degradation during fixation, embedding and dewaxing of sections. Samples that are negative in immunohistochemistry and positive in PCR may originate from animals that are in chronic stages of disease or early stages of recovery, where bacteria are present in small numbers or are absent from the tissue.

ORAL PRESENTATIONS

Session 7 A – Tumour Pathology 2/O

MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF MALIGNANT LYMPHOMA IN PET RABBITS

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Introduction
In Europe malignant lymphoma is the second most common skin neoplasm in pet rabbits. However, little is known about the specific sub- and immunotypes of lymphoma in this species. The goal of this morphologic study was to identify the various subtypes.

Materials and methods
Twenty seven tumours with the diagnosis of lymphoma were reviewed. Specimens were immunostained with CD 79a and CD 3 and subtyped according to the Histological Classification of Hematopoietic Tumors in Domestic Animals.

Results
All neoplasms contained moderately to highly pleomorphic lymphoblastic cells. Seven tumours contained binucleated or multinucleated giant cells. Fourteen tumours were classified as large cell immunoblastic B-cell lymphoma. Eleven tumours were classified as T-cell-rich B-cell lymphoma, while one tumour was classified as epitheliotropic lymphoma. One lymphoma could not be subclassified.

Discussion
This is the first case series of malignant lymphoma in pet rabbits. In contrast to other domestic animals lymphomas were highly pleomorphic and frequently contained multinucleated giant cells. Unexpectedly, the second most common subtype was T-cell-rich B-cell lymphoma, a lymphoma subtype that is rarely diagnosed in man, dog or cat yet frequently diagnosed in equines. Further research to elucidate a possible viral etiology is currently being undertaken; follow up information for this case series is being collected.

DETECTION OF CLONALITY IN FELINE T CELL LYMPHOMAS

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Introduction

Lymphatic neoplasias of cats can be difficult to distinguish from reactive lymphocytic proliferations and lymphoproliferative diseases using histopathological and immunohistochemical methods. Diagnosis in such cases can be supported by methods that detect features that are characteristic for tumour cells, e.g. the presence of a clonal population. Clonality can be detected by analysis of the rearranged T cell receptor gene sequences.

Materials and methods

Two primers from the second and three primers from the third framework region of V gene segments were used together with three primers from the J gene segments. The polymerase chain reaction (PCR) was performed as a multiplex PCR and both native and denatured PCR products were evaluated using horizontal polyacrylamide gel electrophoresis. DNA from formalin-fixed, paraffin-embedded (FFPE) lymphoma samples was used as template.

Results

111/140 (80%) samples produced results suggestive of the presence of a mono- or oligoclonal population of T cells. With the described method, FFPE as well as frozen samples of lymphoid hyperplasia, GALT and thymus were examined as controls and contained polyclonal T cell populations.

Conclusions

With this assay it is possible to detect clonality as an important adjunctive tool for the diagnosis of T cell lymphomas in the cat. Discrepancies between the results of different diagnostic methods need to be discussed further.

IMMUNOHISTOCHEMISTRY FOR ANTI-APOPTOTIC PROTEINS MCL-1 AND BCL-X IN CANINE NORMAL TISSUES AND LYMPHOMA SAMPLES

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Introduction

Bcl-2 family members are important in tumourigenesis but their roles in the genesis of canine lymphoma are yet unexplored. The aims of this study were to select antibodies for immunohistochemical detection of various Bcl-2 family proteins in canine tissues and to analyze expression of the anti-apoptotic members Mcl-1 and Bcl-x in canine normal tissues and lymphoma samples.

Materials and methods

Commercially available antibodies were tested for cross-reactivity and specificity by immunohistochemistry and Western blotting using bacterially expressed canine recombinant proteins. Immunohistochemical protocols for canine Mcl-1 and Bcl-x were optimized using formalin-fixed cultured cells and selected normal tissues and were applied to tissue arrays containing a panel of normal tissues and archival lymphoma samples.

Results

A total of twelve antibodies out of a panel of twenty-one were deemed to specifically cross-react with canine recombinant Mcl-1, Bcl-x, Bcl-w, Bak and Bax. Immunohistochemical expression patterns of Mcl-1 and Bcl-x in normal canine tissues roughly coincided with those reported for human tissues. Lymphomas were shown to often express Mcl-1 and, to a lesser extent, Bcl-x, across a range of different subtypes.

Conclusions

These findings suggest a role for anti-apoptotic Bcl-2 family members Mcl-1 and Bcl-x in the genesis and maintenance of canine lymphoma.

CYTOLOGICAL, HISTOLOGICAL AND IMMUNOHISTOCHEMICAL CORRELATION IN LYMPHOMA OF CARNIVORES

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Introduction
Correlative diagnosis in lymphoma of carnivores offers a complete vision and the possibility to establish an accurate prognosis and adequate treatment.

Materials and methods
Our study was performed on tumour samples from 15 domestic carnivores. The samples were cytologically, histologically and immunohistochemically investigated using May Grünwald Giemsa, Masson trichrome stain and human monoclonal antibodies with the ABC method, respectively.

Results
Cytological examination showed forms with small and undifferentiated cells, centrocitic, centroblastic, immunoblastic, lymphoblastic and large granular lymphoma. The histopathological findings included follicular, diffuse and mantle cell lymphoma. Immunohistochemical findings included B-cell, T-cell and T-cell-rich B-cell lymphomas. Other tumours showed negative reaction to CD79 but positive reactions to CD34, Bcl-2 and vimentin.

Conclusions
Cytological examination is of limited value in the diagnosis of small and undifferentiated cell lymphoma. Histopathology is of use in the diagnosis of incipient lymphoma due to the fact that in the advanced stage of the disease the lymph node structure is diffusely damaged. Immunohistochemical examination is indispensable for the diagnosis of B cell cutaneous lymphoma (CD79+), T cell digestive lymphoma (CD3+) and T-cell-rich B-cell lymphoma. Follicular centrocitic lymphoma (CD79+) is the least aggressive form with 4 years survival. Large granular lymphoma shows maximal aggression with 14 days survival.

CLONAL RECOMBINATION OF T-CELL RECEPTOR Γ V- AND J-REGION VARIANTS IN FELINE LYMPHOMAS

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Introduction

Lymphomas are among the most important neoplasms of domestic cats. As it can be challenging to distinguish lymphomas from reactive lymphatic hyperplasia, molecular methods based on cellular monoclonality are helpful to support the diagnosis of lymphomas. However, for this it is essential to know which variable- and joining-region variants are utilized by neoplastic lymphoma cells.

Material and Methods

Using primers directed against the conserved framework regions 1 and 4 of the T-cell receptor γ, we amplified clonally rearranged variants from DNA extracted from 50 formalin-fixed and paraffin-embedded lymphomas using polymerase chain reaction. Amplicons were sequenced. Additionally we tested the lymphomas for the presence of Feline Leukemia Virus provirus DNA.

Results

All clonally recombined variable-region variants amplified belonged to one of the two known subgroups. Twelve of the joining-region variants belonged to the first subgroup, the other two, however, have also been found three times each. Two neoplasms contained one example of each joining-region subgroup and were thus oligoclonal. 68% of the lymphomas contained Feline Leukemia Virus provirus DNA.

Conclusion

Clonality testing is a useful aid in the diagnosis of formalin-fixed and paraffin-embedded feline lymphomas. Importantly, the assay should include primers for subgroup 1 of variable-region and all subgroups of joining-region genes.

IDENTIFICATION OF BOVINE DENDRITIC CELL PHENOTYPES AND CYTOKINE EXPRESSION IN CATTLE INFECTED WITH BLV

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Introduction

Dendritic cells (DCs) are specialized, potent antigen presenting cells (APCs) with the unique ability to prime effective immune responses. They express higher levels of MHC class II and accessory molecules on their surface than other professional APCs.

Materials and methods

The investigations were performed on peripheral blood mononuclear cells isolated by density gradient centrifugation from the blood of leukemic cows. With the use of paramagnetic microbeads labelled with antibodies to CD14 (Miltenyi Biotech), monocytes were isolated and cultured in the presence of IL-4 and GM-CSF for DC generation. A panel of monoclonal antibodies and flow cytometry were used for DC phenotyping. Cytokines IL-6, IL-10, IL-12p40 and IL-12p70 were determined in culture fluids using ELISA tests. DC morphology was microscopically observed after staining with May Grünwald –Giemsa.

Results

In cell cultures many types of DCs were observed at different stages of maturity. We found that expression of CD11b, CD11c, MHC-I class and MHC-II was much higher in DCs from leukemic cattle than in DCs from healthy cattle.

Conclusions

Infection with BLV causes increased expression of cytokines and changes in the percentage of surface molecules on DCs. In comparison with DCs from healthy cattle, DCs from leukemic cattle have a more delicate structure with numerous cytoplasmic vacuoles and granules.

ORAL PRESENTATIONS

Session 8 B – Viral Pathogenesis

AVIAN INFLUENZA SPECIFIC RECEPTORS EXPRESSED IN THE RESPIRATORY AND GASTROINTESTINAL SYSTEM FROM CHICKENS, TURKEYS, OSTRICHES, PATRIDGE, DUCKS AND QUAIL

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Introduction

In the present study, we investigate the distribution of α2-3-linked SA specific for binding of avian viruses and α2-6-linked SA for binding human viruses, in the upper and lower respiratory tract and gastrointestinal system of chickens, turkeys, ostriches, partridge, ducks and quail.

Materials and methods

Tissue sections of the nasal cavity, trachea, lung, duodenum, cecum and colon were evaluated by lectin histochemistry using Maackia amurensis hemagglutinin for the detection of α2-3 and Sambucus nigra for α2-6. Receptor specificity was confirmed using neuraminidase to remove sialic acid residues. Sections of human, mouse, and pig lung were used as positive controls.

Results

Chicken and quail cells expressed α2-6 and α2-3 virus receptors in the upper and lower respiratory tract, while partridge had both receptors in the nasal cavity. Both types of receptors were also found in quail intestine. Similar to previous reports, α2-6 and α2-3 type receptors were found in the lower respiratory tract from humans and pigs.

Conclusions

This investigation supports the notion that chickens and quail can serve as mixing vessels where human and avian virus could be reassorted. Additionally, we demonstrated that partridge could be an intermediate host where new viruses with human and avian components may arise.

COMPARISON OF INFLAMMATORY RESPONSES AND APOPTOSIS IN THE BRAIN OF THEILER'S MURINE ENCEPHALOMYELITIS VIRUS-INFECTED SJL/J AND C57BL/6 MICE

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Introduction

Theiler's murine encephalomyelitis virus (TMEV) causes a chronic demyelinating leukoencephalomyelitis in susceptible SJL/J mice, similar to multiple sclerosis. In comparison, in resistant C57BL/6 mice the virus is eliminated from the brain after an acute polioencephalitis. The aim of this study was to compare apoptotic changes and inflammation in different brain areas of TMEV-infected SJL/J and C57BL/6 mice.

Materials and methods

60 TMEV-SJL/J and C57BL/6 mice were necropsied at 7, 14, 56, 98, and 196 days post infection (dpi). Immunohistochemistry to detect T cells (CD3), B cells (CD45R), microglia/macrophages (Mac3) and apoptotic cells (caspase-3) was performed on paraffin-embedded brain samples.

Results

Both mouse strains showed T cell-dominated inflammatory responses in the cerebrum at 7 and 14 dpi. Microglia/macrophages were found predominantly in the brain stem of SJL/J mice during the late phase (56, 98, 196 dpi) associated with myelin damage. Increased numbers of apoptotic cells were present in the hippocampus, thalamus and meningeal infiltrates of C57BL/6 mice at 7 and 14 dpi.

Conclusion

Apoptosis in early cerebral inflammatory lesions of C57BL/6 mice may be a consequence of activation-induced cell death. Inflammatory responses in the chronic phase are suggestive of delayed-type hypersensitivity or bystander mechanisms, contributing to demyelination in the brain stem of SJL/J mice.

THE ROLE OF M1 PROTEIN IN THE ACUTE INFECTION OF WOOD MICE WITH MHV-68 (MURID HERPESVIRUS 4)

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Introduction

MHV-68 infection in a natural host, the wood mouse (*Apodemus sylvaticus*), is a good laboratory model for the study of γ-herpesviruses. The aim of this study was to investigate the function of the viral M1 gene in this model.

Materials and methods

Wood mice were infected intranasally with either M1-partially-knocked-out (M1KO) or a marker-rescue control MHV-68 virus and culled after 7 days. Lungs were examined for morphological changes, MHV-68 antigen (immunohistology) and latency-associated tRNA (*in situ* hybridization). Real time PCR was performed to assess viral DNA load in lungs of infected animals and *in vitro* studies were completed to assess growth of the different viruses.

Results

The lungs of wood mice infected with M1KO showed more severe granulomatous infiltration together with more extensive necrosis and a wider spectrum of latently infected cells. This was despite a mild decrease in the viral load in M1KO infected wood mice. The *in vitro* studies revealed that the partial knocking out of the gene did not impair viral replication.

Conclusion

The M1 protein exerts a strong influence on the host's acute inflammatory response most likely by interfering with macrophage activation.

FCΓ RECEPTORS (FCΓRS) AND AUTOIMMUNITY: A STUDY ON DIFFERENT COMBINATIONS OF FCΓRS-DEFICIENT MICE

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Introduction

Fc receptors for Immunoglobulins G (IgGs) are antagonistic receptors for either activation or inhibition via signal transduction upon ligation by immune complexes. Systemic autoimmune diseases, such as lupus, arise as a consequence of the failure of maintenance of immunological self-tolerance for nucleosomal antigens.

Materials and methods

To facilitate the dissection between pathways which culminate in autoimmunity we developed and phenotyped FcγRII-/-, FcγRII-/- RIII-/-, FcγRIII-/-RII-/- RI-/- and FcγR-/- lines. Mice were generated with 129 derived ES cells and subsequently back crossed on C57Bl6.

Results

FcγRII-/- and FcγRIII-/-RII-/- RI-/- mice developed proliferative lupus nephritis, systemic vasculitis and severe lymphoid hyperplasia in secondary lymphoid organs. These mice died because of renal insufficiency. FcγRII-/- RIII-/- line developed milder lesions. Such lesions were not observed in FcγR-/- mice.

Conclusions

Our study confirms that Fc receptors of the FcγRII type are involved in the inhibitory arm of the immune response. Deficiency of FcγRIII or RI alone either attenuates or abolishes the development of autoantibody-mediated disease. By contrast, FcγRII-/-RIII-/- and FcγRIII-/-RII-/- RI-/- lines showed moderate to severe renal pathology. This finding indicates that, despite the combined deficiency of multiple Fc receptors, absence of the inhibitory type FcγRII determines susceptibility for systemic immune complex-mediated autoimmune disease.

MOUSE MODEL OF INFLUENZA VIRUS-ASSOCIATED ENCEPHALOPATHY OF CHILDHOOD

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Introduction

Influenza virus-associated encephalopathy (IAE) of childhood is characterized by brain edema and DIC following clinical signs of influenza. The pathological mechanisms remain unknown, although hypercytokinemia and clotting disorders are possible contributing factors. Here, we attempted to establish a mouse model of IAE using LPS as a cytokine inducer.

Materials and methods

One-week old ICR mice were inoculated with H3N2 subtype influenza A virus (IAV mice) followed in some mice by two inoculations of LPS (IAV+LPS mice). Control mice were injected only with IAV or LPS. The animals were examined using histopathology, viral isolation, cerebral evans blue-leakage test, and by measurement of plasma cytokines.

Results

IAV+LPS mice showed a reduced survival rate. Histopathologically, LPS and IAV+LPS mice showed cerebral microbleeding and edematous changes. These changes were more distinct in IAV+LPS mice than in LPS mice. In IAV and IAV+LPS mice, virus was recovered from the lungs and was absent from the brain. IAV+LPS mice also showed increased cerebrovascular permeability and higher levels of IL-6 and TNF-alpha in the plasma.

Conclusion

The increased cerebrovascular permeability, failure to detect virus in the brain and elevated plasma cytokine levels in IAV+LPS mice accord with the findings in IAE. The mechanisms of hypercytokinemia are now under investigation.

SUPPRESSION OF RABIES VIRUS PROPAGATION IN MICE BRAIN BY INTRACEREBRAL IMMUNIZATION OF INACTIVATED VIRUS

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Introduction

Rabies is one of the classical viral zoonoses. The disease is lethal in many mammals including humans and no effective treatment is available. The objective of this study was to examine the efficacy of intracerebral (IC) immunization against rabies virus propagation in mice.

Materials and methods

Mice were immunized with inactivated rabies virus via the subcutaneous (SC) or intracerebral (IC) route. Lethal doses of live rabies virus, CVS strain, were then inoculated into the brain of immunized mice. Clinical signs and body weights were recorded, serum antibody titers were determined, and histopathological and immunohistochemical studies were performed.

Results

Progressive paralytic neurological signs were observed in all control mice, in 75% of SC immunized mice and in only 20% of IC immunized mice. The neutralizing antibody titer in serum was significantly elevated in both SC and IC immunized mice. Analysis of whole brain lysates showed high levels of total immunoglobulin in IC immunized mice with virus neutralizing abilities. Brains from control mice, but not IC immunized mice, showed severe encephalitis and virus antigen in nerve processes.

Conclusion

The study shows that immunization of mice by the IC route induces a protective humoral immune-response against intracerebrally inoculated rabies virus.

ORAL PRESENTATIONS

Session 11 A – Dog and Cat Pathology

CARDIAC FIBROSIS IN CATS

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Introduction
The pathogenesis of feline endomyocardial fibrosis, clinically classified as restrictive cardiomyopathy, remains uncertain. Only single descriptions are available. The present study aimed to characterize the alterations in more detail.

Materials and methods
22 cats with extensive myocardial fibrosis (mean age 9.3 years) and 36 cordially healthy cats (mean age 7.7 years) were investigated clinically (n = 14), grossly and histopathologically (H&E, picrosirius red).

Results
Heart weight and size were significantly higher ($p < 0.001$) in the fibrosis group compared to the controls. The left (n = 16) and right (n = 10) atria were markedly dilated. Diffuse atrial endocardial fibroelastosis in the fibrosis group was up to 80±23 µm (controls 23 ± 12 µm).

Echocardiographically, the left ventricle showed markedly impaired systolic function (6/14) and hyperechoic areas (10/14) related to extensive myocardial fibrosis (n = 18) which was characterized by interstitial (n = 9), perivascular (n = 7) and/or replacement (n = 10) fibrosis. In 11 cases the endocardium was thickened up to 214 ± 70 µm (controls 26 ± 10 µm) by increased amounts (50%) of collagen III (controls 30%). Marked mitral valve degeneration (n = 12) and angiosclerosis of the intramural vessels (n = 16) were seen in cats with cardiac fibrosis.

Conclusion
The combination of vascular and valvular degeneration and endomyocardial fibrosis suggests a complex pathogenesis of myocardial remodelling.

ABDOMINAL HAEMORRHAGE AND EXERCISE-ASSOCIATED DEATHS IN GREYHOUNDS

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Introduction

The Greyhound Board of Great Britain (formerly the National Greyhound Racing Club) reviewed necropsy data from Greyhounds that died unexpectedly at licensed race tracks and residential kennels since November 2006.

Materials and methods

24 dogs died in an outbreak of respiratory disease in 2008. The significant findings in 37 other cases of sudden, unexpected death are described.

Results

Four deaths resulted from traumatic injury; in 8 dogs changes were consistent with cardiac failure. A variety of lesions affected individual dogs; no specific cause of death was identified in three animals. In 23 cases there was obvious, recent massive haemorrhage, including haemoperitoneum and retroperitoneal haemorrhage, sometimes with extension into sublumbar muscles. In 5 cases muscle histology suggested previous episodes of haemorrhage. The precise pathophysiological mechanism behind the fatal abdominal haemorrhage in these racing dogs is unclear; it is not related to gender, age or weight.

Conclusion

The incidence of fatal abdominal haemorrhage is very low (about 1 in 10 000–20 000 dog-runs) but is an important cause of death in racing Greyhounds. Episodes of non-fatal haemorrhage undoubtedly occur and may be one possible cause of sub-lumbar muscle pain observed in some dogs.

ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY ASSOCIATED WITH SUDDEN DEATH IN TWO CHINESE CRESTED NAKED DOG SIBLINGS

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Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a primary myocardial disease primarily affecting the right ventricle. Gradual replacement of the myocardium by fatty-fibro fatty tissue leads to right ventricular dilatation and right-sided heart failure or fatal arrhythmia. ARVC occurs as an inherited disease in man and Boxer dogs.

Material and Methods

Two Chinese crested naked dog siblings died suddenly at the age of twenty-two and eighteen months respectively. No clinical signs preceded the deaths. Both dogs underwent a complete necropsy.

Results

Myocardial lesions consistent with ARVC were present in both dogs. Macroscopically, severe dilatation of the right and left ventricles was accompanied by diffuse thinning of the ventricular walls. The ventricular myocardium showed patchy discoloration, due to numerous diffusely scattered intramural whitish streaks and spots. Histologically, the myocardium of the right ventricle was extensively intramurally infiltrated and replaced by fat or fibro fatty tissue. Remaining myofibers were often thin and atrophied. Scattered within the myocardium were occasional small infiltrates of lymphocytes.

Conclusion

This is to our knowledge the first report of ARVC in Chinese crested naked dogs. The close relationship of the dogs raises a strong suspicion that ARVC may be a familial disease in this breed.

MELAMINE INDUCED CANINE NEPHROTOXICOSIS IN ITALY

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Introduction

Melamine is an organic base containing a relatively large quantity of non-protein nitrogen. Addition of melamine to animal or human food is illegal in the US and EU.

Material and Methods

Two young dogs from the same kennel died showing clinical signs of renal failure. They were subjected to necropsy and were evaluated for histopathological and toxicological changes. Samples of pet food, urine and renal tissue were analysed for melamine content.

Results

Postmortem investigations revealed severe nephrotoxicosis associated with urolith deposition within renal tubules and pelvis. The predominant crystal type was identical to those observed in the kidneys of animals involved in the 2004 and 2007 melamine associated renal failure epidemics in Asia and US, and stained positively by Oil Red O indicating a plastic or lipid origin. High doses of melamine were detected in the pet food administered to the dogs. Likewise, melamine was identified in renal tissue from one dead dog and in urine samples from both animals. A diagnosis of melamine-related neprotoxicosis was made.

Discussion

This is the first report of melamine intoxication in dogs in Italy. Histological investigation seems to be a valuable tool in the diagnosis of melamine induced nephrotoxicosis. Increased surveillance of raw material and animal feed production at EU level is recommended.

ORAL PRESENTATIONS

Session 12 B – Fish Pathology

INVESTIGATION OF COMMON CARP MUSCLE AND GILLS FOLLOWING EXPOSURE TO FERROUS SULFATE AT ACIDIC PH

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Introduction

Ferrous iron is the most toxic form of iron in aquatic environments but has been little studied.

Materials and methods

190 fish with mean body mass of 15 ± 5 gr. were divided into six treatment groups and exposed to ferrous sulfate. Three replicates and one control group were included, all according to OECD guidelines and the semi static method. All calculations were based on dissolved iron concentrations. For constancy of ferrous concentrations in test solutions, pH was maintained at 5.5 ± 0.1. Ferrous concentrations were maintained constant during the test period using a spectrophotometer and the orthophenontroline method. LC50 was calculated by Probit analysis.

Results

The LC50 at 24, 48, 72 and 96 hours, respectively, was 2.35, 2.15, 1.95 and 1.85 mg/l Fe2+. Pathological findings included hyperemia, hyperplasia and hypertrophy of epithelial cells, telangiectasis, and fusion and cuboidal forms in primary and secondary lamellae in the gills. No changes were detected in muscle tissue.

Conclusion

Ferrous iron toxicity in fish has variable manifestations in different tissues. Gills are more susceptible than muscle because of their closer proximity to environment pollutants.

DEVELOPMENT OF AN RNA-CONTAINING PREPARATION FOR PROPHYLAXIS OF BACTERIAL DISEASES OF HERBIVOROUS FISH

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Introduction

RNA-containing preparations have a specific anti-infectious effect when applied to different species including fish. The aim of this study was to develop an immunomodulator for herbivorous fish using RNA bacteria.

Materials and methods

The activity and specificity of a preparation containing RNA of Aer-121 against two bacterial diseases (aeromonosis and pseudomonosis) was tested in two-year-old grass carp (Ctenopharyngodon idella) and bighead carp (Aristichthys nobilis). The indexes of activity of leukocytes (PhA), phagocytic index (PhI) and bactericidal activity of blood serum (BABS) were established.

Results

The preparation had a positive influence on the immune status of both fish species: PhA increased by 2.3–2.4, PhI by 3.1–7.2 and numbers of leukocytes by 3.8–4.4 thousands/mcl. Intramuscular injection of the preparation (10 mg/kg) increased PhA from 23.0 to 31.0% and PhI from 2.5 to 3.3. The indicated dose had no significant impact on BABS and lysozyme activity.

Conclusions

RNA-AER-121 is effective when used for prophylaxis of infectious diseases of herbivorous fish caused by *Aeromonas hydrophila*, *Aeromonas punctata* and *Pseudomonas fluorescens*. Disease processes caused by these bacteria are inhibited or stopped in fish inoculated with this preparation.

INVESTIGATION OF INTERIOR PITUITARY CELL CULTURE OF ACIPENSER GUELDENSTAEDTII TO PRODUCE GROWTH HORMONE

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Introduction

Fish cell culture has widespread application in virology, toxicology, cytogenetics and biomedical research.

Materials and methods

Pituitary glands from various stages of adult Acipenser gueldenstaedtii were collected for the development of cell cultures using explant methods. Developing gonads from 15–20-year-old male and female sturgeon were collected during spring 2007. The tissues were pooled in cold PBS–antibiotic–antimycotic solution and were evaluated for attachment, growth and ability to produce cell suspensions. Primary cultures were initiated from tissues according to earlier procedures, with certain modifications in the subcultivation procedure. Briefly, tissues were cut into 1 mm^3 fragments and seeded into 25 cm^2 tissue culture flasks. Cell growth was monitored after appropriate semidrying and addition of minimum essential medium supplemented with 15% fetal bovine serum. Seeding density of 1.5×10^5 cells was determined by a haemocytometer.

Results

Acipenser gueldenstaedtii interior pituitary cells were adapted and grown in a CO_2 incubator at 370°C. They were in lag phase for 10 days, in log phase on days 10–22, in stationary phase on days 23–28 days, after which they died. The ability to produce growth hormone from cultured fish pituitary cells was demonstrated.

Conclusion

Our results indicate that the cells need to be passaged regularly on day 21. In this way, continued cell cultures can be maintained and stored for gene banking.

PATHOGENESIS OF RAINBOW TROUT FRY SYNDROME (RTFS)

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Introduction

Rainbow Trout Fry Syndrome (RTFS) is a bacterial disease caused by *Flavobacterium psychrophilum*. Experimental studies have shown that the pathogenicity of F. *psychrophilum* is dependent on fish size and water temperature. The purpose of the present study was to examine the influence of *F. psychrophilum* on nonspecific humoral defence mechanisms in rainbow trout.

Materials and methods

Fingerlings with an average body mass of 10 g were examined after low levels of mortality occurred at a temperature of 10–12°C. Dystrophic lesions were observed only in the gills. *F. psychrophilum* was identified on the gills and in the kidney following routine microbiological examination. When the first signs of disease were noted (time 0), and then 3, 7 and 10 days afterwards, blood from 20 diseased fish and 20 healthy fish was withdrawn for evaluation of nonspecific humoral defence parameters.

Results

High levels of immunosuppression were demonstrated in fish infected with *F. psychrophilum*. Lysozyme activity and total Ig levels were significantly lower ($P < 0.05$) in diseased fish than in control fish. Serum ceruloplasmine activity was significantly higher ($P < 0.05$) in the diseased fish than in the control fish.

Conclusion

The results indicate that mortalities in RTFS are associated with immunosuppression and dystrophic lesions in the gills.

ORAL PRESENTATIONS

Session 15 A – Diagnostic Pathology

SARCOCYSTIS IN BOVINE EOSINOPHILIC MYOSITIS: CONTRIBUTION TO THE PATHOGENESIS

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Introduction

Lesions of bovine eosinophilic myositis (EM) are mainly found at meat inspection, resulting in condemnation of carcasses. Although sarcocyst remnants are often found intralesionally, their role remains unknown.

Materials and methods

Lesions of 97 natural EM cases were histologically and immunohistochemically examined. Laser capture microdissection with subsequent molecular tools were used to identify *Sarcocystis* spp. The capacity of *Sarcocystis* to induce EM was studied by repeated intramuscular injection of calves with adjuvanted sarcocyst antigens.

Results

Sarcocystis hominis, cruzi and hirsuta, as well as unidentified species, were detected in EM lesions. The cellular infiltrate mainly consisted of eosinophils, macrophages, T-cells and B-cells with diffuse, focal and granulomatous accumulations. Lesions were more severe in calves and were characterized by diffuse to granulomatous organization of the inflammatory infiltrate. In both natural and experimental ME, MHCII and COX-2 expression were present on muscle cells and endothelial cells, respectively.

Conclusions

This study supports the hypothesis that *Sarcocystis* antigens can induce lesions of EM in cattle. The findings suggest that EM lesions are maintained by MHCII antigen expression on muscle cells and are reinforced by endothelial expression of COX-2.

DETECTION OF *LEISHMANIA DONOVANI* COMPLEX USING *IN SITU* HYBRIDIZATION

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Introduction

Canine leishmaniosis is a chronic systemic disease of dogs caused by protozoal parasites of the genus Leishmania. L. infantum, the causative agent of canine leishmaniosis in the Old World, is transmitted by blood sucking sand flies. The incidence of the disease in Central Europe is increasing due to tourism and importation of dogs. Diagnosis of leishmaniosis, based on biopsy samples, remains difficult especially in cases where the parasitic load of tissues is low. To overcome this problem a chromogenic *in situ* hybridization (ISH) test was established, which facilitates detection of protozoal parasites directly within the tissue.

Materials and methods

For ISH, a digoxigenin-labelled oligonucleotide probe was designed targeting a segment of 5.8S ribosomal RNA. Hybrids were detected using an anti-digoxigenin antibody followed by an enzymatic colour reaction. The investigations were carried out on paraffin embedded tissues.

Results

Three of 13 tested dogs showed chronic granulomatous inflammation in various tissues by standard histological staining. ISH performed on these cases confirmed the tentative diagnosis of leishmaniosis showing amastigote stages. Cross reactions with other protozoa and fungi were eliminated.

Conclusion

This is the first demonstration that specific and simple detection of Leishmania parasites is possible in paraffin embedded tissues using ISH.

MULTIFOCAL GRANULOMATOUS PANNICULITIS WITH CEROID PIGMENT IN TWO MEDITERRANEAN STRIPED DOLPHINS (*STENELLA COERUELOALBA*): COMPATIBLE WITH NUTRITIONAL PANNICULITIS

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Introduction

Two striped dolphins (*S. coeruleoalba*) died shortly after being found stranded. Both animals showed lesions in the blubber that resembled those of nutritional panniculitis. To our knowledge these lesions have not been described previously in cetaceans.

Materials and methods

Complete necropsies were performed and tissue samples were routinely processed and stained with hematoxylin and eosin (H/E). Skin sections were also stained with periodic acid–Schiff (PAS), Ziehl-Neelsen (ZN), Gram's and Grocott's methenamine-silver. Blubber samples were processed for electron microscopy and brain and lymph nodes were tested for Morbillivirus by immunohistochemistry (IHC).

Results

In both dolphins, the most relevant finding was the presence of non-raised, yellow/orange lesions in the blubber. Microscopically, they corresponded to granulomas composed of macrophages and multinucleated giant cells containing abundant vacuolar intracytoplasmic material that was autofluorescent under blue-light and stained positively with PAS and ZN. Osmiophilic rounded structures were observed in the cytoplasm of macrophages using electron microscopy. No microorganisms were identified within the lesions and the IHC test for Morbillivirus antigen was negative.

Discussion

The occurrence of ceroid pigment in inflammatory lesions of the panniculus is characteristic of nutritional panniculitis and is commonly associated with diets deficient in vitamin-E and/or rich in unsaturated fatty acids. We believe that the blubber lesions observed in the two dolphins are compatible with nutritional panniculitis. However, determination of vitamin-E levels would be necessary to confirm this suspicion.

EMERGENCE OF A *NOVEL SARCOCYSTIS* – ASSOCIATED ENCEPHALITIS AND MYOSITIS IN DOMESTIC PIGEONS TRANSMITTED BY THE NORTHERN GOSHAWK

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Introduction

Five flocks of racing pigeons were found to be infected by a novel Sarcocystis species in Berlin, Germany. The pigeons had severe neurological signs mimicking paramyxovirosis or salmonellosis.

Materials and methods

Necropsy was performed on 14 naturally infected pigeons. Salmonella and Paramyxovirus diagnostics were conducted. For experimental infection, Sarcocystis infested skeletal muscle was fed to potential final hosts (2 falcons, 2 goshawks, 2 dogs, 2 ferrets, 2 rats, 2 mice). Sporocysts were isolated only from intestine of goshawks. Pigeons (n = 21) were reinfected with sporocysts and necropsied sequentially. An electron microscopical and a genetic analysis of the parasite were conducted.

Results

Paramyxovirus and Salmonella could not be isolated. Pigeons had severe lymphohistiocytic encephalitis and myositis. Myriads of Sarcocystis cysts were present in skeletal muscles. Electron microscopy further characterized the parasite. Sequence analysis demonstrated a novel parasitic species with a close relationship to other bird-infecting Sarcocystis species. Experimental infection identified the Northern Goshawk as a definitive host. A reinfection trial in pigeons confirmed the parasitic life cycle and clinical signs.

Conclusion

The novel Sarcocystis species cycles between Northern Goshawks and domestic pigeons following oral infection.

MICROSATELLITE GENOTYPING AND VIRULENCE ASSESSMENT OF *ASPERGILLUS FUMIGATUS* ISOLATES FROM WHITE STORK NESTLINGS AND THEIR ENVIRONMENT

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Introduction

Pulmonary aspergillosis caused by inhalation of spores of *Aspergillus* spp. is a major cause of mortality in captive birds. Recently, greatly increased prevalence of invasive fungal pneumonia has been reported in wild White Stork nestlings (*Ciconia ciconia*) in Germany.

Materials and methods

Necropsy was performed on 103 nestlings in 2007 and 2008. Fungi isolated from nestlings, nests and surrounding nesting material were grown on Sabouraud dextrose agar and in Minimal medium. DNA was extracted from mycelium. For fungal species typing the ITS1-5.8SrRNA-ITS2 region was sequenced. For strain typing of *Aspergillus fumigatus*, nine microsatellite markers were used. Differences in virulence of *A. fumigatus* isolates were assessed by a novel embryonated egg model.

Results

Histopathological examination of lungs identified granulomatous pneumonia associated with filamentous fungi in 41% of 103 nestlings. Molecular species typing predominantly identified *A. fumigatus* and zygomycetes. Strain typing and virulence assessment showed a high variability of *A. fumigatus* strains. The majority of strains irrespective of their origin were highly virulent in an embryonated egg model.

Discussion

A. fumigatus microsatellite strain typing in combination with virulence assessment using an embryonated egg model is a promising approach to understand the emergence of aspergillosis in stork nestlings.

ORAL PRESENTATIONS

Session 16 B – Model Systems

A COMPARISON OF STUDENT LEARNING USING MICROSCOPES AND GLASS SLIDES OR DIGITISED IMAGES

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Introduction

Histopathology has traditionally been taught using microscopes and histology slides but the use of computers to view digitised images is becoming more common. The student experience of using both methods was assessed both objectively and subjectively.

Materials and methods

Students, at either Bristol, where histopathology has traditionally been taught using glass slides, or at Nottingham, that makes use of many digitised images (Digital Slidebox [©Slidepath, Dublin]) completed a simple formative test before and after taking part in a practical class using either microscopes and histology slides or digitised images of the same slides.

Results

In general, the performance of the students in the tests improved after participation in the practical classes; there was little difference between students using glass slides or Digital Slidebox. Use of Digital Slidebox improved performance in questions which required more depth of knowledge beyond simple recognition of tissues or histopathological changes; use of microscopes improved performance in some questions relating to tissue recognition.

A subjective assessment showed no consistent student preference for digitised images or microscopes; the importance of the teacher/instructor during practical classes was emphasised.

Conclusion

Digitised images are useful in teaching veterinary histopathology but are not more effective than teaching using microscopes and histology slides.

COMPARISON OF THE PATHOLOGY OBSERVED IN LAMBS AND ADULT SHEEP EXPERIMENTALLY INFECTED WITH *MYCOBACTERIUM AVIUM* SUBSP. PARATUBERCULOSIS

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Introduction

The objective of this study was to evaluate the susceptibility of adult sheep to Mycobacterium avium subsp. paratuberculosis (Map) infection using different doses of Map.

Materials and methods

24 lambs and 22 adult sheep were orally infected with two doses of Map (1010 and 103 CFU). Fourteen animals remained as uninfected controls. The animals were necropsied at 120 and 210 days post-infection. Numbers of granulomas were counted and the severity of lesions was evaluated in samples of intestine and associated lymphoid tissue. Peripheral cellular immune response was assessed fortnightly.

Results

No gross lesions were observed in any of the animals. Microscopic lesions of paratuberculosis were detected only in the high dose-infected groups. Adult sheep showed small, demarcated focal granulomas restricted to areas of lymphoid tissue. Lesions were larger, more numerous and extended to the lamina propria in the lambs. The peripheral IFN-γ response appeared earlier in the adult sheep than in the lambs.

Conclusions

These results indicate the possibility of infection and development of characteristic lesions of paratuberculosis in adult sheep. Adult sheep seem better able to control the progression of the disease than lambs.

PATHOGENESIS OF CHRONIC GASTRITIS IN AN ANIMAL MODEL OF NON-PYLORI HELICOBACTER INFECTION

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Introduction

The role of non-*H. pylori Helicobacter* spp. in the development of gastric pathology in many domestic animals is unclear. Recently, we isolated and characterized *Helicobacter suis* from the gastric mucosa of pigs.

Materials and methods

Mongolian gerbils were experimentally infected with the newly described species *H. suis*. The development of gastric pathology was studied microscopically at different time intervals after infection.

Results

From three weeks after infection onwards, bacterial colonization of the antrum was associated with gradually increasing infiltrations of T- and B-lymphocytes in the propria mucosae, extending into the submucosa and the tunica muscularis at later stages. The germinal centers of the lymphoid follicle-like structures were expanded. Simultaneously, there was a mild loss of parietal cells at the transition zone between fundus and antrum and an expansion of the dividing epithelial cell population. Ultrastructurally, the helically shaped bacteria were found in close association with parietal cells and in debris of necrotic parietal cells.

Conclusion

Helicobacter suis induces necrosis of parietal cells. We previously found a similar phenomenon with *Helicobacter felis*. This has prompted ongoing *in vitro* studies on necrosis-inducing virulence factors.

A NON-TRAUMATIC PORCINE MODEL OF ACUTE *STAPHYLOCOCCUS AUREUS* INDUCED OSTEOMYELITIS

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Introduction

In juvenile pigs and humans, *Staphylococcus aureus* is a major cause of osteomyelitis. Previous porcine models of osteomyelitis have been traumatic. The aim of the present study was to establish a non-traumatic porcine model of osteomyelitis following intravenous inoculation of *S. aureus*.

Materials and methods

Twelve 8-week-old pigs were challenged intravenously once or twice with 108 bacteria/kg. The animals were euthanized 6 to 48 h after inoculation, and lungs and metaphyses of bones were sampled for histology and bacterial load. Tissue sections were stained histochemically and immunohistochemically.

Results

The bacterial load declined over time in the lungs and rose in the femoral growth plates. Micro-abscesses developed in the lungs and bones. Whereas pulmonary lesions disappeared after 24 h, bone lesions progressed until 48 h. The bone lesions commenced within the metaphyses and spread towards the growth plate. Micro-abscess formation was accompanied by thrombosis, oedema, and the presence of *S. aureus*.

Conclusion

In the present study, a non-traumatic porcine model of acute, suppurative osteomyelitis was developed following haematogenous spread of *S. aureus*.

SYSTEMATIC EXPRESSION ANALYSIS OF PORCINE CFTR AND CLCA1 – INTERACTING PARTNERS IN CYSTIC FIBROSIS?

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Introduction

Cystic fibrosis (CF) is the most common lethal inherited disease in humans. It is based on a genetic defect of the chloride channel CFTR. Currently a pig model of CF is established to better investigate the pathogenesis of this disease. Recent studies have identified members of the CLCA gene family as modulators of CF but little is known regarding the CLCA gene family in the pig and the expression pattern of the porcine CFTR.

Materials and methods

The mRNA and protein expression patterns of the porcine CFTR and CLCA1 were investigated using RT-PCR or immunohistochemistry and immunofluorescence, respectively.

Results

Porcine CFTR showed a broader expression pattern on the mRNA level than on the protein level. Abundant protein was found in juxtanuclear locations and only faintly on apical membranes in the intestine and respiratory tract. Porcine CLCA1 was expressed in mucin producing cells throughout the body and showed an overlapping tissue expression pattern with porcine CFTR.

Conclusions

This co-expression of the two proteins could indicate a modulatory function between pCLCA1 and pCFTR. Further studies will focus on the functional interaction of these proteins and the role of pCLCA1 in CF.

POSTER PRESENTATIONS

Session 1 A – Tumour Pathology 1/P

1. IN SITU HYBRIDIZATION OF THE MAJOR SATELLITE DNA FAMILY (FA-SAT) ON FELINE FIBROSARCOMA CELL CULTURES AND PARAFFIN EMBEDDED FIBROSARCOMA TISSUE SECTIONS

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Introduction

FA-SAT DNA is found in telomers and centromeres of some feline chromosomes, and has been associated with DNA rearrangements in mammalian cells.

Materials and methods

Primary cell cultures of feline neoplastic fibroblasts and feline skin fibroblasts, as well as biopsy specimens from feline fibrosarcomas were used for the detection of the FA-SAT DNA using FISH.

Results

The FA-SAT DNA hybridization pattern in tumour fibroblastic cells showed variations in size and number in comparison with the skin fibroblasts *in vitro*. A similar effect was also observed in the total number of signals in the biopsy tissues.

Conclusions

As the FA-SAT DNA plays a role in some cellular functional mechanisms, and forms part of essential chromosomal structures like centromeres, aberrations at this DNA level could be associated with chromosomal instability. The difference in the hybridization patterns of the FA-SAT DNA observed in this study could reflect rearrangements of this DNA sequence in tumour cells *in vitro* and *in vivo*.

A MAMMARY CARCINOMA IN A MALE GUINEA PIG (*CAVIA PORCELLUS*) WITH IMMUNOHISTOCHEMICAL CHARACTERIZATION

2.

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Introduction

Neoplasms are relatively rare in guinea pigs (*Cavia porcellus*). Mammary tumours are not common, but can occur in both males and females and are most often found in older guinea pigs. Most lesions are benign fibroadenomas and approximately 30% are adenocarcinomas. The adenocarcinomas usually do not metastasize, but aggressive surgery is necessary to prevent recurrence.

This report describes the immunohistochemical features of a mammary carcinoma in a 5-years-old male guinea pig that presented for examination with a 3-months history of masses in the mammary gland.

Materials and methods

Tissues were routinely processed and sections were stained with haematoxylin and eosin and examined immunohistochemically using the avidin-biotin peroxidase complex method and the following monoclonal antisera: pan-cytokeratin (AE1/AE3), cytokeratin-14, E-Catherin (E-cad), β-catenin (β-cat), P63, estrogen receptor (ER), progesterone receptor (PgR), vimentin, muscular α-actin, NSE and synaptophysin.

Results

Histological examination revealed well-circumscribed nodular masses, composed of round to oval luminal epithelial cells, predominantly organized in a solid pattern. This cell population showed positive immunostaining for AE1/AE3, E-cad, β-cat and ER. Neoplastic emboli and lymph node micrometastases were also observed.

Conclusion

The morphological and immunohistochemical findings clearly showed the luminal epithelial nature of the neoplasia. A diagnosis of solid mammary carcinoma with vascular invasion and lymph node metastasis was established.

3.

SUBCUTANEOUS EMBRYONAL RHABDOMYOSARCOMA IN A DOG

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Introduction

Canine rhabdomyosarcomas (RBDs) are classified into embryonal, botryoid, alveolar and pleomorphic forms. Although rare, the embryonal type is the most common and is reported to have a locally invasive behaviour and metastatic potential.

Materials and methods

An 11-year-old Pointer had a subcutaneous mass in the left forearm and an enlarged prescapular lymph node. Cytology, immunocytochemistry (ICC), histology and electron microscopy (EM) were performed.

Results

Cytology of the mass and lymph node showed neoplastic cells with morphologic features consistent with an undifferentiated sarcoma. Cytological atypia was severe. Histology revealed a subcutaneous, unencapsulated neoplasm composed of sheets of cells with the same morphology observed in aspirates. The lymph node was positive for metastasis. Expression of vimentin, desmin and myoglobin was demonstrated using ICC and indicated a diagnosis of RBD. EM revealed numerous mitochondria, large nuclei and nucleoli and rare dense masses of tangled myofilaments, suggestive of poorly differentiated rhabdomyoblasts. The findings were together diagnostic of an embryonal RBD. Six-months after treatment, the dog remains free of disease.

Conclusion

Cytology and ICC are pivotal for RBD diagnosis. Despite the guarded prognosis, in this case of metastatic embryonal RBD the dog responded well to therapy.

PROGNOSTIC VALUE OF INTRATUMOURAL VESSEL DENSITY IN CUTANEOUS MAST CELL TUMOURS

4.

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Introduction

Mast cell tumours (MCTs) are some of the most common cutaneous tumours of dogs. The current grading system established by Patanik *et al.* (1984) is based on histomorphological features. The main disadvantage of the system relates to standardization of histological criteria, especially for MCT grade II. The purpose of this study was to determine whether microvessel density is a useful supplementary marker to Patnaik's system.

Materials and methods

Paraffin sections obtained from 65 cases of CMCT were stained with HE, toluidine blue and classified according to Patnaik's grading system. Assessment of microvessel density was performed in immunostained sections (anti FVIII-RA) according to Weidner *et al.* (1991).

Results

Of the 65 tumours sampled, 19 were graded I, 31 were graded II and 15 were graded III. No significant correlation was observed between Patnaik's histopathologic grade and microvessel density (Spearman $R = 0.03$, $p > 0.8$). Also, there was no significant difference in microvessel density between tumours of different grade (ANOVA $p > 0.1$).

Conclusion

Our results indicate that measurement of tumour angiogensis is not a useful additional tool in histomorphological assessment of CMCT. The release of angiogenic factors contained in secretory granules of neoplastic mast cells is being investigated.

5. SURVIVIN EXPRESSION IN CANINE TUMOURS WITH SEBACEOUS DIFFERENTIATION

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Introduction

Survivin is a member of the inhibitor of apoptosis (IAP) protein family and exhibits low expression in most normal adult tissues. Elevated expression in tissues during embryonic development and in the majority of cancers, as well as in human sebaceous tumours, indicates potential diagnostic utility. In normal adult human skin, survivin has been recently proposed as a marker for keratinocyte stem cells.

Materials and methods

15 canine tumours with sebaceous differentiation (2 carcinomas, 9 adenomas, 1 epithelioma, 3 hyperplasia) and 5 specimens of normal canine skin were evaluated histologically and immunohistochemically. Full-length survivin (0.7 μg/ml, rabbit polyclonal antibody, NOVUS Biologicals) expression was correlated with p53 (1:400, rabbit polyclonal anti-human antibody, CM1, Novocastra) expression and mitotic index (MI).

Results

Survivin positive nuclei in normal sebaceous glands were few and scattered. Pre-neoplastic lesions and benign tumours showed several survivin positive nuclei and, apart from one case, there was no evidence of p53 expression. Numerous survivin positive nuclei occurred in carcinomas together with a higher mitotic index and large numbers of p53 positive nuclei.

Discussion

The present study revealed for the first time that in dogs, as in human beings, nuclear survivin immunohistochemical expression is increased in sebaceous carcinoma cases, compared to normal glands and benign lesions. The findings suggest a direct correlation with biological behavior, consistent with the anti-apoptotic and proliferative functions of the molecule.

IN VITRO INVASIVE POTENTIAL OF CANINE MALIGNANT HISTIOCYTIC SARCOMA CELLS WITH AND WITHOUT VIRUS INFECTION

6.

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Introduction

The Onderstepoort (Ond) strain of Canine Distemper Virus (CDV) is able to infect various cell types including hematopoietic cells. Infection of canine histiocytic sarcoma cells (DH82 cells) with CDV leads to morphological and functional modifications suggesting a less malignant biological behaviour. In addition, recent observations showed a virally altered MMP-expression in DH82 cells. The aim of this study is to compare the invasive potential of CDV-Ond infected and non-infected DH82 cells.

Materials and methods

Invasion assays were performed with non-infected and persistently CDV infected DH82 cells using a transwell system consisting of uncoated or Matrigel-coated inserts in wells of 24 well plates. The number of invasive cells at the bottom of the well was determined after 1, 3 and 6 days.

Results

Persistently CDV infected DH82 cells showed severely reduced migration after 6 days in contrast to non-infected cells using Matrigel-coated or uncoated filters.

Conclusion

This preliminary data indicates that CDV might represent a useful candidate for oncolytic therapy of canine histiocytic neoplasias.

7.

INTESTINAL ADENOCARCINOMAS WITH CHONDROID AND OSSEOUS DIFFERENTIATION IN TWO HORSES

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Introduction

Intestinal adenocarcinomas are rare neoplasms in horses. They occur more often in the large than in the small intestine. The morphological and immunohistochemical features of two small intestinal adenocarcinomas are presented in this report.

Materials and methods

Case 1: A 15-year-old Arabian mare showed signs of colic and an osseous mass almost obstructing the jejunal cavity was surgically removed. Case 2: A 6-year-old warmblooded gelding showed vague abdominal signs for a long time and was euthanized. Necropsy revealed an infiltrative duodenal mass. Both tumours were fixed in 4% buffered formaldehyde, decalcified (case 1), processed routinely and stained with hematoxylin-eosin and picro-sirius red. Furthermore, immunohistochemistry (α-smooth muscle actin, desmin, cytokeratin) was performed.

Results

The histological appearance of both tumours was of infiltrative, tubular adenocarcinoma with extensive chondroid and osseous metaplasia. The epithelial origin of the tumours was confirmed immunohistochemically. The epithelial cells were surrounded by variable numbers of spindle or polygonal cells that reacted positively for α-actin but were negative for desmin.

Discussion

Extensive chondroid and osseous differentiation comprised an uncommonly extensive component of two intestinal adenocarcinomas. Immunohistochemical studies failed to confirm the expected desmoplasia. The histogenesis of the α-actin positive spindle cell component is unclear.

EQUINE GENITAL CARCINOMA IN SITU RESEMBLING HUMAN GENITAL INTRA-EPITHELIAL NEOPLASIA (*BOWENOID PAPULOSIS*)

8.

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Introduction

Squamous cell carcinoma (SCC) is the most prevalent tumour of the external genitalia in horses. In a high number of typical genital SCC, careful examination reveals the presence of concurrent plaque-like mucosal lesions in the vicinity of the invasive SCC.

Methods and Results

Histological examination of 10 penile and 3 vulvar plaques (7 with and 5 without SCC in the same patient) revealed focally extensive and severe irregular epithelial hyperplasia with deep and broad rete ridges. The affected epidermis was severely dysplastic throughout all layers with lack of orderly maturation. Keratinocyte pleomorphism and atypia were present and there was no evidence of basement membrane disruption. The appearance of keratinocytes varied from small and polygonal with oval or flattened hyperchromatic nuclei to large and round with pale cytoplasm and large vesicular irregular nuclei. Mitotic figures were usually observed within all epidermal layers. Scattered koilocytes could sometimes be found. Immunoreactivity for papillomavirus was not observed but papillomaviral DNA was detected by PCR in all of the lesions examined.

Conclusion

The appearance of the intra-epithelial dysplastic lesions is consistent with a diagnosis of carcinoma *in situ*. For this new entity of equine tumours we propose the term genital intra-epithelial neoplasia, analogous to the nomenclature of papillomavirus-induced lesions used in man.

9. LYMPHANGIECTATIC FIBROUS POLYP OF THE TONSIL IN A DOG

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Introduction

In dogs, the most frequent tonsillar lesions are tonsillitis, lymphoid hyperplasia, and malignant neoplasms, especially squamous cell carcinoma and lymphosarcoma. Benign tonsillar proliferations such as inflammatory polyps are uncommon. In human beings, several benign and malignant lesions of tonsils are reported, including a rare entity, the lymphangiectatic fibrous polyp. We report here a case of a single tonsillar polyp in a 13-year-old female Bichon dog with moderate inspiratory dyspnea associated with a pedunculated pink mass growing from the left palatine tonsil and partially obstructing the pharyngeal lumen.

Materials and methods

Tissue specimens were prepared for histology by standard protocols. Immunohistochemical stains were performed using the streptavidin-biotin-complex method and anti-human LYVE-1 and CD3 antibodies.

Results

Histologically, the polyp was covered by a non-keratinized pluristratified squamous epithelium, and was formed by several lymphoid follicles and a central core of numerous severely ectatic lymphatic vessels. Endothelial cells lining the dilated vessels labelled positively for LYVE-1, an antigen specific for lymphatic endothelial cells.

Conclusion

We describe here a case of an unusual tonsillar polyp in a dog sharing strong clinical and histological similarities with the rare human lymphangiectatic fibrous polyp.

IMMUNOHISTOCHEMICAL ANALYSIS OF BCL-2 PROTEIN IN CANINE MAMMARY TUMOURS

10.

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Introduction

Development of canine mammary tumours involves accumulation of mutant cells caused by excessive proliferation and insufficient apoptosis. Bcl-2 is an oncoprotein and plays a role in blocking programmed cell death. Moreover, p53 gene products have been linked to apoptosis pathways.

Materials and methods

Samples of 112 canine mammary tumours were fixed in 10% buffered formalin. Histopathological diagnosis was based on the WHO classification system using sections stained with HE. Expression of Bcl-2, p53 protein, Ki67 antigen, ERα and PR receptors was evaluated by immunohistochemistry.

Results

Positive immunostaining for Bcl-2 and p53, respectively, was noted in 73% and 38% of the tumours. While immunostaining of p53 showed nuclear localization, Bcl-2 was found in the cytoplasm of tumour cells. The Bcl-2 protein was detected in 81% of benign tumours and in 40% of grade I carcinomas. Bcl-2 was significantly correlated with ERα, but not with PR or p53. Proliferative activity showed a significant positive correlation with histological type, tumour grade and p53.

Conclusion

The positive correlation between Bcl-2 and ERα suggests that estrogen may be a regulator of Bcl-2 in canine mammary tumours. Prospective determination of Bcl-2 and p53 status may be useful in tumour grading and prognosis.

11.

RELATIONSHIP BETWEEN RECEPTORS FOR INSULIN-LIKE GROWTH FACTOR-I, STEROID HORMONES AND PROLIFERATIVE ACTIVITY IN CANINE MAMMARY TUMOURS

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Introduction

Disturbances in expression of estrogen (ERα) and progesterone (PR) receptors together with changes in insulin-like growth factor-I receptors (IGF-IR) play a role in human breast cancer development and progression. In-depth investigations of these relationships in canine mammary tumours have not been completed.

Materials and methods

The investigations were carried out on 112 canine mammary tumours. The tumours were examined histologically (HE stain) and Ki67 antigen, ERα, PR and IGF-IR receptors were demonstrated using immunohistochemical methods. The level of significance was set at $p < 0.05$.

Results

Positive immunostaining for Ki67, ERα, PR, IGF-IR, respectively, was noted in 80%, 36%, 69% and 67% of the tumours. IGF-IR expression was significantly higher in carcinomas than in adenomas. The IGF-IR immunostaining was significantly associated with ER status, but not with PR status. Nuclear expression of ERα and PR, respectively, was detected in 47.6% and 76% of adenomas. High index values of Ki67 and MI showed a positive correlation with histological type and histological grade.

Conclusion

The findings suggest that oestrogen influences the expression of IGF-IR in canine mammary tumours. The proliferative index may be a useful prognostic indicator in canine mammary tumours.

TYPES AND INCIDENCE OF TUMOURS IN CATS IN CROATIA

12.

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Introduction

Types of tumours and their incidence in cats in Croatia were analysed as part of the effort to improve tumour surveillance in various animal species. The data relating to cats from the sea coast (SC) and cats from inland (IN) areas was compared to evaluate changes possibly associated with varying levels of UVB radiation and environmental pollution. Generally, it can be assumed that UVB radiation is higher and environmental pollution is lower in coastal areas than in the rest of country.

Materials and methods

The analysis related to all cat tumours examined in the Veterinary Pathology Department between January 1st 2005 and December 31st 2008. The diagnoses were established using the AFIP tumour classification system.

Results

The total number of tumours was 154. The most important differences in the frequency of tumours of the organ systems were in the ratio of tumours of the endocrine and exocrine glands (0.8% SC and 6.9% IN), hematopoietic system (3.4% SC and 19.2% IN) and skin (58.6% SC and 36.8% IN). The incidence of mammary gland tumours in cats from the two areas was not significantly different.

13.

EFFECTS OF PROGESTERONE, MIFEPRISTONE AND ONAPRISTONE ON PROLIFERATION OF THE PROGESTERONE RECEPTOR-POSITIVE CANINE MAMMARY CARCINOMA CELL LINE CMTU-27

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Introduction

In human mammary tumour cell lines with progesterone receptors (PR), progesterone (P) exerts genomic proliferative actions that are reversed by PR antagonists.

Materials and methods

CMTU-27 cells were incubated with 10-6 M P (24 h, 48 h, 72 h) alone and after incubation with 10-6 M mifepristone or 10-6 M onapristone (24 h, 48 h, 72 h). Incubations with 10-6 M mifepristone, 10-6 M onapristone or vehicle were used as controls. Formalin-fixed, paraffin-embedded cells were stained with the avidin-biotin-peroxidase immunohistochemical method using PR10A9 and MIB-1 monoclonal antibodies.

Results

Thirty four percent of control cells expressed PR. Proliferation index (pi, percentage of MIB-1-stained nuclei and/or nucleoli) was similar in P-incubated and control cells (pi 24.0–30.0, 25.0–39.2 and 33.4–26.9, respectively, at 24 h, 48 h and 72 h). Mifepristone alone (pi 61.2%) and combined with P increased pi at 48 h ($p > 0.05$) and 24 h ($p > 0.05$), respectively.

Conclusion

The lack of effect of P on proliferation of CMTU-27 cells may be related to the low number of PR-positive cells in this cell line. The proliferative effect of mifepristone was unexpected and will be further investigated.

MYXOID LIPOSARCOMA IN A CAT

14.

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Introduction

Liposarcoma is a rare tumour of domestic animals and is most frequently described in dogs. We describe the first case of myxoid liposarcoma in the proximal humerus of a cat.

Materials and methods

A 15-year-old male cat presented with a gradually expanding swelling in the proximal region of the left humerus. The left forelimb, including the humerus and scapula, was amputated. Tumour samples were processed routinely and stained with HE, Oil Red O, Alcian Blue (pH = 2.5) and Masson trichrome stains, and immunostained for vimentin, pancytokeratin and S-100.

Results

Grossly, the tumour was tan to white, soft and multilobulated. Areas of hemorrhage and necrosis were prominent. Microscopically, round to polygonal cells, arranged in sheets, contained numerous clear, round, variable sized and well defined intracytoplasmic vacuoles. Nuclear atypia and frequent mitosis were present. Oil Red O verified fat in the cytoplasmic vacuoles of tumour cells. Alcian blue showed a prominent background of myxoid extracellular substance. Additionally, tumour cells were strongly positive for vimentin, but negative for pancytokeratin and S-100.

Conclusion

The gross, microscopic, histochemical and immunohistochemical findings are consistent with a diagnosis of myxoid liposarcoma.

15.

PURE CUTANEOUS MAST CELL TUMOUR IN A HOLSTEIN HEIFER

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Introduction

Mast cell tumours are uncommon in cattle and account for only 3% of cutaneous and subcutaneous bovine tumours. Scant data suggest that cutaneous tumours in cattle are associated with visceral mast cell aggregates, although purely cutaneous tumours have been reported.

Materials and methods

An 8 month-old Holstein heifer presented with nodular pruritic skin lesions on the face and neck. The nodules measured 2–4 cm in diameter, were non-encapsulated, had a rubbery texture and grey to light yellow colouration. Excision biopsies of skin nodules were fixed in 10% buffered formalin and stained with HE and Giemsa. The animal was necropsied and visceral organs were examined histologically.

Results

Histological examination of the cutaneous nodules showed sheets of round cells and occasional eosinophils in the dermis and subcutis. The round cells had uniform, centrally located round to oval nuclei surrounded by clear to amphophilic cytoplasm and well-defined cell borders. Cytoplasmic granules in Giemsa stained sections were few. The appearance of the tumour was of a grade II mastocytoma. No visceral mast cell aggregates were detected.

Conclusion

A pure cutaneous mast cell tumour was diagnosed in a Holstein heifer.

IMMUNOHISTOCHEMICAL EXPRESSION OF CYCLOOXYGENASE-2 (COX-2) IN FOUR CASES OF EQUINE MAMMARY GLAND TUMOURS

16.

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Introduction

Cyclooxygenase (Cox-2) is an inducible enzyme linked to tumour growth and angiogenesis. Cox-2 is expressed in a variety of canine and feline carcinomas and a strong Cox-2 expression may confer a worse prognosis in mammary tumours of this species. As Cox-2 expression has never been evaluated in equine mammary gland carcinoma, the purpose of this study was to evaluate its expression in two healthy equine mammary glands and four equine mammary carcinomas.

Material and methods

Paraffin-embedded sections of two healthy equine mammary glands, 4 mammary gland carcinomas and their metastases, were immunohistochemically investigated for Cox-2, cytokeratins (AE1/AE3, CK-19) and Ki67.

Results

Only one carcinoma and its metastases were positive for Cox-2, one carcinoma had weak Cox-2 positivity, and the remaining two were negative. Cytokeratins were expressed in all tumours and in the epithelium of normal glands, whereas all neoplasms and metastases had a high proliferating index (Ki67+). Cox-2 immunoreactivity was not observed in normal mammary gland tissues.

Conclusion

Cox-2 expression could be an immunomarker linked to the type of carcinoma and the phenotype of neoplastic cells.

17. EXPRESSION OF ENDOTHELIAL GROWTH FACTORS, THEIR RECEPTORS, AND ANGIOGENIC HOMEOBOX GENES IN SIX MURINE XENOGRAFTS OF CANINE HAEMANGIOSARCOMAS

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Introduction

For investigation of the malignant growth of haemangiosarcoma (HSA), we established canine HSA xenograft lines and analyzed the expression of growth factors, their receptors, and angiogenic homeobox genes, which regulate the transcription of angiogenic molecules.

Materials and methods

Six canine HSA xenografts were subcutaneously established in nude mice, and serially transplanted thereafter. Subsequently, the expressions of VEGF-A, bFGF, flt-1, flk-1, FGFR-1, and angiogenic homeobox genes were investigated in original and xenograft tumours using histopathological analyses, immunostaining, and RT-PCR.

Results

All the xenograft tumours comprised vascular-like structures and expressed CD31, vWF, VEGF-A, bFGF, flt-1, flk-1, FGFR-1, HoxA9, HoxB3, HoxB7, HoxD3, Pbx1, and Meis1 mRNA and proteins. Intense expression of bFGF mRNA was observed in three of the xenograft lines.

Conclusion

We established six canine HSA xenograft lines expressing angiogenic growth factors and their receptors were similar in spontaneous HSAs. We also detected the expression of angiogenic homeobox genes; thus, xenograft models may be useful to analyze the malignant growth in HSA.

PRIMARY DIAPHRAGMATIC PLEOMORPHIC LIPOSARCOMA IN A DOG

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Introduction

Primary tumours of the diaphragm are extremely rare in man as well as in animals, the most commonly reported being lipomas/liposarcomas.

Material and Methods

A 10-year-old female dog was submitted for abdominal ultrasound which revealed a 4 cm mass between the liver and diaphragm, interpreted as a liver mass. CT-guided FNA and biopsy were performed.

Results

Histologically, the biopsy was composed of cells arranged in a perivascular pattern with eosinophilic cytoplasm and round central hyperchromatic nuclei. A hepatocellular carcinoma was suspected, but IHC for Hep par 1 was negative. At surgery, the mass was identified within the diaphragm and completely resected. Microscopically, the tumour was composed of sheets of closely packed cells with distinct cell borders and eosinophilic, foamy or microvacuolated cytoplasm. Occasional large cells with large clear cytoplasmic vacuoles distorting the nucleus were present. Differential diagnoses were liposarcoma and adrenocortical carcinoma. The IHC pattern was CK-, Vim+, S-100-, Melan-A+ and Inhibin-, consistent with adrenocortical carcinoma. Nevertheless, TEM findings (indented nuclei, large non-membrane bound cytoplasmic lipid vacuoles, mitochondria with parallel lamellar cristae) were suggestive of liposarcoma. At necropsy, multifocal to coalescing nodular infiltration throughout the diaphragm was found without any other recognizable mass.

Conclusion

The final diagnosis was pleomorphic liposarcoma with epithelioid features. To our knowledge, this is the first reported case of primary diaphragmatic liposarcoma in the dog.

19. THE EXPRESSION OF P63 AND CALPONIN IN CANINE MAMMARY TUMOURS – HISTOGENETIC AND PROGNOSTIC CONSIDERATIONS

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Introduction

Identification of myoepithelial cells plays an important role in classification of canine mammary tumours (CMT). The aim of this study was to compare immunoreactivity of calponin, a smooth muscle-specific protein, with p63 expression, a regulatory cell cycle element which has been demonstrated in myoepithelial cells.

Material and Methods

Tissues samples from 10 benign and 32 malignant CMT were evaluated. The myoepithelial phenotype of cells was confirmed by using complementary antibodies including alpha-smooth muscle actin, cytokeratin 14 and vimentin.

Results

Co-localisation of p63 and calponin was demonstrated in identical cell populations, with p63 signal restricted to the nucleus and calponin to the cytoplasm. A gradual decrease of p63 immunoreactivity was observed during myoepithelial cells transformation from pre-existing through hypertrophic and spindle-stellate to rounded cells. The antibody to p63 protein used had the highest specificity to myoepithelial cells among all tested antibodies as myofibroblasts or vascular smooth muscle cells lacked p63 expression.

Conclusion

p63 is a sensitive and specific myoepithelial cell marker and may be included in immunohistochemical panels aiming at identification of myoepithelial cells in CMT.

POSTER PRESENTATIONS

Session 2 B – Naturally-Occurring Diseases

20. MYOFIBROBLASTS IN CAT LIVER FIBROSIS

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Introduction

Chronic cholangiohepatitis is characterized by a mixed inflammatory infiltrate in portal areas and bile duct proliferation. In terminal stages it may progress to liver fibrosis. We describe the distribution of hepatic stellate cells (HSC) and portal myofibroblasts that express α-smooth muscle actin (α-SMA) and vimentin in the liver of cats with varying degrees of fibrosis.

Materials and methods

Formalin-fixed and paraffin-embedded liver samples from 13 necropsied Persian cats were examined by light microscopy, using H&E and Masson-trichrome stains and immunohistology for α-SMA and vimentin.

Results

Liver fibrosis was confirmed microscopically in 13 Persian cats with terminal chronic cholangiohepatitis. The inflammatory reaction was dominated by neutrophils, lymphocytes, and plasma cells. Severe liver fibrosis was characterized by connective tissue septae that divided the liver parenchyma into irregular lobules. Numerous α-SMA and vimentin positive cells of different shape and size were located in fibrous septae and perisinusoidal spaces.

Discussion

The immunopositivity of perisinusoidal HSC to α-SMA and vimentin varied depending on the degree of fibrosis and was strongest in cats with cirrhosis. Activated fibroblasts which develop myofibroblastic characteristics play an essential role in hepatic fibrogenesis. Inflammation in cat liver is connected with the activation of periductal myofibroblasts.

SCREENING OF CHITINOLYTIC MICROORGANISMS IN SHRIMP FARMING WATER AND WASTEWATER

21.

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Introduction

Chitinases produced by pathogens of many marine organisms can assist penetration of the host exoskeleton and can provide nutrients both directly in the form of amino-sugars, and indirectly by exposing other host material to enzymatic digestion. Chitinolytic pathogens may establish their parasitic relationship by thenselves or by being partner for another invading microorganism in a mutualistic approach.

Materials and methods

Microorganisms isolated at different locations in southern Iran were screened on agar plates containing 1% colloidal chitin, 0.1% K_2HPO_4, 0.05% $MgSO_4$, $7H_2O$, and 1.5% agar powder. The organisms were subcultured in liquid media and the culture broth supernatants were collected for measurement of chitinase activity.

Results

These experiments resulted in the isolation of a number of chitinolytic Eubacteria. Colloidal chitin, prepared from residues of shells of some crustaceans such as lobster and shrimp, was supplemented in synthetic solid media. The results represent basic data that will be a useful starting point for subsequent studies.

Conclusion

The findings indicate that shrimp breeding industries are at risk from pathogenic microorganisms. Further studies on characterization of culprit microorganisms and their relative enzymes are underway.

22.

COLLAGEN PARAMETERS IN NORMAL AND HYPERPLASTIC CANINE PROSTATE GLANDS

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Introduction

Previous reports of changes in prostatic collagen in dogs related to ageing and pathology are few and controversial.

Materials and methods

Samples of prostate gland were collected during routine necropsy from 42 dogs aged 1 to 17 years. The dogs were divided into three different age groups: group I, 1–5 years; group II, 6–10 years and group III, 11 years and older. Histomorphometric studies was performed using the Quantiment 520 computer image analysis system. The percentage volume of collagen, perimeter of collagen area and number of collagen fibers in the sight field were determined.

Results

The percentage volume of interstitial collagen was highest in dogs in group II (11.1%). The amount of collagen fibers in a sight field differed between the groups: I – 544.2, II – 446.1, III – 395.3 ($p < 0.01$). The perimeter of collagen area was largest in group I (14.5 mm/mm^2), and smallest (12.8 mm/mm^2) in group III ($p < 0.01$). The percentage volume of collagen was largest (12.1%) in prostates with normal histological appearance, and smallest in cases of epithelial cystic hyperplasia ($p < 0.01$).

Conclusions

The volume of collagen in canine prostates remains almost constant with age. Collagen fibers unite into the thicker, larger fibers as dogs get older.

CONCURRENT UVEODERMATOLOGICAL (VOGT-KOYANAGI-HARADA-LIKE) SYNDROME AND POLYMYOSITIS IN A JACK RUSSELL TERRIER

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Introduction

We describe the concurrent presence of uveodermatological (Vogt-Koyanagi-Harada-like) syndrome and polymyositis in a dog.

Materials and methods

Histopathological examination was carried out on the right globe of a 1-year-old male Jack Russell Terrier after a history of bilateral anterior uveitis and bilateral enucleation in 2006. Three years later, the dog presented with skin irritations, progressive weight loss, dysphagia and stiff gait. The dog was euthanized because of poor prognosis and was submitted for a complete post mortem examination.

Results

The enucleated globe showed marked granulomatous panuveitis with numerous melanin-laden macrophages. The inflammation extended into extraocular muscles. At necropsy the dog had generalized muscle atrophy. Microscopic examination revealed mononuclear polymyositis affecting skeletal muscles of the rump, extremities and oesophagus. Skin and mucous membranes showed mononuclear lichenoid interface inflammation with intralesional melanomacrophages. Examined lymph nodes contained numerous plasma cells and melanomacrophages.

Conclusion

To our knowledge this is the first report of concurrent uveodermatological syndrome and polymyositis in a dog. In this case, autoimmune polymyositis was suspected due to the absence of a detectable infectious cause and the widespread distribution of the muscle lesions. Epitope spreading is one possible cause for the development of two or more immune-mediated diseases in one patient.

24.

CUTANEOUS ANGIOCENTRIC LYMPHOMA (LYMPHOMATOID GRANULOMATOSIS) IN A CAT

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Introduction

Lymphomatoid granulomatosis (LG) is an unusual round cell tumour with angiocentric extravascular growth behavior. The tumour is well known in human medicine and is rarely diagnosed in animals. It occurs most commonly in the lung, but cutaneous and renal involvement are observed. A report of LG in a cat with the pulmonary form represents the only previous report in this species.

Materials and methods

A 12-year-old female domestic short hair cat showed deep necroses of the facial skin. Euthanasia was chosen.

Results

Histological examination revealed round cell accumulations with angiotropism and extensive infiltration of the walls of large and medium-sized vessels. Immunohistochemical studies showed that cells infiltrating the walls of blood vessels labelled positively for CD3.

Discussion

The findings are consistent with lymphomatoid granulomatosis and represent the first report of cutaneous manifestation of the disease in a cat.

CAUDA EQUINA POLYRADICULONEURITIS IN A DOG

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Introduction

Polyradiculoneuritis is an inflammatory condition primarily involving multiple nerve roots. It is extremely rare in dogs. Here we report the characterization of the inflammatory infiltrate in a typical case.

Materials and methods

A 6-year-old, neutered male, Welsh Springer Spaniel was referred with a 4-days history of sudden urinary and faecal incontinence and tenesmus. The animal did not respond to treatment and was euthanised. A necropsy was performed. The spinal cord was examined histologically and immunohistochemically (CD3, CD20, Mac387, MHCII).

Results

Some nerve roots presented extensive mononuclear cell infiltration. The most severely affected axon bundles showed swollen and demyelinated axons. Infiltration of T-lymphocytes was most prominent in the epineurium. B-lymphocytes were more diffusely spread in the perineurium. A small number of reactive macrophages was found at the periphery of the affected roots. In the affected nerve roots, lymphocytes and macrophages all stained MHC-II positive.

Conclusion

This is, to our knowledge, the third case of cauda equina neuritis to be reported in dogs. The inflammatory infiltrate has the same composition as that seen in polyneuritis equi. The etiology of the condition remains unknown.

26.

A HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF ARCHIVED OVINE NATURAL SCRAPIE CASES

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Introduction

Lesion profiling and patterns of disease-specific prion protein (PrPd) accumulation were evaluated in ovine natural scrapie cases diagnosed in Castilla y León (Spain).

Materials and methods

Tissue sections of medulla oblongata (obex area) from 198 archived ovine scrapie cases were examined histologically and immunohistochemically (IHC), the latter using P4 monoclonal antibody (mAb) against PrPd. Single and double IHC, using Ab against bovine glial fibrillary acidic protein, synaptophysin, human neurofilament and CD68 were performed to investigate the distribution of PrPd in astrocytes, neuronal synapses or processes, and microglial cells, respectively.

Results

In 192 of the 198 cases, intense intraneuronal and intraglial PrPd immunolabelling was detected. Intraneuronal PrPd was most intense in the olivary nuclei. Intraglial and neuropil PrPd deposits were marked in the nucleus of the spinal tract of the trigeminal nerve. The pattern of PrPd deposition was similar in 66 cases from the same herd with different genotypes.

Conclusion

PrPd double labelling is useful for identification of PrPd-positive cells in brain tissue. The profile of PrPd deposition in the brain may help in the characterization of scrapie strains in sheep.

IMMUNOHISTOCHEMICAL INVESTIGATION OF FELINE HYPERTENSIVE RETINOPATHY

27.

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Introduction

Feline hypertensive retinopathy (HR) is a well-known, poorly investigated disease that occurs mostly secondary to chronic renal failure.

Materials and methods

Eyes from six cats affected by systemic hypertension (SH), with an ophthalmoscopic diagnosis of HR and normal intraocular pressure were histologically and immunohistochemically investigated. The eyes were processed for histology and sections were stained with hematoxylin and eosin, PAS and Mallory, and immunostained with antibodies against metalloproteinases (MMP) 2 and 9, smooth muscle actin (αSMA) and glial fibrillary acidic protein (GFAP). Eight normal feline eyes served as negative controls (NCs).

Results

Histologically, retinal vessels of hypertensive cats (HCs) had variable degrees of arteriolosclerosis with occasional luminal occlusion. Apart from a case of complete unilateral retinal atrophy, retinal changes were limited to mild, multifocal perivascular edema. Immunohistochemically, MMP2 and 9 stained vessel walls and Müller cells more intensely in HCs than in NCs. αSMA staining was reduced in HCs when compared to NCs. GFAP stained Müller and retinal glial cells more intensely and diffusely in HCs than in NCs.

Conclusions

Immunohistochemical staining of eyes from cats with hypertensive retinopathy revealed severe retinal distress paralleled by minimal retinal morphological changes. The findings indicate that the morphological changes are preceded by biochemical, and possibly functional, alterations.

28. INHERITED JUNCTIONAL EPIDERMOLYSIS BULLOSA IN GERMAN BLACK HEADED MUTTON SHEEP

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Introduction

Epidermolysis bullosa (EB) is a group of heritable mechanobullous skin disorders occurring in man and different species of animals. Based on ultrastructural findings, EB is divided into different forms: EB simplex, junctional EB (EBJ) and dystrophic EB. In this investigation, the first cases of inherited EBJ in sheep are described.

Materials and methods

Skin and mucosal tissue samples from 16 German Black Headed Mutton lambs and one crossbred lamb were examined histologically, by transmission electron microscopy and immunohistochemically using antibodies to laminin 5 and collagen VII.

Results

Macroscopically, all lambs had blisters and erosions or ulcerations of skin and mucous membranes. Alterations of hoof horn were present in several animals. Separation of the dermoepidermal junction was seen histologically. Electron microscopy showed that the separations of the dermoepidermal junction were located in the lamina lucida of the basement membrane. Hemidesmosomes were present in reduced numbers and, in most cases, showed alterations. Expression of laminin 5 was markedly reduced whilst expression of collagen VII appeared to be normal. Reproducing the disorder in a trial breeding proved heritability.

Conclusion

The findings are consistent with EBJ in other species and represent the first reports of inherited junctional epidermolysis bullosa in sheep.

ANENCEPHALY IN A GERMAN SHEPHERD PUPPY

29.

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Introduction

True anencephaly is a very rare condition in veterinary medicine and has not been reported in dogs so far.

Material and Methods

The puppy presented here was born dead by caesarean section at the 62nd day of gestation.

Results

The examined dog had a hypoplastic calvarium, a thickened and flattened skull base, and shallow orbits in combination with protruding eyes. The brain was completely absent macroscopically. Histopathologic examination of the cranial bone and stroma filling the rudimentary cranial cavity revealed a loosely arranged stroma that contained cross and tangential sections of well-differentiated peripheral nerves, fragments of cerebellar folia, and small nests of glial cells with single interspersed neurons. Immunohistochemically, single cells within structures resembling cerebellar folia and stroma stained positive for NeuN (mature neurons), whereas other neurons within the stroma, most neurons within ganglia of cranial nerves and peripheral nerve fibers had mild expression of doublecortin. Doublecortin is a marker for immature neurons and neurons within the migratory phase. Spinal cord lesions resembled syringomyelia had compressed and immature nervous tissue, and well differentiated spinal ganglia.

Discussion

Anencephaly develops as a consequence of closure defects of the neural tube early during gestation. The presence of many immature and only a few mature neurons in this case, indicates a complete failure of brain development and differentiation.

30. INFANTILE NEUROAXONAL DYSTROPHY (INAD) IN MICE RESEMBLING NEUROAXONAL DYSTROPHY IN CHILDREN

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Introduction

Neuroaxonal dystrophy (NAD) is a primary neurodegenerative disorder of axons. In humans and animals several variants have been defined. The aim of this study was to determinate the neurohistological lesions and their distribution in a spontaneous disorder in mice resembling human infantile neuroaxonal dystrophy (INAD).

Material and methods

Young wildtype C57BL/6N (MP) mice displaying a motor disturbance were investigated to determine the type and pathogenesis of the nervous disorder. Brain and spinal column were routinely processed and stained with HE, Luxol Fast Blue, and Bielschowsky's (B) method. Amyloid precursor protein (APP), neurofilaments (NF), and ubiquitin (Ub) were investigated immunohistologically.

Results

Numerous spheroids were found in the white and grey matter of cervical, thoracic and lumbar spinal cord, the reticular formation, and rarely in the brain. Spheroids were positive for B, APP, NF, and Ub. The most severely affected areas were ventral horns, ventral and ventro-lateral funiculi of cervical and thoracic spinal cord and the reticular formation. A few chromatolytic neurons were present in the mesencephalic and pontine nuclei.

Conclusion

The cause of the nervous disturbance was a neuro-axonal dystrophy. Due to the histopathological findings, this spontaneous murine disease seems to be a suitable model for other animal and human neuro-axonal disorders.

LIPID AND GLYCOGEN CONTENT OF THE LIVER AND THYROIDAL STATUS IN DAIRY COWS WITH HEPATIC LIPIDOSIS

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Introduction

Hepatic lipidosis develops when the rate of hepatic lipid uptake exceeds that of hepatic lipid oxidation and secretion. Excess lipids are stored as triacylglycerol in the liver and are associated with decreased liver glycogen content. Hepatic lipidosis of cows is also associated with decreased blood concentrations of thyroid hormones.

Materials and methods

Ketotic Holstein cows (n = 10) were chosen. Blood serum triiodthyronine and thyroxine concentrations were determined by radioimmunoassay (RIA). Liver sections were stained with HE, Best's glycogen stain and Sudan III for lipids. The hepatocyte lipid and glycogen content was determined by the Leica Q500 MC computer image analysis (Software Q Win).

Results

Various degrees of hepatic lipidosis (32.91 ± 13.23%) were estimated. The hepatocyte glycogen content was 28.75 ± 10.45%. Hepatic lipidosis in cows was negatively associated with blood concentrations of triiodthyronine ($r = -0.51$, $p < 0.05$) and thyroxine ($r = -0.55$, $p < 0.05$).

Conclusion

These investigations showed that a rapid decrease in glycogen and an increase in lipid content of liver in dairy cows were prerequisites for susceptibility to ketosis. The study suggested that the hypothyroidal status in the cows may be a major factor in developing hepatic lipidosis.

32. FATAL APLASTIC ANAEMIA WITH HAEMORRHAGIC DISEASE IN CALVES IN GERMANY

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Introduction

A haemorrhagic disease occurred in 52 calves from 42 farms in Germany. At the age of about 2 to 3 weeks, calves had conspicuous, spontaneous transcutaneous petechiae and haemorrhages in mucosal surfaces as well as excessive bleeding associated with trauma.

Results

Blood analysis revealed a marked thrombocytopenia, leucopenia, and granulocytopenia. Severe haemorrhages in the skin and gastrointestinal tract were the major findings at post-mortem examination. Histological investigation indicated a severe bone marrow hypoplasia/aplasia. Infections with bacteria, bovine viral diarrhoea virus, or bluetongue virus were ruled out. Specific toxins such as Furazolidone, DCVC metabolites or mycotoxins were not detected. Pedigree analysis gave no indication for heredity of this syndrome. Using a broad-spectrum PCR, a circovirus with high similarities to porcine circovirus type 2b (PCV2b), was detected in several of the affected calves.

Conclusion

The distinct cause of the disease still remains unknown. Potentially, the pathogenesis is complex and includes components such as infection, hereditary disposition, and immune-mediated destruction of blood cell precursors. Further investigations are necessary to clarify the role of PCV2.

ENDOMETRIOSIS GENITALIA INTERNA AND MASTITIS IN COWS-NEW DISEASE SYNDROME?

33.

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Introduction

The precise diagnosis of some diseases may need the microscopic examination of the target organ. Previously described endometriosis genitalia internal of bovine uterus has led to further studies of pathological effects of this endocrinopathy.

Material and Methods

The histopathological examination of uterine horns and mastitic mammary glands from 45 slaughtered cows were performed. Tissue sections were formalin-fixed, paraffin-embedded and stained with HE.

Results

In all examined cows, mammary glands had inflammatory changes. They were of different types and intensity, from acute purulent inflammation to chronic scirrhosing lymphocytic inflammation. In the same cows, endometriosis genitalia interna was also present.

Conclusion

This study provides the first data with a correlation between the development of bovine mastitis and endometriosis genitalia interna. These findings suggest the same etiopathogenesis of both disease processes. However, the exact pathogenesis of endometriosis is still not resolved, and the endocrine disturbances are mainly discussed. The decrease of the mammary glands' defence mechanism due to disturbances in cell homeostasis is also discussed as a predisposing factor in the pathogenesis of mastitis.

34. PATHOLOGICAL STUDY OF NATURALLY OCCURING OVINE PULMONARY ADENOCARCINOMA IN SHEEP

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Introduction

Ovine Pulmonary Adenocarcinoma (OPA) is a contagious and transmissible lung cancer of sheep resembling human bronchiolar-alveolar carcinoma.

Materials and methods

The lungs of 9400 sheep slaughtered in Fars Province, Iran, were examined. Multiple appropriate samples from the suspected lungs and mediastinal lymph nodes were fixed in 10% neutral buffered formalin, processed routinely and stained with haematoxylin and eosin (HE).

Results

OPA was diagnosed in the lungs of 21 sheep (0.22%). The frequencies of involvement of different lobes in the affected lungs were apical lobe (62%), cardiac lobe (33%), middle lobe (33%), diaphragmatic lobe (62%) and accessory lobe (5%). In 12 sheep, the classical form was observed as firm, white to grayish coalescing masses mostly in the cranioventral lobes. In 9 sheep, atypical lesions were observed as small clearly demarcated nodules mostly in the diaphragmatic lobes. Histopathological changes were almost similar in the two forms. They consisted of acinar or papillary growth of neoplastic cells in the alveoli, and polypoid proliferation of bronchiolar epithelium. There were variable amounts of fibroplasia, myxomatous foci and infiltration of lymphocytes and plasma cells in the interstitial tissue of affected alveoli. No metastatic lesions were observed in the lymph nodes.

Conclusion

The findings of this study support the hypothesis that atypical and classical forms represent the different stages or manifestations of a single disease spectrum.

DISTRIBUTION OF STELLATE CELLS IN CATTLE LIVER WITH PARASITIC FIBROSIS AND CIRRHOSIS

35.

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Introduction

Increase of extracellular matrix in humans, as well as in animals, occurs as a consequence of hepatic stellate cell (HSC) activation. The role of these cells in the development of liver fibrosis and cirrhosis, which occurs as a consequence of migration of *Fasciola hepatica* and *Dicrocoelium dendriticum*, is the subject of this paper.

Materials and methods

Formalin-fixed and paraffin-embedded liver samples from 30 slaughtered cows, infected with flukes, were examined by light microscopy, using HE and Masson-trichrome stains and immunohistochemistry for α-smooth muscle actin (α-SMA), vimentin and desmin.

Results

Distribution of HSCs is dependent on the degree of liver fibrosis. Actively synthesisizing HSCs were most prominent in the peri-sinusoidal areas. In chronic cases with liver cirrhosis and formation of hyperplastic pseudolobules, HSCs were located at the periphery. Cells of different shapes and size were positive for α-SMA, desmin and vimentin. Numerous septal and portal myofibroblasts also stained positive with these antibodies.

Conclusion

HSCs play an important role in the synthesis of extracellular matrix components in the development of parasitic fibrosis and cirrhosis in the liver of cattle. Activated HSCs, as well as portal and septal myofibroblasts, correlate to the degree of liver fibrosis.

36. STROMAL CYSTS IN THE CANINE OVARY

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Introduction

Ovarian cystic disease in the bitch includes paraovarian, ovarian epithelial and stromal cysts (SC). The latter, although uncommon, are clinically relevant for their potential hormone secretion.

Materials and methods

462 female genital tracts were clinically, grossly and microscopically evaluated. Cavitatory structures over 8 mm, without egg cells, and lined by granulosa and/or luteinic cells were considered to be SCs.

Results

95 and 36 bitches had ovarian and paraovarian cysts respectively and 32 presented with both. The prevalence of ovarian cysts had a linear relationship to the age of the bitch. Most ovarian cysts were epithelial and asymptomatic. SCs (n = 17) were found in young bitches (< 2 years) with a prevalence of 4%, and in elderly bitches (8–12 years) with a prevalence of 6–7%. In 53% of SCs, an irregular oestrous cycle with hormone-dependent lesions in other organs was evident, while in the remaining 47%, the cysts were associated with functional corpora lutea. 70% of SCs showed a partial to complete luteinization of the wall. SCs were always positive to α-inhibin and negative to cytokeratins.

Conclusion

Considering the low prevalence of SCs and pre-ovulatory luteinization, a distinction between follicular and luteal cysts is not useful in the bitch. The pathologic significance of SCs has to be deduced when associated with functional corpora lutea.

ACARDIUS ACEPHALUS IN A KID

37.

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Introduction

Acardius acephalus is a form of monozygous twins in which the parasitic twin is joined by the chorionic or cord vessels to the other one.

Materials and methods

A malformed foetus, delivered by an adult black Messinese goat, was radiologically and grossly examinated.

Results

The foetus had only a single pair of limbs. External genitalia were represented by an inhabited scrotum. Radiological examination revealed the presence of morphologically unremarkable hind limbs and a malformed pelvis. The vertebral column appeared malformed, turned at an angle of 90° and ventrally shifted, with clear morphological anomalies of lumbo-sacral vertebral bodies. Skinning revealed absence of subcutaneous fat and muscular hypotrophy. Omphalocoele and a probable preputial sheath, internally connected to a rudimentary urethro-penile structure leading to the urinary bladder, was detected. The intestine was constituted only by ileum, an atretic, dilated and blind caecum and colon. No other abdominal or thoracic organ was detectable.

Conclusion

On the basis of gross and radiological findings the malformation was classified as *Acardius acephalus*. In human medicine, acardia is a rare and severe abnormality reported as unique complication of monozygotic twin pregnancies, known as twin-reversed arterial perfusion (TRAP). Only a few cases of *A. amorphous* in bovines and a single case of *A. acephalus* in ovines have been reported.

38. VENTRICULAR CANDIDIASIS IN A LOVE BIRD (*AGAPORNIS FISCHERI*)

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Introduction

Candidiasis, is caused by a yeast-like fungus, *C. albicans*. Avian candidiasis of the digestive tract is considered to be rare.

Materials and methods

A young Fisher's lovebird, had clinical signs of asymmetric nares, weakness, diarrhea prior to death. Collected organs were preserved in 7.2% buffered formalin. At the laboratory, microscopic sections were prepared and stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS) and Gram.

Results

Gross necropsy revealed a large quantity of hemorrhage in the gizzard and duodenum. Histopathologically, pseudohyphae were detected in haemorrhagic parts of the gizzard and the surface of the intestine and most often extended deep into the submucosa and muscularis layers. Severe haemorrhages and vasculitis with invasion of fungal organisms into vessel walls were observed.

Conclusion

Enlargement of the nares may have predisposed this bird to the disease. On the other hand, candidiasis could have acted as a primary pathogen if the bird's resistance was lowered by environmental stress.

POSTER PRESENTATIONS

Session 5 A – Tumour Pathology 2/P

39. OVARIAN ARTERIOVENOUS HAEMANGIOMA IN A FEMALE DOG

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Introduction

Haemangioma is a vascular tumour, which occasionally may affect the ovary. Vascular tumours have to be differentiated from vascular abnormalities (hamartomas). The presence of normally organized arterioles and nervous structures in the lesional context may be indicative of a vascular hamartoma.

Material and Methods

A 12-year old female German Shepherd dog, with no heat in the previous two years, was admitted for abdominal distension and presence of a multicavitated structure difficult to evaluate by ultrasound. At laparotomy, the genital tract was removed including a large left ovarian neoplasm (31 × 20 × 7 cm). The mass (weight 4 Kg) had a spongy and congested appearance and, at sectioning, had multiple cavernous blood-filled structures. The uterus was congested and had endometrial cystic hyperplasia. The right ovary had paraovarian cysts and an early granulosa cell tumour.

Results

Histologically the mass was characterized by newly-formed vessels, including small arterioles with scant and abnormal elastic lamina, irregularly displaced into a fibromuscular stroma. No nerves were identifiable by means of S100 and PGP9.5 immunohistochemistry. A diagnosis of arteriovenous haemangioma was made.

Conclusion

The reported case shows the differential approach which enables the distinction between a haemangioma and a vascular hamartoma. The term arteriovenous haemangioma indicates the presence of a mixture of neoplastic arterioles with a scant elastic lamina and veins.

PROLIFERATION RESPONSE *IN VIVO* TO AGLEPRISTONE IN CANINE MAMMARY CARCINOMA: RELATIONSHIP WITH PROGESTERONE RECEPTOR EXPRESSION AND PHOSPHORYLATION

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Introduction

The progesterone receptor antagonist, aglepristone, may have a role in the neoadjuvant or adjuvant therapy of canine mammary carcinoma.

Materials and methods

Twenty two bitches with malignant mammary tumours were injected subcutaneously with 40 mg/kg of aglepristone (17) or vehicle (5). Core biopsies taken before treatment (day 1) and excisional biopsies (day 15) were fixed in 10% formalin, embedded in paraffin wax and used for tumour classification and immnohistochemistry for progesterone receptor (PR), MIB-1 antigen and phospho-PRser294 expression.

Results

All tumours were classified as carcinomas. 61% and 80% of experimental and control tumours expressed PR respectively. 20–30% of these PR-positive tumours were phospho-PRser294-positive. Plasma progesterone was >15 ng/ml in 15% of the cases. A decrease of MIB-1 index by more than 30% between the two paired biopsies was more frequently observed in PR-positive tumours (75%) but differences in MIB-1 index decreased with PR-negative tumours (66%) or control tumours (50%) and were not statistically significant.

Conclusion

The proliferation response to aglepristone in mammary carcinoma is linked to the low PR activation by plasma progesterone.

41. IMMUNOHISTOCHEMICAL CHARACTERISATION OF A HEPATIC CARCINOID IN A DOG

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Introduction

A hepatic mass was identified in a 10-year-old male mixed-breed dog with a clinical history of frequent vomiting, anorexia, lethargy and seizures.

Material and Methods

After necropsy, tissue samples were fixed and five micron sections were stained with HE.

Results

Radiographically, a large mass was detected in the liver. At necropsy, the liver had a $6 \times 2 \times 4.5$ cm, multilobulated, firm, mass involving the whole caudate lobe. On cut section, the tumour mass was grey to sanguinous. Microscopically, there were neoplastic proliferations with a trabecular to rosette pattern, separated by a fibrovascular stroma. The neoplastic cells were round to oval, with granular, eosinophilic, cytoplasm and hyperchromatic nuclei. Metastases were observed in lungs, kidneys and mesenteric lymph nodes. Immunohistochemically, neoplastic cells were immunoreactive for NSE and S100 protein and they were negative for CEA, Chromogranin A, CD34, CK20 and Hepatic antigen.

Conclusion

Negative CEA, CK20, CD34 and Hepatic antigen ruled out the probability of cholangiocarcinoma, metastatic carcinoma, hematopoietic/vascular origin and hepatocellular origin of this tumour, respectively. Immunohistochemical demonstration of NSE and S100 protein supported the diagnosis of neuroendocrine carcinoma and a negative reaction for Chromogranin A does not necessarily disprove this diagnosis either.

AN OUTBREAK OF PRIMARY ALIMENTARY LYMPHOMA IN A SHEEP FLOCK

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Introduction

Leukaemia is a rare occurrence in sheep. Mucosal-associated lymphoid tissue of the gastrointestinal tract can be the primary site of lymphoma.

Materials and methods

Sixteen adult ewes (over two years old) in Sarakhs, Northeast of Iran, showed signs of an alimentary disorder. The affected animals were anorexic and cachectic with ascites and abdominal pain. Symptomatic treatment was done but the condition of five animals deteriorated and these animals were euthanized.

Results

At necropsy, there was marked ascites and thickening of the intestinal wall. Most of the mesenteric lymph nodes showed notable enlargement. There were also multiple, grayish, white nodules in the liver, renal cortices and spleen. Histologically, the lesions in the lymph nodes consisted of a neoplastic proliferation of lymphoid cells that had obliterated the normal architecture. The neoplastic cells had indented, round or ovoid, vesicular nuclei with prominent nucleoli. There were a few mitotic figures with neoplastic cell necrosis. Clusters of neoplastic cells were found in the hepatic parenchyma and renal cortex. There was also enlargement of the splenic corpuscles due to proliferation of neoplastic cells.

Conclusion

Both macroscopic and histopathological findings confirmed the primary lymphocytic type of alimentary lymphoma in affected sheep.

43. VAGINAL LEIOMYOSARCOMA IN A DAIRY COW DURING PREGNANCY

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Introduction

Reported neoplasms of the ruminant female reproductive tract are rare. An estimated 10–50% of such tumours are of smooth muscle origin, of which approximately 10% are considered malignant. Leiomyosarcomas in these species are reported to display a growth pattern of low-grade malignancy, with slow invasion and rare metastasis. To our knowledge, there is no report about occurrence of vaginal leiomyosarcoma in the cow and in this report, we describe gross and histopathological features of the tumour.

Materials and methods

During rectal examination of a 5-year-old pregnant cow, a single multinodular vaginal mass was encountered and removed surgically after parturition. Tissue samples of the mass were fixed in 10% neutral buffered formalin, paraffin-embedded, sectioned at 5–6 μm, and stained with hematoxylin and eosin.

Results

Grossly, the mass had two convex and flat surfaces. Multiple firm nodular masses and ulcers were observed in the convex and flat surfaces, respectively. On cut section, the tumour was solid, non-encapsulated and gray-white with small yellowish foci. Histological examination of the mass revealed round to spindle cells with cellular and nuclear pleomorphism. The tumour cells had indistinguishable cytoplasmic borders and prominent nucleoli. Necrotic debris and infiltration of inflammatory cells around bacterial colonies were present. There was diffuse fibrous connective tissue arranged in interwoven fascicles.

Conclusion

According to macroscopic and microscopic features, the mass was diagnosed as vaginal leiomyosarcoma.

ATYPICAL CUTANEOUS FORM OF LYMPHOSARCOMA IN HOLSTEIN-FRIESIAN COWS

44.

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Introduction

Sporadic bovine leukosis is a rare lymphosarcoma with unknown etiology. The cutaneous form affects cattle of all ages with localized skin lesions and occasional generalised lymphadenopathy.

Material and Methods

A 2-year-old Holstein-Friesian cow, which had given birth 3 months previously, developed small cutaneous tumours around the neck and scapular regions which were associated with lymphatic vessels. Tumours then started to appear and grow in several body regions. They varied in size from 1 to 10 cm in diameter and necrosis, ulceration and suppuration were very common. Clinical examination revealed enlargement of the superficial lymph nodes. Rectal examination demonstrated slight enlargement of the mesenteric lymph nodes. The serological examination excluded BLV infections. Two of the neck tumours were taken by surgical biopsy, fixed in 10% buffered formalin, paraffin-embedded, and stained with HE. Based on the poor prognosis, the cow was euthanised one month after the first tumours appeared.

Results

At necropsy there was enlargement of superficial, mesenteric and hepatic hilar lymph nodes and spleen. Histologically, all samples had a similar appearance with pleomorphic, highly immature lymphoid cell infiltration effacing normal tissue architecture. Lymphosarcoma cells were round to polygonal with large, irregularly-round nuclei of varying size, a clumped chromatin pattern, prominent nucleoli, a moderate amount of cytoplasm and frequent mitoses. Slight proliferation of connective tissue and reticular fibers were seen.

Conclusion

A diagnosis of atypical cutaneous lymphosarcoma was made.

45. HEPATOBLASTOMA IN A CAT

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Introduction

Hepatoblastomas are benign neoplasms originating from putative pluripotential stem cells of the liver and have only rarely been reported in domestic animals. We report histopathological and immunohistochemical characteristics consistent with hepatoblastoma in a feline.

Results

A hepatic mass of 8mm in diameter was surgically resected from a spayed Abyssinian cat aged 8.2 years. Histologically, the majority of tumour cells had sparse, uniform, cytoplasm with round or ovoid nuclei resembling immature hepatocytes. The neoplastic cells were arranged in cords and sheets, although they sometimes formed rosettes and small ductal structures. Mitotic figures were rare. The tumour mass also contained primitive mesenchymal cells with spindle-shaped nuclei. Immunohistochemically, some tumour cells with uniform round nuclei were weakly positive for α-fetoprotein, and ductal structures were positive for cytokeratin 7, 8/18, CAM5.2 and vimentin. Some tumour cells also had a positive reaction for neuronal markers such as PGP9.5, CD56 (NCAM), and synaptophysin, but were negative for Grimelius stain and chromogranin.

Conclusion

The morphological and immunohistochemical characters of this tumour were suggestive of hepatoblastoma. Rosette formation and a positive reaction for neuronal markers and synaptophysin suggested neural differentiation. A diagnosis of carcinoid was ruled out due to negative Grimelius and chromogranin staining.

A CASE OF BASAL CELL TUMOUR IN A HORSE: HISTOLOGICAL AND IMMUNOHISTOCHEMICAL PATTERN

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Introduction

Basal cell tumour is an infrequent neoplasm in horses. Information on the immunohistochemical phenotype has not been documented. The purpose of this study was to investigate potential epithelial surface markers for this neoplasm.

Materials and methods

A 15-year-old Thoroughbred, gelding was presented with a 1 cm diameter cutaneous nodule on the base of the right nostril. Surgical excision, histological (HE) and immunohistochemical investigations were performed. A panel of antibodies was applied using IHC protocols as indicated in the respective datasheets: CK 5/6, CK, CK10, CK17, CK20, CK AE1/AE3, CKHMW, VIM.

Results

Histopathology revealed an incomplete surgical excision and surgery was performed to clean the margins two weeks later. Histology revealed a dermal medusoid pattern without epidermal connection. Cells formed irregular clusters of compact, pseudostratified to columnar shape with prominent clear cytoplasm. In areas, there were radiating arms of peripheral small clusters, surrounding lumina filled with amorphous material. The cells were strongly positive for CK5/6 and CK AE1/AE3. A six month follow-up clinical examination revealed no post-operative complications, recurrence or metastases.

Conclusions

In the nodule, the cells were arranged around lumina giving the impression of an adnexal neoplasm. Positivity for CK5/6, expressed in the basal layers of stratified epithelium together with positivity for CK AE1/AE3, supports the diagnosis of basal cell tumour.

47. IMMUNOHISTOCHEMICAL STUDY OF HORMONE RECEPTORS AND ANGIOGENESIS IN HIGHLY MALIGNANT CANINE MAMMARY CANCER

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Introduction

Inflammatory mammary cancer (IMC) is a distinct clinical type of mammary cancer in man with particularly aggressive behaviour and an unfavourable prognosis. It is characteristically angioinvasive and angiogenic. Several studies on human breast cancer suggest that steroid hormones have angiogenic properties inducing the synthesis of angiogenic factors *in situ*. The aim of this study was to evaluate the expression of hormone receptors in relation to angiogenic factors in highly malignant canine mammary tumours, including IMC cases.

Material and Methods

Twenty-two cases with IMC (group IMC) and 20 histological grade III non-inflammatory malignant mammary tumours (group MMT) were included. The expression of REα, REβ, RP, RA, CD31, VEGF-A, VEGF-D, COX-2, and Ki 67 was performed by immunohistochemistry; microvascular density (MVD) and lymphangiogenesis (Ki 67 index) were calculated.

Results

REβ was significantly related to VEGF-D and COX-2 (all tumours) and with DMV and VEGFR-3 (IMC group). RP was associated with COX-2 (all tumours) and with VEGF-D, VEGFR-3, and COX-2 (IMC group). RA was significantly associated with VEGF-A, VEGF-D, VEGFR-3, and COX-2 (all cases), VEGF-D and COX-2 (IMC group), and VEGF-D (MMT group).

Conclusion

According to our results, there is a possible hormonal regulation of angiogenesis in highly malignant canine mammary tumours, especially in inflammatory mammary cancer.

IMMUNOHISTOCHEMICAL EXPRESSION OF THE HER-2 RECEPTOR IN MAMMARY GLAND NEOPLASMS OF FEMALE DOGS

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Introduction

In veterinary medicine, there are some reports on the amplification of the c-erbB-2 oncogene and overexpression of the HER-2 protein in dogs with mammary tumours.

Materials and methods

Material for the investigation comprised mammary gland tumours collected from dogs attending veterinary clinics for surgical procedures or archival samples. Altogether there was a total of 18 adenomas, 32 adenocarcinomas simplex, 31 adenocarcinomas complex, and 12 solid carcinomas. Histopathological and immunohistochemical examinations were performed.

Results

HER-2 expression was greater in carcinomas than adenomas. There was no correlation between carcinoma type and tumour grade or the expression of HER-2 receptor. A positive correlation was observed between proliferative activity and there was a negative correlation with oestrogen receptor-α expression. There was no correlation with p53 expression.

Conclusion

HER-2 expression occurred more frequently in malignant carcinomas than in benign adenomas and was associated with high proliferative activity in these neoplasms.

49.

ESTABLISHMENT AND CHARACTERISATION OF A NEW CELL LINE DERIVED FROM CANINE MAMMARY ANAPLASTIC CARCINOMA

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Introduction

Canine mammary anaplastic carcinoma (CMAC) proliferates with diffuse infiltration. Surgical treatment is not effective and prognosis is poor. We established and characterised a new cell line (designated 2000MC) derived from CMAC.

Materials and methods

2000MC cells were derived from CMAC of a 10-year-old female Labrador retriever. Homogenised tumour tissue was placed into Dulbecco's minimal essential medium with 10% foetal calf serum. The cells were passaged over 100 serial generations. The expression and activitiy of MMP-2 and MMP-9 were determined by RT-PCR and gelatin zymography (GZ). Migration of the 2000MC cells was analysed using a migration assay kit.

Results

The 2000MC cells were polyhedral and weakly adherent to each other andthe culture dish. Expression and activity of MMP-9 was detected by RT-PCR and GZ while MMP-2 was not identifiable. In the migration assay, 2000MC cells exhibited high activity, which was inhibited by MMP-9.

Conclusion

The 2000MC cell lines were highly infiltrative, similar to that observed in spontaneous CMAC. The activity of MMP-9 appears to be predominantly associated with the infiltrative nature of these cells.

OVER DIAGNOSIS OF MALIGNANCY IN TUMOURS OF THE CANINE MAMMARY GLAND: IS THERE A ROLE FOR MYOEPITHELIAL CELL MARKERS?

50.

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Introduction

Histological evidence of malignancy in canine mammary tumours does not invariably imply a malignant clinical course. In human breast cancer, an intact myoepithelial cell layer is a feature of *in situ* carcinomas.

Materials and methods

Tissue samples from 59 routinely-processed canine mammary carcinomas were stained with H&E for tumour classification and histological grading. Calponin expression was used to evaluate integrity of the myoepithelial cell layer. The percentage of tumour nodules surrounded by a single layer of calponin-positive spindle cells was evaluated in eight microscopic fields per tumour at 20× magnification.

Results

Histological grade of the tumours included 4 grade 0, 31 grade 1, 17 grade 2, and 7 grade 3 carcinomas. All 31 tumours with histological grade 1 had more than 70% of tumour cell nests surrounded by an intact calponin-stained myoepithelial cell layer, while this percentage was 76.4% and 14.2% for grade 2 and 3 tumours, respectively.

Conclusion

This correlation study between histological grade of malignancy and calponin expression indicates that most histological grade 1 canine carcinomas may be *in situ* neoplasms.

51. RARE CASE OF THIRD EYELID MAST CELL TUMOUR IN A HORSE

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Introduction

Mast cell tumours (MCT) are uncommon in horses. They usually occur as benign, solitary masses on the skin of the head, neck, trunk and legs. In the present study, we describe a rare case of an equine mast cell tumour located in the third eyelid.

Materials and methods

An 18-year-old mare presented with a 4 cm diameter tumour in the left third eyelid. Other ocular examinations were unremarkable and the right eye was normal. The tumour was surgically removed with the third eyelid and paraffin sections were stained with HE and toluidine blue. Immunohistochemical methods for the detection of cytokeratin, vimentin, and CD-117 were also applied.

Results

Microscopically, tumour cells were round to ovoid with slightly pleomorphic nuclei. Fine cytoplasmic granules stained weakly with toluidine blue. The tumour cells were immunopositive for CD-117 and vimentin and cytokeratin negative.

Conclusion

A neoplasm in the third eyelid of an adult horse was diagnosed as a mast cell tumour according to Patnaik's classification for cutaneous MCTs. MCTs should be considered in the differential diagnosis of third eyelid tumours in horses.

OCCURRENCE OF NEUROFIBROMA IN THE SPINAL CORD OF A GERMAN SHEPHERD DOG

52.

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Introduction

Neurofibroma is one of the sub-classified tumours of peripheral nerve sheath (PNSTs). Tumours of this type are rare in dogs and are observed most commonly unilaterally in the spinal nerves with high frequency in the brachial plexus and lower frequency in the lumbosacral plexus.

Materials and methods

A one and a half years old male dog with posterior paralysis was referred to the University Clinic. Using USG and MRI, an unusual mass was observed in the vertebral column along the left side of the spinal cord between T12 and L2. The dog was euthanised and the tumour was removed for macroscopic and microscopic examinations.

Results

Macroscopically, the tumour was soft and gelatinous, and was white to gray with a smooth surface. Histologically, the tumour cells were uniformly elongate and fusiform and lacked obvious cytoplasmic borders. They were arranged as bands, whorls, and palisades. Antigenic markers diagnostically specific for Schwannomas are not available.

Conclusion

The appearance of the tumour was of a neurofibroma. This tumour should be differentiated from other neoplasms such as meningioma, fibroma, and haemangiopericytoma by histological and immunohistochemical patterns.

53. BIOCOMPATIBILITY AND ACTIVITY OF OXIDISED SILICON MICROPARTICLES TREATED WITH CHITOSAN

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Introduction

The effect of oxidised silicon microparticles, treated with chitosan, was investigated in Wistar rats. Changes in skin and muscle at sites of inoculation were examined together with weight gains and survival rates. Levels of the molecular biological markers P450-cytochrome and glutathion-S-transferase (GST) were assayed.

Results

Local irritative effects at skin level disappeared 48 hours after injection. After 24 hours, small powdery deposits in the muscular area had an acicular and/or crystalloid appearance. These deposits were dispersed and partially resorbed at 48 hours. Weight gains and survival rates were similar for both control and experimental animals, suggesting good biocompatibility of the tested microparticles. Changes in concentration of P450-cytochrome and GST-activity indicated the induction of dynamic changes resulting from activation of detoxication mechanisms.

Conclusion

This study represents an intermediary step in the development of oxidised silicon particles both as possible carriers of antitumour agents and as factors expressing synergic effects. The finding of good biocompatibility bodes well for the future use of the particles as drug carriers.

EXPRESSION OF MULTIDRUG RESISTANCE-ASSOCIATED P-GLYCOPROTEIN IN FELINE TUMOURS

54.

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Introduction

Several studies in normal and neoplastic canine tissues indicate that P-glycoprotein (P-gp) is involved in multidrug resistance, as described in human medicine. In this study we evaluated the expression of P-gp in a range of spontaneous feline tumours from patients that had not previously received chemotherapy.

Materials and methods

In 100 feline patients with spontaneous tumours, biopsies were taken during surgery or at necropsy and fixed in formalin for P-gp immunostaining using the antibody C494.

Results

Tumours (apocrine gland carcinoma, bile duct carcinoma, meningioma, pulmonary carcinoma, intestinal adenocarcinomas, haemangiopericytoma, and haemangiosarcoma) arising from tissues with intrinsic P-gp expression showed consistently positive labeling for P-gp in a cellular pattern identical to that described for normal feline tissue. Tumours with both C494 positive and negative cells included mammary gland tumours, lymphomas, mastocytomas, and squamous cell carcinomas. Mammary gland tumours in particular showed strong membranous staining for P-gp in areas with infiltrative growth and highly malignant cells.

Conclusion

Different feline tumours show different P-gp expression. This may have consequences with respect to treatment options.

55. A PERIPHERAL PRIMITIVE NEUROECTODERMAL TUMOUR IN A DOG

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Introduction

Neuroectodermal tumours are rarely reported in human beings and in animals. This report describes an 8-year-old male German shepherd dog, which was referred to the veterinary clinic of Tehran Azad University.

Materials and methods

Following surgery, representative samples of lesions were fixed in 10% buffered formalin. After decalcification and paraffin embedding, sections were prepared and stained with HE.

Results

Clinical evaluation showed a pedunculated ulcerative mass $8 \times 5 \times 6$ cm in the mandible. Radiographically, the mass was associated with lysis of the surrounding bone and there was no evidence of metastases to other organs. Histologically, the mass was composed of broad sheets of uniformly small, round to oval cells with thin rims of basophilic cytoplasm, pale chromatin and indistinct nucleoli. The tumour cells labelled positively for S100, NSE and GFAP, and negatively for synaptophysin.

Conclusions

The positive reaction for NSE and S100 showed that the tumour did not originate from haematopoetic stem cells or muscle and the positive reaction for GFAP indicated an origin in nervous system. Immunohistochemical methods are indispensable in the differential diagnosis of neuroectodermal tumours.

CD117 AND MMP-9 IMMUNOREACTIVITY IN 40 CASES OF MAST CELL PROLIFERATION (MAST CELL TUMOURS AND MASTOCYTOSIS) IN THE CAT

56.

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Introduction

Mast cell proliferations in felines assume various patterns, from nodules to diffuse lesions.

Materials and methods

Forty cases of feline mast cell proliferation were selected: 27 cutaneous mast cell tumours (CMCT), four extracutaneous mast cell tumours (lip, vulva, stomach, and mesentery) (EXCMCT), and nine cases of mastocytosis (MTCS). Samples were routinely processed. Immunohistochemistry for CD117 and MMP9 was performed.

Results and discussion

The mean age of diseased cats was 7.6 years (range 2 to 16), mastocytosis being more frequent in younger cats. No sex or breed predisposition was identified. Of the 27 cases of CMCT, 19 were well differentiated, seven were moderately to poorly differentiated and one was pleomorphic. The four cases of EXCMCT were poorly differentiated except for one case involving the lip, which showed epithelial infiltration. CD117 was irregularly expressed in CMCT, MTCS and negative in EXCMCT. Strong expression of MMP9 was generally seen in mast cells from localised and diffuse lesions. The fact that many other stromal cells also expressed MMP9 indicates that invasive behaviour may not depend exclusively on mast cell production of metalloproteinases.

57. CONCOMITANT APPEARANCE OF RIGHT ATRIUM MYXOMA AND LEFT ATRIUM FIBROMA IN A DOG

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Introduction

Primary and secondary cardiac tumours are extremely rare in humans and dogs. A case of concomitant occurrence of different cardiac tumours in a dog was described.

Materials and methods

Corpse of the male, 10-year-old, Dachshund dog was presented to the Clinic. Before the death, the dog displayed long-term respiratory distress, fatigue, exercise intolerance, and systolic murmur at the mitral and tricuspid valves. The dog was autopsied and samples of the heart were taken for histopathological examination. The samples were fixed in 10% formalin, embedded in paraffin, and then sections stained with haematoxylin and eosin were prepared.

Results

At autopsy, the right and left atrium and left ventricle chamber were dilated and tumour changes in the both atria were observed. Microscopically, the tumour of the right atrium was found to be myxoma. It was composed of faintly eosinophilic myxoid matrix and spindle shaped fibroblast-like cells with elongated nuclei and stellate cells. The changes in the left atrium were diagnosed as fibroma. The tumour was composed of interweaving bundles of spindle cells extending into surrounding myocardium. This case shows the unusual concomitant appearance of cardiac myxoma infiltrating from the right atrium, and fibroma arising from the left atrium, which were responsible for myxomatous degenerative valvular disease.

Conclusion

To our knowledge, we presented for the first time a concomitant occurrence of both tumours in the canine heart.

POSTER PRESENTATIONS

Session 6 B – Experimentally-Induced Disease/Toxicological Pathology

58.

COMPARATIVE PATHOLOGY OF THE CAECA OF ANTICOCCIDIAL IMMUNIZED CHICKS INFECTED WITH A NIGERIAN ISOLATE AND HOUGHTON STRAIN OF *EIMERIA TENELLA*

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Introduction

This study compares the lesions seen in immunized and unimmunized chicks infected with a Nigerian (Local – L) isolate and Houghton (H) strain of *Eimeria tenella*.

Materials and methods

Ninety day-old dominant black chicks were assigned to 9 groups of 10 birds each. Group 1, unimmunized, uninfected; group 2, unimmunized, infected with H-strain; group 3, unimmunized, infected with L-isolate; group 4, immunized with Livacox®, infected with H-strain; group 5, immunized with Livacox®, infected with L-isolate; group 6, immunized with Immucox®, infected with L-isolate; group 7, immunized with Immucox®, infected with H-strain; groups 8 and 9 were immunized controls. Immunization was done at 5 days of age by oral gavage and infection was with 1.7×10^4 *E. tenella* of either H-strain or L-isolate at 4 weeks of age. Caeca of moribund birds were examined for gross and microscopic changes 6 days post-infection.

Results

Grossly, the L-isolate of *E. tenella* produced severe necrohemorrhagic tyhilitis in unimmunized and Immucox® immunized infected chicks. Microscopically, H-strain and L-isolate infected chicks had severe mucosal and crypt damage with more than 300 oocysts or schizonts per low power field (LPF). Livacox® and Immucox® immunized chicks infected with either the H-strain or L-isolate showed variation in caecal damage and numbers of oocysts or schizonts.

Conclusion

Immunization of chicks with Livacox® and Immucox® produces variable levels of protection against the H-strain and L-isolate of *Eimeria tenella*.

INFLUENCE OF MILD HYPOTHERMIA INDUCED BY SURFACE COOLING ON HYPOXIC LESIONS IN THE PIG CEREBELLUM

59.

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Introduction

Sudden cardiac arrest leads to neuronal injury and poor neurologic outcome due to hypoxia and formation of oxygen radicals during reperfusion.

Materials and methods

16 swine were put to cardiac arrest for 10 min. followed by 8 min. of conventional life support. After restoration of spontaneous circulation the swine were randomized into two groups (normothermia control, hypothermia). The hypothermia group was cooled to 33.0°C and kept for 14 h. At day 9 of the experiment the animals were killed, the brains perfused with formalin and embedded in paraffin. Coronal slices of the cerebellum were stained with HE and GFAP.

Results

By means of HE-staining, a reduction of hypoxic lesions (eosinophilic degeneration of Purkinje cells, loss of Purkinje and granule cells, gliosis of molecular layer) was detectable in the hypothermia group. All control animals showed a marked astrocytosis in the granule cell and molecular layers.

Conclusion

Mild hypothermia induced by surface cooling during reperfusion has a protective effect on hypoxic cerebellar lesions.

60. OCHRATOXIN A (OTA): CELL-MEDIATED IMMUNE RESPONSE, HISTOPATHOLOGICAL FEATURES AND PROTECTIVE ROLE OF VITAMIN E IN EXPERIMENTALLY INTOXICATED PIGLETS

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Introduction

Immunosuppression of the immune system by Ochratoxin A (OTA) can be detected as an increased amount of apoptosis in lymphoid tissues. Vitamin E is a natural antioxidant molecule that can counteract the toxic effect of OTA. The aim of this study was to establish if a vitamin E supplement in natural OTA-contaminated food can mitigate against the toxic effect of OTA.

Materials and methods

Twenty-four 28-day-old piglets, were randomly sub-divided into 6 groups and daily fed with: OTA free feedstuff, OTA free feedstuff supplied with vitamin E, or feedstuff contaminated with 200 ppb of OTA supplied with vitamin E. Blood samples were collected for phenotypic characterization of T lymphocytes, a MTT assay and RT-PCR. Tissue specimens were also taken for histopathological evaluation.

Results

Expression of IL-6 and TNF-α was increased in the groups treated with OTA compared with control groups. Histopathology highlighted apoptotic lymphocytes in lymphoid tissues and multifocal degeneration of convoluted distal tubules of all piglets fed with OTA-contaminated feedstuff.

Conclusion

It can be concluded that 200 ppb of OTA in feedstuff does not compromise the immune response, but can increase the production of pro-apoptotic cytokines which can be influenced by using a supply of vitamin E in the diet.

HISTOPATHOLOGICAL STUDY OF THE EFFECT OF DOXYCYCLINE ON EXPERIMENTAL AA AMYLOIDOSIS IN MICE

61.

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Introduction

Amyloid is a pathological extracellular proteinaceous material arising from different mechanisms in different tissues. Systemic amyloidosis can be distinguished by amyloid deposition in different tissues. Recent investigations show that some antibiotics such as doxycycline destroy the amyloid fibrils.

Material and methods

In this study, 24 syrian mice were divided into two groups of 12 animals. In the control group, animals received bovine serum albumin (25 mg in 0.5 ml normal saline) subcutaneously as one injection per week for 4 weeks to induce experimental amyloidosis. In the test group, animals received bovine serum albumin in the same manner and doxycycline was added at 100 mg/kg to the drinking water. After four weeks, mice of both groups were euthanised. Tissue samples of kidney, liver, spleen, heart, brain and lung were collected, fixed in 10% buffered formalin and processed routinely. The slides were stained with HE and Congo Red.

Results and conclusion

In the control group, histopathological findings comprised various degrees of amyloid deposition in renal glomeruli, hepatic parenchyma and vascular walls of lung and spleen. In the test group, mild amyloid deposition was only observed in renal glomeruli and occasionally in the vascular wall of other tissues. It is suggested that doxycycline could reduce amyloid formation and accumulation in mice.

62. THE IMMUNE RESPONSE IN MICE FOLLOWING INTRA-CEREBRAL VACCINATION AGAINST PSEUDORABIES VIRUS

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Introduction

In a previous study, intracerebral (IC) immunisation of inactivated pseudorabies virus (PRV) was more effective at inducing a protective immune response against PRV than subcutaneous (SC) immunisation. In this study, we investigated the mechanism of immune response to IC immunization.

Materials and methods

We compared the immune response in both peripheral blood and central nervous system (CNS). We analysed the level of serum anti-PRV antibodies (Abs) with ELISA and a neutralization test, and spleen cytokine levels with RT-PCR. The levels of IgG and PRV within the CNS were also analysed with real time RT-PCR and immunohistochemistry.

Results

Anti-PRV Abs and cytokines (IFN-r, IL-4, and IL-10) induced by IC immunization were relatively higher than those induced by SC immunization. The results of real time PCR and immunohistochemistry for IgG showed Ab-secreting cells (ASCs) within the CNS after IC immunisation, but not after SC immunisation. Furthermore, the IC immunised mice had more efficient protection than SC immunised mice when challenged with live PRV by IC innoculation. The former had more IgG and less PRV than the latter in CNS.

Conclusion

IC immunisation induced a stronger immune response, not only in peripheral blood but also within the CNS. We consider that ASC recruitment into the CNS contributed to PRV clearance.

EVALUATION OF THE LOCAL IMMUNE RESPONSE TO *FASCIOLA HEPATICA* IN LIVER AND HEPATIC LYMPH NODES (HLN) OF GOATS IMMUNIZED WITH SM14

63.

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Introduction

The Sm14 is a fatty acid binding protein from Schistosoma mansoni which has shown cross protection against *F. hepatica*.

Materials and methods

The goats used consisted of: group 1 (unimmunised and uninfected); group 2 (infected control – immunised with QuilA); group 3 (infected and immunised with Sm14 in QuilA). Group size was n = 7. Goats were infected with 200 metacercariae. Protection and hepatic damage were evaluated by histopathology and morphometry. The local immune response was assessed by studying the distribution of inflammatory cells in the liver and HLN by immunohistochemistry.

Results

Fluke burden and microscopic morphometric study did not show significant statistical correlations. Gross and microscopic hepatic changes had high individual variability, with lower levels in group 3. Gross hepatic morphometric study revealed a reduction of 56.6% ($P < 0.001$), in group 3 (21.7% ± 8.3) with respect to group 2 (50.0% ± 14.9). Cellular infiltration in liver and HLN showed a reduction in Group 3 for CD2+, CD4+, CD8+, γδ+ T-lymphocytes, IL-4+ and IFN-γ+ ($P < 0.05$).

Discussion and conclusion

This is the first report of ruminant immunisation with the complete molecule of recombinant Sm14 against *F. hepatica*. Immunisation reduced hepatic damage; however, further studies are required to improve the vaccine efficacy.

64.

EVALUATION OF THE LOCAL IMMUNE RESPONSE TO *FASCIOLA HEPATICA* IN LIVER AND HEPATIC LYMPH NODES (HLN) OF GOATS IMMUNISED WITH CATHEPSIN-L1 (CL1)

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Introduction

There is increasing interest in developing an effective vaccine or other control method for fascioliasis.

Materials and methods

Twenty-one Florida-breed goats were used for the study: group 1 (n = 7) was used as the uninfected control; group 2 (n = 7) was immunised twice with CL1; group 3 was inoculated with adjuvant (QuilA). Goats were infected with 200 metacercariae. Hepatic damage was evaluated by histopathology and the local immune response was evaluated by immunohistochemistry in liver and HLN.

Results

Fluke burden revealed a 38.7% decrease in the immunized group with respect to the infected control group, although without significant differences. With liver immunohistochemistry, the values in group 3 were lower compared with group 2 for CD2+, CD4+ and CD8+ ($P < 0.001$) and IL-4+ ($p < 0.05$). In the cortical areas of the HLN, there was a reduction in the CD2+, CD4+, CD8+, γδ+ T-lymphocytes, IL-4+ and IFN-γ+ ($p < 0.001$) and in the medullary areas for CD2+, CD8+, IL-4+ and IFN-γ+ with $p < 0.001$, and for γδ+ T-lymphocytes with $p < 0.05$.

Discussion and conclusion

These results suggest that CL1 in QuilA adjuvant produced a reduction in the local immune response compared to the group 3 adjuvant only animals. Further studies are necessary to confirm and improve these results.

THE EFFECT OF INFRA-RED LASER IRRADIATION ON WOUND HEALING IN HAMSTERS

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Introduction

Acceleration of the wound healing process after surgical incision is important in cosmetic surgery. In this study, the effect of infra-red laser therapy on vascular growth and fibroblast proliferation in hamsters was evaluated.

Materials and methods

Thirty male and female adult Syrian hamsters were selected randomly. Under general anaesthesia, a 1cm incision were made on the back and sutured with a simple interrupted stitch. Animals were divided into two groups (n = 15) (experimental and control). The laser group (experimental), were treated with Mustank Infra Red laser (0.5 J/cm^2), for one minute for six days. Biopsies were taken at 48 h and on day 5 and 10 and were sent for histopathology. Statistical analysis was done using software version 13.0, Kolmogrov-Smirnov. Mann Whitney U and T study tests were used.

Results

The mean number of vessels in the experimental group was significantly higher than the control group at 48 h. There was a significant difference between the two groups with regard to vascular growth on day 5. The fibroblast growth was also more prominent in the experimental group in comparison with the control group. On day 10, the growth of fibroblasts was higher in the experimental group compared with controls.

Conclusion

The rate of wound healing in the control group was slower than in the IR laser irradiation group. Fibroplasia and angiogenesis were significantly different between the two groups. It appears that the use of IR Laser irradiation accelerates the wound healing process in hamsters.

66.

HISTOLOGICAL EVALUATION OF EFFECT OF INVASIVE AND SEMI-INVASIVE METHODS OF ELECTROSTIMULATION ON HEALING PROCESS IN RABBITS' FEMUR

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Introduction

Semi conduction and piezoelectricity are important properties of bone. Current passing through callus enhances transport of mineral substances, thus stimulating regeneration of bone structure and function. Against this background, electrostimulation of bone union is investigated

Materials and methods

The aim of this study was to evaluate the impact of invasive and semi-invasive electrostimulation with direct and alternating currents on the development of synosthosis in the femur of rabbits. The most recent stimulators and metallic implants with passive-diamond layers developed by IMIiB of Politechnika Śląska were used. Routine histological examinations were enhanced by non-demineralised processing methods. Immunohistochemistry was assessed using a computer system measuring cumulated optical density.

Results

A range of values of electrical stimulation accelerated synosthosis in rabbits, influencing both the number and activity of cells involved in this process.

Conclusion

The results provide encouragement for further studies on electrostimulation in clinical practice as a supportive method for synosthosis.

INVESTIGATION OF PATHOLOGICAL EFFECTS OF DISSOLVED IRON ON KIDNEY TISSUE OF COMMON CARP (*CYPRINUS CARPIO*) IN ACIDIC PH

67.

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Introduction

Toxicology is one of the important branches of science in aquatic environments and fish are regarded as good indicators for research in this area.

Material and Methods

Research was started in May 2007 to evaluate the toxicity of dissolved iron and its pathological effects on kidney tissue of common carp. 190 fish with mean body mass of 15 ± 5 g. were divided into six treatment groups (10 fish in each group) in three replicates with one control group and exposed to ferrous sulfate hepta-hydrate, according to OECD guideline and semi static method. To maintain constancy of ferrous concentrations in test solutions, pH was maintained at 5.5 ± 0.1. A spectrophotometer and the orthophenontroline method were utilised to ensure desirable ferrous concentrations remained constant during the test period.

Results

The LC50 calculated by Probit analysis at 24, 48, 72 and 96 h, respectively, was 2.35, 2.15, 1.95 and 1.85 mg/l Fe2+. Pathological changes in the treatment groups after 96 h exposure included necrosis and blockage of renal tubules, hyperplasia of glomerular cells, decrease of Bowman's capsule and accumulation of melanin pigment in kidney tissue.

Conclusion

Ferrous iron is the most toxic form of iron in aquatic environments. The kidney accumulates heavy metals and is a suitable tissue for pathological investigations.

68. THE EFFECTS OF LEAD ACETATE ON HEART MUSCLE CELLS IN RABBITS

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Introduction
The toxic effects of lead acetate on many tissues including the nervous and cardiovascular systems are well established. Despite this, few reports exist of the effects of lead acetate on the myocardium. The purpose of the present study was to characterize the histological and ultrastructural changes caused by lead administration in heart muscle cells in the rabbit.

Materials and methods
20 New Zealand White rabbits were randomly selected and were assigned into experimental and control groups. The experimental group and the control group, respectively, received 6.5 mg/kg of lead acetate and the same volume of deionized water by intraperitoneal injection every other day for 7 weeks. The animals were then euthanised and samples from the left ventricular wall were prepared for histological and ultrastructural examination.

Results
Heart muscle cells in rabbits receiving lead acetate were accompanied by fibrosis and showed hyperchromasia, electron dense figures, increased numbers of mitochondria, cytoplasmic degeneration and nuclear pleomorphism. The differences in morphological findings in the treated and control groups were statistically significant using the t-test.

Conclusion
Heart muscle cells in rabbits undergo significant degeneration in chronic lead toxicity.

ALTERATION OF THE PULMONARY ARTERIES IN A PUPPY TREATED WITH SHAMPOO CONTAINING CHLORHEXIDINE. A CASE REPORT

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Introduction

Chlorhexidine is a disinfectant that is used in veterinary medicine for cleansing wounds, skin, instruments and equipment. We present a case of sudden death of one dog shortly after washing its hair with shampoo containing chlorhexidine diacetate.

Materials and methods

A four-month-old, female, Yorkshire terrier, suffered from oiliness of the skin and hair and was treated with shampoo containing chlorhexidine. The puppy died very shortly after the fourth bath. Samples of all internal organs were stained with HE, PAS and VG staining, and immunohistochemical detection of vimentin, actin, and von Willebrand factor was performed on lung sections.

Results

Gross pathology revealed acute hyperaemia of the lung. Histologically, there was hydropic degeneration of epithelium in the bronchioli, oedema and/or hyperplasia of the subendothelial connective tissue and oedema and/or hyperplasia of adventitia in predominantly small and medium size arteries. Oedema and hyperplasia of the subendothelial connective tissue caused stenosis or obturation of the vessel lumen. Veins were not affected.

Conclusion

On the basis of the clinical presentation and pathological findings, we consider chlorhexidine as the causal agent of the arterial lesions.

70. BACKGROUND LESIONS OF THE ADRENAL GLAND INPRIMATES FROM TOXICOLOGICAL STUDIES

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Introduction

Adrenal gland lesions in primates can occur spontaneously or can also be induced by a chemical substance or drug. Therefore, a precise knowledge of the naturally occurring changes as well as spontaneous frequencies is necessary when making a judgment as the toxicologist/toxicological pathologist, on the significance of a finding in a toxicological study. The presentation is intended to assist and support the interpretation of these studies in primates.

Methods

Data presented are derived from a review of slides of 200 control marmosets (Kaspareit *et al.*, 2006) or from the historical data base of histopathology findings of our institution including control cynomolgus (n = 449) and rhesus monkeys (n = 83). All animals were part of control groups in toxicological studies. A complete necropsy was performed at terminal kill and organs were fixed in 10% neutral formalin. Samples of tissues (including the adrenals) were embedded in paraffin wax, sectioned at a nominal thickness of 5 µm and stained with HE.

Results and discussion

Lesions observed included capsular extrusion of adrenal cortex, ectopic cortical tissue, osseous metaplasia in the cortex, amyloidosis, cortical fatty vacuolation, eosinophilic cortical foci, focal cortical cell hypertrophy, diffuse cortical cell hypertrophy, cortical necrosis, cortical/corticomedullary mineralization, inflammatory cell foci, extramedullary hematopoiesis, capsular adhesion and lipofuscin pigmentation. The incidences of the different lesions in different primate species will be reported and the features of the histopathological lesions will be described.

EXPOSURE OF OVINE BLASTOCYSTS TO POLYCHLOROBIPHENYL (AROCLOR 1254): ULTRASTRUCTURAL MODIFICATIONS AND EMBRYOTOXICITY

71.

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Introduction

The potential embryotoxic and foetotoxic effects of polychlorobiphenyls (PCBs) have been reported only in rabbit, rat, mouse, mink and gerbil. No data are available concerning the effects of PCBs (in particular, Aroclor 1254) on ovine reproduction and offspring growth rate and the ultrastructural modifications of blastocysts due to exposure to these toxic compounds. The aim of this study was the analysis of ultrastructural anomalies induced in ovine blastocysts by PCB (Aroclor 1254) at different concentrations.

Materials and methods

The ovine blastocysts were fixed in 2.5% glutaraldehyde, post-fixed in OsO4, dehydrated, rinsed in propylene oxide and embedded in epoxy resin. Semi-thin and ultrathin sections were stained with toluidine blue and uranyl acetate and lead citrate, respectively.

Results

All treated blastocysts were characterized by increased lipid droplets, severe cytoplasmic vaculation (single membrane-bound vacuoles and empty lacunae, containing whorled membrane, granular osmiophilic material or laminated membranes), marked mitochondrial swelling with loss of cristae and pyknosis, and a few autolysosomes containing mitochondrial remnants.

Conclusion

Embryos treated with PCBs show severe ultrastructural modifications not dependent on PCB concentration and correlated to the progressive increase of cell mortality rate indicated by *in vitro* studies. These results contribute to the knowledge of harmful effects of these compounds on reproduction and embryonal growth rate.

72. INDUCTION OF LIVER PRENEOPLASTIC FOCI IN F344 RATS SUBJECTED TO 28-DAY ORAL ADMINISTRATION OF DIHEPTYL PHTHALATE AND ITS *IN VIVO* GENOTOXIC POTENTIAL

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Introduction

Diheptyl phthalate (DHP) has been categorized as a PPARα agonist and induces hepatocellular swelling in rats. Accordingly, DHP, like other PPARα agonists, probably has a hepatocarcinogenic potential in rats, but it is unclear as to the genotoxicity and carcinogenicity of DHP. On the other hand, there are a lot of reports demonstrating that PPARα agonists induce DNA damage secondary to oxidative stress resulting from the generation of reactive oxygen species (ROS).

Materials and methods

Male F344 rats were subjected to repeated oral administration of DHP at dose levels of 0, 2.5 or 5 g/kg for 28 days, and were subjected to one or 14 times oral administration of 5 g/kg/day of DHP in an alkaline single-cell gel electrophoresis (comet) assay. Furthermore, we performed *in vivo* liver initiation assays by one, three times, or 14 times oral administration of DHP in rats.

Results

In the 28-day study, the mean area and number of glutathione S-transferase placental form (GST-P) positive foci increased in DHP-treated groups compared with the control group. In the comet assay, cell migration was induced in the liver of rats subjected to 14 times oral administration of DHP at 24 hr after administration. In the *in vivo* initiation assay, an increase in the number and area of GST-P positive foci was observed in DHP-treated groups subjected to 14 times oral administration of DHP.

Conclusion

The results of these studies suggest the possibility that DHP is a genotoxic carcinogen in the liver of rats.

GIANT MITOCHONDRIA IN PANCREATIC ACINAR CELLS OF ALLOXAN-INDUCED DIABETIC RATS

73.

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Introduction

The pathomorphological features of eosinophilic granules encountered in pancreatic acinar cells of alloxan-induced diabetic rats were investigated.

Materials and methods

Diabetic conditions were induced in a total of 5 male F344 rats by single intravenous dosing with 35 mg/kg of alloxan at 6 weeks of age. Rats were sacrificed after 25 weeks.

Results

Histologically, pancreatic acini were diffusely atrophied with loss of islets. Eosinophilic granules in varying size were observed in the basal area of acinar cells and were negative following PAS, Alcian blue, Oil red O and Sudan III stains. Ultrastructurally, giant granules corresponding to eosinophilic granules were surrounded by double membranes with irregular cristae. Small zymogen granules with low electron density accumulated near the lumen, and a large number of small lipid droplets were present at the basal area. Immunohistochemical analysis of prohibitin, a protein located in the mitochondrial inner membrane, was partially positive in the marginal area of some eosinophilic granules, but negative in the central areas. A positive reaction for succinate dehydrogenase, one of the mitochondrial enzymes, showed a similar localizing pattern to prohibitin by enzyme histochemistry.

Conclusion

These findings confirmed that the eosinophilic granules in the exocrine pancreas of alloxan-induced diabetic rats were giant mitochondria.

74. GLOMERULAR THROMBOTIC MICROANGIOPATHY AS AN INDICATOR OF LETHAL RICIN INTOXICATION IN DOGS

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Introduction

Ricinus communis (i.e. castor bean) is an ornamental plant containing ricin. Ricin is one of the most potent plant toxins and may cause hypotension, gastroenteritis, depression, and death. The residual plant material from the oil extraction, castor bean cake, is largely used as fertiliser. Thus, intoxication can occur indirectly through ingestion of this product.

Materials and methods

Two dogs suspected to have been poisoned with ricin were brought for necropsy. The dogs died within 24 and 48 h respectively following clinical signs of sudden, uncontrollable vomiting, haemorrhagic diarrhoea and terminal shock. Necropsies were performed and samples taken for toxicological examination. Exposure to ricin was confirmed by the identification of the biomarker ricinine by solid phase extraction and liquid chromatography tandem mass spectrometry. Samples of the heart, lymph nodes, spleen, intestines, liver, and kidneys were taken for histopathology.

Results

The toxicological examination confirmed the presence of the biomarker ricinine. Together with the standard histopathological lesions described in literature, we also found prominent renal changes (glomerular thrombotic microangiopathy) that, till now, have only been described in experimental rats and mice receiving a lethal dose of ricin.

Conclusion

This is the first report of lethal castor bean fertiliser poisoning in dogs in Belgium which has been associated with glomerular thrombotic microangiopathy.

DIMETHYLBENZ[A]ANTRACENE-INDUCED HEPATOTOXICITY AND EXPRESSION OF THE MITOCHONDRIAL 18 KDA TRANSLOCATOR PROTEIN IN RAT LIVER

75.

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Introduction

Metabolic activation and detoxification of DMBA *in vivo* occurs primarily in the liver. In three bioassay experiments, we studied whether 7,12 dimethylbenz[α]antracene (DMBA) triggered oxidative stress in the liver.

Materials and methods

Different DMBA regimes in three groups of rats were used: a single dose of 10 mg DMBA (DS 10); a single dose of 20 mg DMBA (DS 20), and four doses of 5 mg DMBA (DR 20) in weekly intervals. All animals were sacrificed 18 weeks after the first DMBA administration.

Results

Regarding the liver histology, hepatocytes were glossy in appearance with heterochromatic nuclei. Erythrocytes were also clumped. These changes were more pronounced in group DR 20. DMBA appeared to decrease translocator protein expression (TSPO) in the liver of all groups treated with DMBA.

Conclusions

Changes in TSPO expression correlated well with increased protein oxidation products in liver tissue and visible cell swelling in hepatocytes.

76.

ELECTRON MICROSCOPIC STUDY OF THE TRANSLOCATION PATHWAY OF INTRA-TRACHEALLY INSTILLED DIESEL EXHAUST PARTICLES IN MOUSE

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Introduction

Ultra fine particles (UFPs) have the potential to translocate from the lung into the blood circulation through the air-blood barrier (ABB). Diesel exhaust particles (DEPs) are the major components of suspended particle material. The purpose of this study was to demonstrate the precise translocation pathway of intra-tracheally instilled DEPs at the ABB.

Materials and methods

Mice were instilled intra-tracheally with PBS suspension containing 625 μg of DEPs (SRM2975). Lung and pulmonary lymph nodes taken at 0, 5 and 30 min after the instillation examined histologically, immunohistochemically (IL-6, TNF-α), and electron microscopically.

Results

Aggregated DEPs were seen in capillary lumina in the lung and pulmonary lymph node immediately after instillation. The number of macrophages containing DEPs in alveoli increased as time advanced, and some macrophages showed positivity with IL-6 and TNF-α antibodies. Electron microscopy demonstrated increased number of alveolae-like vesicles containing some particulate substances in the alveolar epithelial cells (AEC) type 1 and endothelial cells (ET). In addition, particles were observed to pass through the structure of the ABB (diffusion). Degeneration and loss of the alveolar wall components including AEC type 1 and ET were frequently observed.

Conclusion

DEPs may pass the ABB by endocytosis and diffusion.

DECREASED HEPATIC P-GLYCOPROTEIN EXPRESSION ASSOCIATED WITH *SENECIO* INTOXICATION IN HORSES

77.

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Introduction

The mode of action of pyrrolizidine alkaloids in liver pathology associated with Senecio is incompletely understood. Being involved in the active transmembrane transport of a variety of exogenous and endogenous molecules, P-glycoprotein (P-gp) has a role in the protection against xenobiotic injury. In this study we evaluated the difference in hepatic P-gp expression in horses with Senecio intoxication compared to normal hepatic immunoreactivity in the bile canalicular membranes of the hepatocytes.

Materials and methods

Liver biopsies of four horses with confirmed Senecio jacobaea intoxication and one horse without liver pathology were fixed in formalin and examined microscopically. They were submitted for P-gp immunostaining using the antibody C219.

Results

All four samples showed a decreased expression of P-gp in areas with typical lesions of chronic Senecio intoxication. Intrahepatic bile accumulation was associated with complete loss of P-gp expression in the surrounding bile canaliculae.

Conclusions

Senecio intoxication in horses is associated with cholestasis and decreased P-gp expression in hepatic bile canaliculae. It might be that biliary P-gp excretion, induced by alkaloids, is a modulating factor in canalicular membrane expression as described by Accatino *et al.,* 1996. As a consequence there is a loss of hepatocyte protection by P-gp. Decreased P-gp expression in bile canaliculae may contribute to the pathology and clinical signs of *Senecio* intoxication.

78. EFFECT OF DIFFERENT LEVELS OF CADMIUM ON THE HISTOPATHOLOGICAL CHANGES AND RATE OF LYMPHOID CELL APOPTOSIS IN THE BURSA OF FABRICIUS IN CHICKENS

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Introduction

Cadmium (Cd), a toxic heavy metal, occurs in nature in small amounts and is used increasingly in the metal and plastic industries. This study was designed to investigate the effects of Cd on the bursa of Fabricius of chickens.

Materials and methods

Eighty-four-day-old male broilers were divided randomly into four groups. Groups 1, 2, and 3 had rations containing 25, 50, and 100 ppm of Cd as CdCl2, respectively. A control group received no Cd. At days 14, 28, and 42, seven chicks from each group were randomly selected and sacrificed. The bursa of Fabricius was fixed in 10% buffered formalin and processed for histopathology and assessment of lymphoid cell apoptosis. Paraffin sections were stained with HE and the TUNEL (Terminal deoxynucleotidy transferase dUTP Nick End Labeling) method.

Results

Numbers of apoptotic lymphoid cells was significantly increased in groups 1, 2, and 3 ($P \leq 0.01$). Plicas and lymphoid follicles of chicks in groups 2 and 3 were smaller and more hypocellular than in controls, and some were oedematous. Compared to controls, in groups 2 and 3 on days 28 and 42, there was an increase in the number of intraepithelial cysts. Atrophy of bursal epithelium was observed in 42-day-old chickens of group 3.

Conclusion

High concentrations of Cd in the ration (50 and 100 ppm) have detrimental effects on the bursa of Fabricius of chickens under experimental conditions.

TOXICOPATHOLOGICAL EFFECTS OF ENDOSULFAN (THIODAN® EC 35) IN MALE JAPANESE QUAIL

79.

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Introduction

Experimental studies of endosulfan toxicity in avian species are scarce. This study describes the toxicopathological effects of endosulfan in male Japanese quails (*Coturnix japonica*).

Materials and methods

Four-week-old male Japanese quail divided into six groups (n = 20) were offered endosulfan contaminated feed at rate of 0, 5, 25, 50, 100, and 500 mg/kg, respectively, for 60 days. Clinical signs, body mass, and feed intake were recorded. Samples of blood and tissues were examined for haematological, serum chemistry, and pathomorphological parameters.

Results

Quail of 25 and 50 mg groups had mild depression while 100 and 500 mg groups showed nervous excitement. A dose related delay occurred in onset of puberty. Feed intake, body mass, haematological parameters, and serum total proteins in endosulfan fed quails were significantly decreased ($P < 0.05$). The livers of endosulfan fed birds exhibited fatty change of hepatocytes and necrotic foci. Endosulfan fed groups had a significant decrease in testis weight and volume and diameter of seminiferous tubules ($P < 0.05$). Seminiferous tubules of endosulfan fed quails exhibited degenerated spermatids and multinucleated cells.

Conclusion

The toxicopathological effects of endosulfan in male Japanese quails are comparable to those reported in mammalian species.

80.

AFLATOXICOSIS IN BROILER CHICKS: A RELATIONSHIP BETWEEN DIETARY AFLATOXIN, AGE, AND CLINICAL DISEASE

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Introduction
The aim of this study was to determine the effects of age and dietary aflatoxin levels upon different pathological responses in broiler chicks.

Materials and methods
Aflatoxicosis was induced in 7, 14, and 28-day-old broiler chicks by dietary administration of 1 600, 3 200, and 6 400 ng/g of aflatoxin B1 for seven days. Clinical, pathomorphological, and serum biochemical parameters were determined until day 21 when the experiments were terminated.

Results
Clinical signs and mortality (%) were most severe in 7-day-old birds, were lower in 14-day-old birds and were lowest in 28-day-old birds. The time taken to return to normal behaviour following aflatoxin withdrawal increased with dietary toxin levels but decreased with age. Scores for lesions in the liver and kidneys significantly ($P \leq 0.05$) increased with dietary toxins levels and were significantly ($P \leq 0.05$) lower in 28-day-old than in 7-day-old birds. Serum alanine transferase and alkaline phosphatase concentrations significantly ($P \leq 0.05$) increased with dietary increase of aflatoxin but this increase was less severe in older birds than in younger birds.

Conclusions
Comparison of scores of clinical signs, mortality, pathomorphological alterations, and concentrations of serum enzymes suggested an age related decrease and dose related increase in susceptibility of broiler chicks to aflatoxicosis.

POSTER PRESENTATIONS

Session 9 A – Infectious Diseases

81. PATHOGENESIS OF HIGH AND LOW PATHOGENIC AVIAN INFLUENZA VIRUSES IN PARTRIDGES

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Introduction

In the present study, an experimental infection with HPAI and LPAI viruses was carried out in partridges to study clinical signs, gross and microscopic lesions, viral distribution in tissues and viral shedding.

Materials and methods

Partridges (*Alectoris rufa*) were intranasally infected with H7N1 (HPAI) and H7N9 (LPAI) viruses. Daily, clinical signs were recorded and oropharyngeal and cloacal swabs and feather pulp samples were obtained from all birds. At 3, 5, 10 and 15 dpi, three birds from each infected group were euthanized. Gross lesions were evaluated and tissue samples were obtained for histopathological studies.

Results

Partridges infected with HPA1 first showed clinical signs (ruffled feathers, inactive birds) at 3 dpi and mortality started at 4 dpi. At 8 dpi, surviving birds showed severe nervous signs. Immunohistochemistry and ReTi PCR confirmed the presence of viral antigen in tissues and viral excretion, respectively. No clinical signs were observed in LPAI virus infected partridges, but viral shedding was detected in some birds.

Conclusion

The present study demonstrates that HPAI viruses cause severe disease and mortality in partridges.

MOLECULAR PATWAYS UNDERLYING BOVINE PAPILLOMAVIRUS INDUCED UROTHELIAL CARCINOGENESIS

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Introduction

Urinary bladder tumours in cattle are caused by chronic ingestion of bracken fern and infection with bovine papillomavirus type 2 (BPV-2). Mechanisms underlying urothelial cell transformation by the two viral oncogenes E5 and E7 are still largely unknown. Recently, it has been shown that the physical binding of E5 with the β subunit of the platelet-derived growth factor receptor (PDGFβ-r) is the first step of spontaneous urothelial carcinogenesis. The aim of the present study was to investigate the signal transduction pathways underlying PDGFβ-r activation in naturally occurring bovine urinary bladder tumours.

Materials and methods

Four urinary bladder tumour samples and two samples of normal urinary bladder mucosa (controls) were investigated by co-immunoprecipitation and western blotting to evaluate: 1) the expression of the phosphorylated PDGFβ-r (p- PDGFβ-r); 2) the physical binding of the PDGFβ-r with the p85 subunit of the phosphoinositol-3 kinase (PI3-K); 3) the PI3-K-Akt- cyclin D3 pathway; 4) phospho-JUN (p-JUN) and phospho-JNK (p-JNK) expression. The protein levels were quantified by densitometry and statistical analysis was performed.

Results

p-PDGFβ-r was overexpressed in the tumour samples. Additionally, the p-PDGFβ-r co-immunoprecipitated the PI3-K, thus activating the PI3-K-Akt-cyclin D3 pathway. The phosphorylation status of p-Akt, p-Jun and p-JNK was higher in cancerous tissue than in the controls.

Conclusion

Our study opens a new scenario for further understanding of the molecular mechanisms underlying bovine urinary bladder tumours and BPV induced carcinogenesis.

83. MYCOTIC FLORA IN UTERINE LAVAGE OF DONOR COWS AND IMPLICATIONS IN INFERTILITY DISORDERS

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Introduction

Endometrial cytology has been shown to be valuable in equine and canine reproductive disorders and fertility evaluation. The key for excellent fertility in dairy herds is a healthy uterine environment, optimal estrus detection efficiency and ideal timing for breeding. The objective of this study was to investigate the mycotic flora of the uterus in donor cows intended for embryo transfer procedures and the related uterine cytological findings.

Materials and methods

66 uterine flushings were performed at the time of embryo collection. All samples were cytologically examined using the Cytospin technique and stained with Diff Quick®. 21 samples were processed for mycological culturing on Sabouraud medium at 25° and 37°C.

Results

Fungal elements were detected in 9/66 (14%) cytological samples. 8/21 samples (38%) were positive for yeasts in mycological culturing; 3 for *Rhodotorula* spp. and 5 for *Cryptococcus* spp.

Conclusion

The detection of yeasts by both cytological examination and mycological culturing in uterine lavage from donor cows shows good correlation. It is hypothesized that the presence of fungi might be associated with prolonged antimicrobial therapy or endometrial disorders such as subclinical/chronic endometritis. Practitioners are encouraged to submit uterine lavages from donor cows for mycological examination to increase knowledge of uterine microflora and to determine the role of isolated mycetes.

PNEUMOMEDIASTINUM AND SUBCUTANEOUS EMPHYSEMA IN A CAT ASSOCIATED WITH NECROTIZING BRONCHOPNEUMONIA CAUSED BY FELINE HERPESVIRUS-1

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Introduction

Pneumomediastinum is a rare condition in which free gas is present in the mediastinum. It may result from sharp penetrating trauma to the neck, or from mediastinal infection with gas-producing bacteria, but is usually secondary to accidental or iatrogenic trauma to the lower airways, the marginal alveoli, or the esophagus. In humans several infectious agents have been identified as a cause of pneumomediastinum. In cats, little is known about infectious agents causing this condition.

Materials and methods

A 1-year-old neutered male Siamese cat was presented with severe subcutaneous emphysema and pneumomediastinum. The animal was euthanized and necropsied. Samples of lung and trachea were taken for histopathological and immunohistochemical examination.

Results

Histopathology revealed a necrotizing bronchopneumonia and tracheitis. Amphophilic intranuclear inclusions in sequestered bronchial and bronchiolar epithelial cells labelled positively for FHV-1.

Conclusion

In cats, as in human beings, pneumonia should be considered as a possible cause of pneumomediastinum.

85.

THE FIRST CASE OF CANINE *STRONGYLOIDES STERCORALIS* INFECTION IN FINLAND

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Introduction

Strongyloides stercoralis is a small intestinal threadworm of dogs, cats and primates. It is mostly encountered in tropical and subtropical countries.

Materials and methods

A 10-week-old Yorkshire terrier puppy was submitted for necropsy after three weeks of intermittent diarrhoea, vomiting and pain on defecation followed by sudden death. The puppy was born and raised in a Finnish kennel. Samples for histology were collected during necropsy and routinely processed and stained with haematoxylin and eosin. Faecal samples from the other dogs in the kennel were examined using the Baerman method and collected nematodes were examined by light microscopy and scanning electron microscopy.

Results

Numerous intramucosal nematodes and larvae with moderate lymphoplasmacytic infiltrates were located in the small intestine. The adult nematodes were 2–2.5 mm long and had a rhabditiform oesophagus, paired genital tract, platymyrian meromyrian musculature, an intestine composed of uninucleate cells and a hexagonal mouth with six papillae. The larvae had a prominent genital primordium.

Conclusion

This case shows that *Strongyloides stercoralis* can complete its life cycle and cause disease in dogs also in Northern Europe.

A NEW RAPID AND SENSIBLE IN SITU HYBRIDISATION PROTOCOL TO DETECT AFRICAN SWINE FEVER VIRUS

86.

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Introduction

African Swine Fever Virus (ASFV) is a large double-stranded DNA virus that causes haemorrhagic disease. At present no effective vaccine exists against ASFV and control of the disease is based on efficient diagnosis and slaughter of infected pigs. Electron microscopy (viral particles) and immunohistochemistry (specific antigens) have been utilised to study the distribution of ASFV in infected tissues. In situ hybridisation (ISH) protocols to determine viral genetic material in tissues and cells have been developed but are tedious and time-consuming. The objective of the present study was to develop an alternative rapid and simple ISH protocol to detect ASFV DNA.

Material and Methods

ISH was performed on samples of liver, lymph nodes, tonsil and spleen obtained from animals naturally and experimentally infected with different virulent strains. Two DIG-labeled ASFV DNA probes, a 18 kb DNA fragment and total viral DNA were tested in two assay conditions (PK and pepsin digestion).

Results

Highly specific ASFV DNA was detected in many cells (macrophages, Kupffer cells and endothelial cells) in all infected tissues using total genome as a probe with PK digestion. Irregular finely granular nuclear staining was observed in neutrophils in all infected tissues using the same ISH protocol.

87.

BOVINE HERPES MAMMILLITIS IN A SWISS DAIRY HERD

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Introduction

Bovine Herpes Mammillitis, a local disease of the bovine teat and udder caused by bovine Herpesvirus-2 (BHV-2), occurs worldwide. However, it is a sporadic disease and may be misdiagnosed as pseudocowpox, one of the most important differential diagnoses. An etiological diagnosis is rarely established. Herein, we describe a natural infection detected in Switzerland.

Materials and methods

Three animals from a small dairy herd were presented with a ten-day history of moderate to severe ulcerative dermatitis of the teat and udder.

Results

Histological examination revealed a multifocal necrotizing dermatitis with formation of numerous syncytial cells within the epidermis. Syncytial cells contained large numbers of intranuclear eosinophilic inclusion bodies. Electron microscopy revealed intranuclear viral particles averaging 100 nm in diameter with the typical morphology of Herpesvirus. Lesions healed within four weeks leaving depigmented focal scars. No additional cases of the disease occurred over the next 12 months.

DETERMINATION OF APOPTOSIS IN SHEEP NATURALLY INFECTED WITH PESTE DES PETITS RUMINANTS VIRUS

88.

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Introduction

Peste des petits ruminants (PPR) is an infectious disease specific for small ruminants. PPR virus (PPRV) is classified as the fourth member of the genus Morbillivirus of the family Paramyxoviridae. In recent years, an increasing number of viruses have been found to induce apoptosis in their host cells.

Materials and methods

Tissues from 14 sheep from 3 PPR outbreaks were examined. All 14 animals were positive for PPRV RNA by RT-PCR. Tissue samples were fixed in 10% neutral-buffered formalin and embedded in paraffin wax. Viral antigens and apoptosis were detected using the avidin-biotin-peroxidase technique and TUNEL methods, respectively. Dual-colour fluorescence was used to observe apoptotic nuclei and viral antigens in the same sections.

Results

Strong immunolabelling of viral antigens was seen in the lungs in alveolar macrophages, pneumocytes and syncytial cells. Intense diffuse or granular immunolabelling of viral antigen was seen in the spleen, tongue, buccal mucosa, lips and soft palate. TUNEL positive signals were seen in the same tissues. In dual-colour fluorescence microscopy, TUNEL positive cells had viral antigens in their cytoplasm.

Conclusion

Apoptosis of infected cells appears to play an important role in the pathogenesis of PPR in naturally infected sheep.

89.

PORCINE CIRCOVIRUS TYPE 2 INFECTION IN WILD BOARS IN GERMANY

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Introduction

Porcine circovirus type 2 (PCV2) is a widespread virus in domestic pigs. The aim of this study was to determine the prevalence of this virus in wild boars.

Materials and methods

Samples of 170 wild boars were collected from all over Germany during the hunting season 2006/2007. Tonsils, mesenteric lymph nodes, spleen and lung with pulmonary lymph nodes were investigated by microscopic examination (HE) and immunohistochemistry (monoclonal antibody against ORF2).

Results

Positive immunohistochemical signals were seen in three wild boars.

The histopathological examination yielded PCV2-specific changes in two of the three cases (animal no. 1 and no. 2). Animal no. 1 had depletion of lymphocytes and proliferation of histiocytes in the spleen. Similar findings were detected in the pulmonary lymph node of animal no. 2, with additional multinucleated giant cells (MGC). Furthermore, there was an infiltration of histiocytes, MGC and intracytoplasmic inclusion bodies in the tonsils.

Conclusion

Of the 170 wild boars examined, only three were immunohistochemically positive for PCV2-antigen and presented with post-weaning multisystemic wasting syndrome (PMWS).

ASSOCIATION BETWEEN PLACENTAL LESIONS AND PRESENCE OF *COXIELLA BURNETII* IN CATTLE

90.

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Introduction

Coxiella burnetii is a well-known cause of placentitis and abortion in ruminants. However, it may also be present in the pregnant uterus without causing abortion, even when excreted in large numbers at calving.

Materials and methods

Cotyledonal specimens of 186 bovine placentas were sampled at calving. ¼ of a cotyledon was frozen at -20°C and the adjacent ¼ cotyledon was fixed in formalin. The frozen specimens were analysed by RT-PCR. 123 formalin-fixed samples, representing RT-PCR results from 0 to 5.3×10^8 DNA copies per ml, were evaluated histologically and immunohistochemically.

Results

RT-PCR demonstrated Coxiella burnetii in numbers grouped as 0 (80 samples), 1–500 (68), 501–1000 (15) and >1000 (23) DNA copies per ml. Lesions were demonstrated infrequently and apparently without any association with RT-PCR results. Lesions consisted mainly of mild focal inflammation. Distended trophoblasts were not observed. Specific immunostaining was only observed in one case having a high RT-PCR value.

Conclusions

The study demonstrates the level of placental infection in cows at calving. It also shows that histology and immunohistochemistry are mostly insufficient to demonstrate infection at a normal calving.

91. PREVALENCE OF BVDV-PERSISTENT INFECTION IN ADULT CATTLE BRAIN

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Introduction

Infection with bovine viral diarrhea virus (BVDV), a pestivirus in the Flaviviridae family, is a major cause of loss of productivity and imposes an enormous health and economic burden on the cattle industry worldwide. This is the first report of the prevalence of BVDV-persistent infection (PI) in the brain of cattle.

Materials and methods

261 cattle were received at NVRQS between 2008 August and 2009 February for brain analysis. Brains were from cattle showing the Downer Cow Syndrome and all were more than 2 years old. We used standard laboratory procedures such as histology, PCR, and immunohistochemistry (IHC).

Results

BVDV-specific-antigen was found in 38 cases by PCR and 9 of these were also positive by IHC. BVDV antigen was detected in brain with or without histological lesions. The intensity and distribution of BVDV antigen in neurons was variable throughout the brain. The histopathological findings were perivascular cuffing, neuronophagia and non-suppurative encephalitis.

Conclusion

In the 261 cases, BVDV antigen was 14% positive by PCR and 3% positive by IHC. BVDV antigen can be detected in brain with or without histologic lesions. The BVDV antigen was only detected in neurons.

INVESTIGATION OF AN OUTBREAK OF BOVINE TUBERCULOSIS CAUSED BY *MYCOBACTERIUM CAPRAE* IN A BAVARIAN FLOCK

92.

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Introduction

In this study an outbreak of bovine tuberculosis, caused by Mycobacterium caprae, a member of the *Mycobacterium tuberculosis*, complex was investigated.

Material and methods

Thirty-eight intra-dermal tuberculin skin test-positive cattle were necropsied. Macroscopically evident lesions were examined histopathologically (HE, Ziehl-Neelsen stain), immunohistochemically (rabbit anti-*Mycobacterium tuberculosis* antibody) and bacteriologically (microscopic, cultural, PCR).

Results

Tuberculosis-like lesions (focal lesions with extensive caseous areas and mineralization) were found in at least one organ of 23/38 animals. Lesions involved the intestine (1/23), mesenteric lymph nodes (19/23), lungs (4/23), mediastinal lymph nodes (8/23), liver (3/23), portal lymph nodes (6/23) and the peritoneum (1/23). Bacteriologically, *M. caprae* was isolated by cultivation in all 20 animals tested. Histologically, granulomatous inflammation was found, which differed in its cellular composition and in the occurrence of caseous necrosis and mineralization. In 13 cases, acid-fast bacilli were detected. The number of them varied from extremely low to copious. They were mainly observed in caesous debris and less frequently within macrophages or Langhans multinucleated giant cells. Immunohistochemically, bacterial antigen could be detected as a small granular reaction product within the cytoplasm of macrophages surrounding the necrotic areas and as large granular foci in the periphery of the necrosis.

93. RAM EPIDIDYMITIS: CASE REPORTS

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Introduction

Epididymitis is an important cause of hypofertility in rams. Depending on the causative agent and sexual experience of the affected animals, two pathological entities have been reported: ram epididymitis (RE) and lamb epididymitis (LE).

Materials and methods

The study was performed on 150 rams. Gross examination of testis and epididymis was made for each case. Significative specimens were processed according to routine histological techniques. Blood and lesional tissue samples were often available for serological and bacteriological examinations.

Results

11 cases (7%) of epididymitis were found, unilateral in 91% and localized to the tail of epididymis in 27%. The testicular parenchyma generally showed different degrees of degeneration up to atrophy. Serology showed a positive reaction for *Brucella* spp in 4 rams. Among these, *B. melitensis* was isolated in 3, and *Histophilus somni* in 1. In another case, which was serologically negative for *Brucella* spp, *Streptococcus* spp was isolated.

Conclusion

The prevalence of ram epididymitis (7%) was in agreement with the literature. Spermatogenesis was impaired in most cases. However since the lesion is predominantly unilateral, epididymitis-affected rams may be fertile and/or sexually active and transmit venereal infections. The isolation of *B. melitensis* in 3 cases together with its serological prevalence in Sicily, suggests the absence of *B. ovis* in Southern Italy.

TESTICULAR INFLAMMATORY DISEASES IN THE BUCK 94.

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Introduction

Orchitis and epidydimitis have been poorly studied in the buck. Bacteria and protozoa have been reported as main causative agents.

Materials and methods

A retrospective study was performed on caprine testes registered in the archive of the Department. Testes were grossly and microscopically evaluated. Serological and bacteriological investigations were carried out where possible.

Results

13 orchitis and 25 epidydimitis cases (with 8 exhibiting both pathologies), were found in a survey of 165 bucks. Six cases of necrotizing orchitis involving the whole parenchyma were also recognized. In another case, an intratubular orchitis was observed. Interstitial orchitis was diagnosed in six cases. In seven cases, sperm granulomas were detected mainly in the epidydimal head. In another 18 cases, infectious epididymitis including sclerosing, necrotizing and suppurative forms were diagnosed. In five cases of necrotizing orchitis/epidydimitis, a *Brucella melitensis* infection was suggested by serology, bacteriology and type of lesions. In two cases of interstitial orchitis and sclerosing epidydimitis, a *Chlamydophila* sp. infection was suspected by serological titres before and after castration. In another case, *Staphylococcus* sp. was isolated.

Conclusion

Bucks may occasionally develop both orchitis and epidydimitis. A genetic predisposition to sperm granuloma at the epidydimal head is confirmed. *Brucella melitensis* and *Chlamydophila* sp. were important causative agents of these lesions.

95.

GENERALIZED SARCOIDOSIS IN A TURKMAN HORSE

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Introduction

Equine sarcoidosis is a rare disorder of unknown etiology characterized by exfoliative dermatitis and sarcoidal granulomatous inflammation of multiple organ systems. This report describes generalized cutaneous and systemic sarcoidosis in a Turkman mare.

Materials and methods

A 9-year-old Turkman mare was referred to the Veterinary Clinic of Islamic Azad University of Karaj Campus, with a two-month history of subcutaneous nodules on the neck, abdomen, back and around the tail. The horse was lethargic and showed exercise intolerance. Bacteriological examination of skin scrapings and mycological culture of hair samples were negative. The animal failed to respond to treatment and was euthanised. Necropsy and histopathological examinations were performed.

Results

Small firm subcutaneous nodules and plaques were found in all regions of the skin. Similar firm multifocal nodular lesions were observed in the lungs and liver. Histopathological studies revealed extensive lymphohistiocytic infiltrates within a fibrotic matrix admixed with large multinucleated giant cells of the Langhans-type and extensive epidermal hyperkeratosis. Numerous histiocytes and lymphocytes were admixed with plasma cells and neutrophils. These findings were consistent with a diagnosis of generalized sarcoidosis.

Discussion

Equine sarcoidosis, also known as "equine idiopathic systemic granulomatous disease", is postulated to be the result of an exaggerated immune response to an exogenous infectious agent or allergen. The disease is characterized by exfoliative dermatitis, severe wasting, and granulomatous inflammation of one or more internal organs.

DESIGN AND SUCCESSFUL APPLICATION OF AN OLIGONUCLEOTIDE PROBE FOR DETECTION OF PARASITES OF THE ORDER TRICHOMONADIDA BY CHROMOGENIC *IN SITU* HYBRIDIZATION

96.

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Introduction

Infections with protozoal parasites of the order Trichomonadida are frequently observed in veterinary medicine. Detection of these parasites by *in situ* hybridisation has the advantage of localising the parasite directly in the tissue. Design of one single probe targeting virtually all representatives of the order Trichomonadida was attempted.

Materials and methods

The probe consisted of a digoxigenin-labelled olignonucleotide, targeting 18S ribosomal RNA. Detection was carried out using an anti-digoxigenin antibody on paraffin-embedded tissue.

Results

Successful detection of parasites of the order Trichomonadida could be shown using the designed probe. These were *Monocercomonas colubrorum*, *Histomonas meleagridis*, *Hypertrichomonas acosta*, *Trichomonas gallinae*, *Tetratrichomonas gallinarum*, *Tritrichomonas foetus*, *Tritrichomonas augusta*, *Pentatrichomonas hominis* and *Trichomitus batrachorum*.

Discussion

The probe provides an important tool for diagnosis of all relevant protozoal parasites of the order Trichomonadida. Furthermore it paves the way for further development of species-specific probes.

97. KI 67 EXPRESSION IN THE CEREBELLUM OF DOGS WITH DISTEMPER

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Introduction

Canine distemper virus (CDV) may induce multifocal demyelination in the central nervous system of dogs. The purpose of the present study was to investigate the the presence of Ki 67 immunopositive cells in demyelinated areas, non-demyelinated areas and perivascular areas of the cerebellum in naturally infected dogs with CDV.

Materials and methods

The cerebellum was collected from all dogs for histopathological and immunohistochemical purposes and embedded in paraffin. Sections were immunostained for CDV and Ki 67. The presence of CDV infection was confirmed on the basis of histopathology and immunohistochemistry. Based on histopathological evaluation, CDV infection was classified as acute or chronic. Acute and chronic cases were compared to control dogs for the number of Ki 67 immunoreactive cells.

Results

Ki 67 was expressed in glia, gitter cells, gemistocytes, endothelial cells and perivascular lymphocytes. The number of Ki 67 immunoreactive glial cells in demyelinated areas in chronic cases was significantly higher compared to acute cases and control dogs ($p < 0.05$). Ki 67 immunoreactivity was significantly higher in chronic cases compared to that of acute cases and controls in non-demyelinated areas ($p < 0.05$).

Conclusion

The results suggest that Ki 67 expression was associated with invasion and persistence of CDV by means of increased glial cell proliferation.

PCV2 AND PRRSV CELL CO-INFECTION AND REPLICATION IN PROLIFERATIVE AND NECROTISING PNEUMONIA

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Introduction

Infection with Porcine circovirus type 2 (PCV2) and Porcine reproductive and respiratory syndrome virus (PRRSV) is reported as the cause of proliferative and necrotizing pneumonia (PNP). The aim of this study was to investigate cell co-infection or replication of both viruses.

Material and methods

Double staining was performed in lung from 23 natural cases co-infected with PRRSV and PCV2 with typical PNP histopathological findings. *In situ* hybridisation with two different oligonucleotide probes for detection of PCV2 was performed. A complementary probe detected viral ssDNA and a replicative form probe detected dsDNA, cells which support replication. After colour development, without counterstaining, slides were immersed in 0.1M Tris-buffered saline. Then, IHC for PRRSV detection with anti-PRRSV monoclonal antibody, labelled polymer-HRP test and DAB chromogen was performed.

Results and conclusion

Strong co-infection was detected in most macrophage-like cells in alveolar lumina, necrotic debris and a few bronchial epithelial cells. In some of the macrophage-like cells, PRRSV infected, PCV2 replication was detected. Since, PCV2 replicates in cells which are "target" cells for PRRSV replication and already infected, this suggests the replication of both viruses in the same cell.

99. IMMUNOPATHOLOGICAL STUDY OF CNS LESIONS IN NATURALLY OCCURRING CASES OF OVINE VISNA

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Introduction

The objective of this work was to define the pathology of ovine Visna and its relationship to the local immune response.

Materials and methods

A systematic histopathological study of the central nervous system (CNS) of twenty sheep (15 of them with neurological signs), was performed. Immunohistochemistry was carried out using antibodies raised against CD3, CD4, CD8, CD79, and γδ.

Results

All animals had different CNS lesions which were characteristic for Visna Type 1. These were moderate to severe lymphocytic infiltration of the choroid plexus and meninges, with (1b) or without (1a) parenchymal injury. In the Type 2 variant, there were severe lesions in the parenchyma with large numbers of lymphocytes and areas of malacia (2a), or predominantly macrophages. Type 1a and 2a lesions had a predominance of CD4+ lymphocytes, mainly forming perivascular cuffs. Conversely, macrophages, CD8+ and B lymphocytes predominated in type 1b and 2b lesions.

Conclusion

These features could be related to the individual immunological response and resistance to disease. The proposed classification could be useful in future studies of ovine Visna.

POSTER PRESENTATIONS

Session 10 B – Fish Patholgy and Others

100. RECIPROCAL RELATIONSHIP OF BLOOD PROFILES AS AN IMMUNOLOGICAL BIOINDICATOR IN GREAT STURGEON (*HUSO HUSO*)

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Introduction

Recognition of blood profiles and awareness of ongoing related reciprocal relationships is important in aquaculture, especially in diagnosis of disease and abnormal condition. The aim of this study was to measure blood profiles in 4 to 5-year-old Great Sturgeon cultured in brackish water pools in BAFGH-Iran.

Material and methods

Blood samples were collected from the caudal vein every three months and plasma was frozen for later analysis. Hormone levels were measured by radio immunoassay (RIA), glucose with an autoanalyser, calcium and magnesium with a spectrophotometer and sodium and potassium with a film photometer.

Results and conclusion

Significant correlations were detected between the following biochemical parameters: glucose/calcium; sodium/potassium; sodium/magnesium; potassium/calcium and potassium/magnesium. Of the hormonal profiles, testosterone/estradiol had significant direct correlation. Significant correlations also occurred between cortisol and glucose, and between sodium and potassium. Levels of cortisol showed no correlation with those of other hormones. Blood profiles in Great Sturgeon are similar to those reported in other sturgeons.

THE EFFECT OF OXYTETRACYCLINE AND LYSOZYME DIMER ON THE MORPHOLOGY OF GILLS IN SIBERIAN STURGEON

101.

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Introduction

The influence of oxytetracycline (OTC) therapy with immunomodulation on the morphology of gills in Siberian sturgeon has not been previously reported.

Materials and methods

This study was undertaken on 180 Siberian sturgeons (2400 ± 100 g b.m.) divided into 6 groups (1–3, K1–K3; n = 30). Fish from the groups 1–3 were injected i.p. with 50, 100, 150 mg/kg b.m. of OTC. The fish were then bathed in water with addition of lysozyme dimer (KLP-602) (100 μg/l) for 30 min. Fish in group K2 were given only OTC (100 mg/kg b.m.), those in group K3 were only bathed in KLP-602 and those in group K1 received no treatment. Six fish from each group were slaughtered after the bath in KLP-602 and 6 more after 3, 7, 14 and 21 days. Gill samples were collected and sections were stained with HE.

Results

Fish inoculated i.p. with OTC at 100 mg/kg b.m. and 150 mg/kg b.m. showed accelerated exfoliation of respiratory epithelium and degenerative changes in the gills. Lesions occurred more frequently in fish from group K2 than in fish from group 2.

Conclusion

Bathing Siberian sturgeon in an aqueous solution of KLP-602 (100 μg/l) for 30 min. has a protective effect against OTC induced morphological changes in the gills.

102. CHARACTERIZATION AND COMPARISON OF SERUM PROTEINS IN VARIOUS GROWTH STAGES OF *CTENOPHARYNGODON IDELLA* IN IRAN FISH FARMING

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Introduction
Determination of serum proteins is important in investigations of physiological conditions and disease in fish.

Materials and methods
In this project, proteins and immunoglobulins of *Ctenopharyngodon idella* were evaluated under different weather conditions in Iran. Serum protein levels were evaluated using electrophoretic methods and an autoanalyser. Blood samples were collected from the caudal veins of *Ctenopharyngodon idella*, aged between 8–12 months, from five different aquaculture farms in Northern Iran.

Results
Plasma protein levels in fish normally vary between 30–50 mg/ml and immunoglobulins between 2–7 mg/ml. The levels of total protein, albumin, a1, a2 and immunoglobulin determined in serum from *Ctenopharyngodon idella* were 19, 5, 6.5, 4 and 3.46 mg/ml, respectively.

Conclusion
Quantities of serum proteins and immunuglobulins in *Ctenopharyngodon idella* are dependent on seasonal temperature and the feeding regime.

SEROLOGICAL COMPARISON OF *ONCHORHYNCHUS MYKISS* IN VARIOUS GROWTH STAGES IN IRAN FISH FARMING

103.

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Introduction

The association between increased severity of disease and immunodeficiency in fish is well understood.

Materials and methods

In this project, proteins and immunoglobulins of *Onchorhynchus mykiss* were evaluated under conditions prevailing in Iran. Serum protein levels were calculated using an autoanalyser and by electrophoresis. Blood samples were collected from the caudal veins of *Onchorhynchus mykiss* aged between 7–16 months from two aquaculture farms. Polyvalent antiserum against fish serum was produced and evaluated using immunoelectrophoretic and immunoglobulin precipitation methods.

Results

Plasma protein levels in fish normally vary between 30–50 mg/l and immunoglobulins between 2–7 mg/l. The levels of total protein, albumin, a1, a2 and immunoglobulin determined in serum from Onchorhynchus mykiss were 38.5, 12, 17.3, 4.4 and 4.91 mg/l, respectively.

Conclusion

Quantities of serum proteins and immunoglobulins in *Onchorhynchus mykiss* are dependent on seasonal temperature and the feeding regime.

104. THE EFFECT OF PROBIOTIC (*BACILLUS* SPP) ON GROWTH, SURVIVAL, AND INNATE IMMUNITY OF RAINBOW TROUT (*ONCHORHYNCHUS MYKISS*) FRY DURING THE FIRST TWO MONTHS OF FEEDING

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Introduction

A commercial *Bacillus* spp. probiotic was tested on rainbow trout fry during the first two months of feeding.

Materials and methods

Probiotic was introduced into diets at five different levels, (T1: 4.8×10^8, T2: 1.2×10^9, T3: 2.01×10^9, T4: 3.8×10^9, T5: 6.1×10^9 CFU g-1). The effects were compared with those of a control diet containing no probiotic.

Results

Survival in treatment groups was significantly ($P < 0.05$) higher than in controls despite slightly increased mortality during the first week. Bacterial counts in the intestine in all treatment groups were significantly ($P < 0.05$) higher than in controls and *Bacillus* spp. was not detected in controls. Specific growth rate, condition factor, protein efficiency ratio were significantly ($P < 0.05$) higher and feed conversion ratio was lower in groups receiving probiotic than controls. Growth performance in the treatment group receiving 3.8×10^9 CFU g-1 showed the best results.

Conclusion

Supplementation of diets with probiotic appears to stimulate digestive development and enzymatic activity in fish. Dosage of probiotic should be assessed before application on a large scale to avoid any undesired effects. The supplementation of trout starter diets with *Bacillus* spp. is probably beneficial for rearing conditions.

HISTOLOGICAL EVIDENCE FOR EPITHELIOTROPIC LYMPHOPROLIFERATIVE DISEASE IN A ZEBRAFISH

105.

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Introduction

A lymphoproliferative disorder with epidermal invasion was found in a single zebrafish (*Danio rerio*).

Materials and methods

84 adults were exposed to the flame-retardant tetrabromobisphenol-A (0–3.2 μM), euthanized, fixed in Bouin's and routinely processed.

Results

One female (0.75 μM) had an enlarged thymus extending along the gill lamellae. Groups of uniform round cells with a limited amount of distinctly bordered, basophilic cytoplasm, and central round, occasionally indented, equally sized nuclei with dense chromatin and infrequently prominent nucleoli invaded the branchial epithelium, adjacent musculature and adipose tissue. Mitotic figures were rare. Multiple aggregates of lymphocytic cells invaded the dorsal cranial epidermis, with occasional emperipolesis in club cells.

Discussion

Lymphosarcoma may occur in various fish. A contagious origin with thymus involvement has been indicated in medaka and coho salmon. Presently, no other fish, including tankmates and 144 offspring were affected. TBBPA is not a known carcinogen. Although reported fish lymphomas typically show more pleiomorphism, invasion of various tissues in the absence of primary tissue damage indicates a neoplastic nature. Epitheliotropism consistent with well-differentiated neoplastic T-cells has not been reported in zebrafish.

Conclusions

Our observations indicate the spontaneous occurrence of epitheliotropic lymphosarcoma in zebrafish at a low rate. Further characterisation of incidental zebrafish lymphoproliferative diseases will benefit from development of immunological markers for lymphocyte subsets.

106.

IMMUNO- AND ENZYMOHISTOCHEMISTRY IN ORGANS OF COPPER-EXPOSED SEA BREAM (*SPARUS AURATA*)

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Introduction

Catalase (E.C. 1.11.1.6) is an essential enzyme found in the peroxisome of nearly all aerobic cells, capable of the cleavage of hydrogen peroxide to oxygen and water without the production of free radicals.

Materials and methods

In the present study on copper (Cu) metabolism, sea bream were exposed to 0.5 ppm Cu for 2 (T1), 4 (T2) and 7 (T3) weeks. Samples of liver, posterior kidney, gills and heart were immunohistochemically stained with anti-catalase antibody, whereas catalase enzymohistochemistry was performed on frozen specimens of gills and liver.

Results

Catalase immunohistochemistry on fixed samples showed different amounts of cytoplasmic positivity in the different organs with no difference between exposed subjects and controls. Enzymohistochemistry on frozen livers and gills highlighted enzyme activity at the base of the secondary lamellae of gills and in scattered, perivascular and peripancreatic histiocytes in the liver. Quantitative analysis of enzymatic activity in the gills showed a significant decrease in treated fish compared to controls, at T1 and T2 sampling times (ANOVA, $P < 0.05$). Enzymatic activity at T3 reached the same levels as in controls (ANOVA, $P > 0.05$).

Conclusion

In gills, Cu initially reduces catalase activity and this is followed by recovery to control levels with increased time of Cu exposure.

EFFECT OF CYPRINID HERPESVIRUS-3 (CYHV-3) ON INNATE IMMUNITY IN CARP, TENCH AND SHEATFISH

107.

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Introduction

CyHV-3 is responsible for a highly contagious viral disease, which causes significant mortality in common carp, koi carp, and ornamental fish. The genome of CyHV-3 is larger than those of the other members of the Herpesviridae. The virus attacks epithelial cells, especially in skin and gills. This results in excessive mucus shedding, dry skin, and necrosis of gill cells. Additionally, interstitial nephritis is observed. This study reports the influence of CyHV-3 on spleen phagocyte and head-kidney lymphocyte activities, and on the ceruloplasmin and lysozyme activities in serum. In addition, IL-1-like and IL-6-like protein production was analysed.

Material and methods

The CyHV-3 isolate from carp fingerlings at concentrations 1×10^6 pfu/mL of medium was used and quantified by plaque assay using koi fin cell (KFC) culture incubated at 24°C for 96 h. Fifty carp, tench and sheatfish were intra-peritoneally infected with CyHV-3.

Results and conclusion

Results showed that CyHV-3 significantly decreased the spleen phagocyte and head-kidney lymphocyte activities in carp compared to the control and tench or sheatfish ($P<0.05$). In sheatfish and tench, we did not observe the immunosuppressive effect of CyHV-3. In addition, CyHV-3 significantly reduced lysozyme activity and IL-1 and IL-6-like protein production in carp, compared with tench and sheatfish.

108.

INFLUENCE OF ANTIBIOTICS ON IMMUNOCOMPETENCE AND VACCINE EFFECTIVENESS IN RAINBOW TROUT

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Introduction

Protection against diseases by vaccination has been an important concept for many years. The present study analysed the influence of the antibiotics oxytetracycline and ciprofloxacin on spleen macrophage and lymphocyte activity and specific immune responses represented by the level of antibody-secreting cells (ASC) and specific Ig levels in rainbow trout (*Oncorhynchus mykiss*) after immunisation against Yersiniosis (Yersivax).

Material and methods

Healthy rainbow trout weighing 50–70 g were used. The fish were given antibiotics *per os* in pellets at therapeutical doses once a day for five days. The blood, spleen, and pronephros were collected after application of antibiotics (before vaccination) and 7, 14, 21, 28, and 35 days after vaccination. The ELISPOT assays were used for quantification of ASC, and the ELISA test for levels of specific antibody.

Results and conclusion

The results of this study showed that oxytetracycline and ciprofloxacin significantly decreased macrophage and lymphocyte activity ($P < 0.05$), but a higher suppressive influence with oxytetracycline was observed. Oxytetracycline and ciprofloxacin also decreased the ASC and specific Ig levels, and had a negative effect on vaccination effectiveness.

PATHOGENESIS OF IRIDOVIRUS: INFLUENCE ON MACROPHAGE AND LYMPHOCYTE ACTIVITY AND CYTOKINE-LIKE PROTEIN PRODUCTION IN FISH – A COMPARATIVE STUDY

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Introduction

Iridoviruses infect different insect and vertebrate hosts. The major structural component of non-enveloped icosahedral virus particles is the major capsid protein, which appears to be highly conserved among members of the family Iridoviridae, Phycodnaviridae, and African swine fever virus. Vertebrate iridoviruses are found in fish, amphibians, and reptiles. An experimental study in fish showed that the most severe lesions were found in the spleen, vascular system, and pronephros, with necrosis of renal haematopoietic tissue. Phagosomes of macrophages in the interstitial tissue contained virus particles.

Material and methods

Macrophage activity was analysed by respiratory burst activity (RBA) and potential killing activity (PKA) in rainbow trout and sheatfish. Lymphocyte activity was analysed by proliferative response to the mitogens, ConA and LPS, in a spectrophotometric assay.

Results and conclusion

This study reports the *in vitro* influence of iridovirus on macrophage and lymphocyte activity, and IL-1- and IL-6-like protein production in rainbow trout and sheatfish. The comparative study demonstrated a strong inhibitory influence of iridovirus on pronephric macrophage activity and the proliferative response of lymphocytes in rainbow trout and sheatfish. A strong inhibitory influence of iridovirus on IL-1 and IL-6-like protein production by pronephros leukocytes, and an immune suppressive effect, depending on fish species, was also observed.

110. MOLECULAR PROFILE OF *AEROMONAS* AND *PSEUDOMONAS* ISOLATED FROM SKIN MUCUS AND DIGESTIVE TRACT OF TROUT (*ONCORHYNCHUS MYKISS*) IN AQUACULTURE

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Introduction

Aeromonas sp. and *Pseudomonas* sp. strains are common microorganisms in intensive fish farming. They are pathogenic for fish and human beings with compromised immune systems and cause food spoilage during processing.

Materials and methods

Skin mucus and digestive tract contents from five trout was serially diluted and cultured on selective media. After incubation, the bacterial strains were biochemically tested and nucleic acids were isolated. Affiliation to *Pseudomonas fluorescens*, *Ps. Aeruginosa,* and *Aeromonas hydrophila* species was confirmed by the presence of 16S rDNA-PCR marker. Selected molecular analysis for *Aeromonas hydrophila* was directed for the presence of aerolysine (*aerA*), haemolysine (*hlyA*), and aerolytic activity complex marker (AHCYTOGEN). Finally, multiplex-PCR for cross-selective primers were applied and compared with molecular identification of bacteria, without culturing (*in situ*).

Results

Pseudomonas sp. strains were isolated in all specimens, especially *Ps. fluorescence* and *Ps. aeruginosa* in two samples. *Aeromonas hydrophila* samples showed the presence of *hlyA* with no marker for aerolysine/ aerolytic complex. Classical and *in situ* approaches yielded convergent results.

Conclusions

Simple and fast multiplex-PCR for the detection of *Aeromonas* and *Pseudomonas* were utilised for bacteriological examination of skin mucus and digestive tract contents in trout. This molecular approach is useful for assessing the health status of fish and their suitability for food processing.

AEROMONAS HYDROPHILA IN FISH AQUACULTURE

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Introduction

Aeromonas hydrophila exists in all inland water environments, benthic sediments and water organisms and dominates in freshwater fish microflora, especially in skin mucus and within the digestive tract. Aeromonas represents a frequent menace to fish health particularly in high-density aquaculture where intensive feeding is applied. Motile Aeromonas include three species: *A. hydrophila*, *A. caviae*, and *A. sobria*. They are usually saprophytic, but are potentially pathogenic for fish. Pathogenic aeromonads are aetiological factors in several fish diseases including erythrodermatitis and motile aeromonad septicaemia (MAS). Spread of infection occurs from direct contact with sick fish or environmental contamination and represents a health hazard during food processing.

Materials and methods

The quantity and quality of bacteria in water and fish was evaluated in various fish aquaculture systems.

Results

Aeromonas hydrophila was isolated from both environments. *Aeromonas hydrophila* was isolated from water (11.3%), skin mucus (22.1%), digestive tract (27.5%) in a catfish (*Silurus glanis* L) pond aquaculture system and from water (24.1%), skin mucus (12.1%), and digestive tract (2.2%) in a Siberian sturgeon (*Acipenser baerii*, Brant) aquaculture system with closed water circulation tanks.

Conclusion

The number of *Aeromomonas hydrophila* strains depends on aquaculture system type, fish species that analysed and type of sample that took for analysis (water, skin mucous or fish digestive tract).

112. MICROBIAL QUALITY OF TENCH AND WHITEFISH FINGERLINGS CULTURED IN A POND PERIODICALLY FED TREATED WASTEWATER

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Introduction

Excreta, crude sewage after sedimentation, treated wastewater, or contaminated river water are used in fish ponds. The bacterial flora of fish reflects the bacteriological quality of the water body inhabited by fish.

Materials and methods

A pond with a surface area of 0.94 ha and a maximum depth of 1.6 m, situated on the premises of a wastewater treatment plant in Olsztynek (Poland), was filled with water from underground springs. Twice during the production season (from April to October 2008), the pond was fed with sewage, which was biologically treated in sequencing batch reactors. Feed was not administered, and natural pond feed was the only source of nutrition for the fish. Medicinal products were not administered. Heterotrophic plate counts at 22°C and 37°C of coliforms; faecal coliforms, *Streptococcus faecalis*, *Salmonella* sp., and *Aeromonas hydrophila* were determined in samples of digestive tract content, mucus from the skin, and muscles.

Results

Tench tissues were more contaminated than common whitefish tissues, but the microbial quality of both species was satisfactory.

Conclusion

The results of this study indicate that the microbial quality of fingerlings can be improved by periodical fertilisation of fish ponds with treated sewage effluent.

ELEVATION OF NON-SPECIFIC RESISTANCE OF CARP

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Introduction

The influence of Izatyzon (2% solution of metysazone), Tryman (derivative of pyridine), and Dyvostym (produced from natural compounds) preparations on non-specific resistance of carp (*Cyprinus carpio* L.) was investigated.

Material and methods

All preparations were used in separate ponds with an independent water supply and respective controls. Izatyzon (0.5 ml/kg of fish) was administered in the fodder at daily intervals for a month (group 1) and Tryman (2.5 mg/kg of fish) was similarly administered at daily intervals for 14 days (group 2). The stimulant complex Dyvostym was added directly into the water to a final concentration of 0.05 g/L (group 3). Control fish (group 4) received mixed fodder without any added preparations.

Results

The average mass of fish treated with the used preparations was higher than that of control fish: group 1 – 13%, group 2 – 18.2%, and group 3 – 11.4%. Fish treated with the preparations showed elevated levels of hemoglobin and total blood protein. The functional activity of T- and B-lymphocytes, phagocytic activity of neutrophils, and bacteriostatic activity of blood serum were improved.

Conclusion

The use of Izatyzon leads to improvement of the immune status of carp.

114. PATHOMORPHOLOGICAL PATTERN OF THE LIVER OF THE BLACK-STRIPED FIELD MOUSE (*APODEMUS AGRARIUS*, PALLAS 1771) AS A TOOL IN THE MONITORING OF ENVIRONMENTAL HEALTH

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Introduction

Increased DDT levels in cormorants and grey herons grazing close to the pesticide tomb (PT) indicated environmental pollution.

Materials and methods

Macroscopic, microscopic, and ultrastructural studies were conducted on 240 black-striped field mice, caught during 2002–2004 (the final period of existence of the PT) and during 2006–2008 (after its liquidation). The mice were from a unique ecosystem in northeastern Poland, which for 30 years had been under the influence of a poorly secured PT.

Results

Compared with mice from the control area, livers of black-striped mice living close to the PT showed parenchymatous degeneration, necrosis, and changes in mitochondria and rough endoplasmic reticulum. Damage caused during detoxification of environmental pollutants apparently accounted for the changes in the livers.

Conclusions

Striped field mouse represent a good biomarker for the assessment of environmental pollution. Four years after the pesticide tomb was liquidated, its effect on the natural environment persists.

MODIFICATIONS OF VISCERAL LYMPHOID TISSUE INDUCED IN CHICKENS BY AN ORALLY DELIVERED LECTIN

115.

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Introduction

Lectins are carbohydrate-binding proteins and are described in almost all living organisms. They are involved in many biological processes, including immunomodulation. Some plant lectins are also known as antinutrients due to their pathological effects on the mucosa of the gastrointestinal tract. The aim of the present study was to evaluate the morphological changes in chickens following oral administration of potato shoots lectin.

Materials and methods

A potato shoots lectin preparation was obtained by affinity chromatography and administered in the drinking water to 3-day-old chickens for 7 days. The chickens were then slaughtered and submitted for necropsy examination. Samples of intestine, kidney, spleen, liver and myocardium were processed for histopathological evaluation using trichromic Masson staining.

Results

No gross lesions were detected. Increased reactivity of gut associated lymphoid tissue and of the visceral lymphoid tissue of the liver was noticed in all treated chickens. The presence of visceral lymphoid nodules in the myocardium was an unusual finding.

Conclusion

Administration of potato shoots lectin to chickens stimulates the formation of visceral lymphoid nodules, especially in the liver and myocardium.

116. EVALUATION OF THE TISSUE REACTIONS AND CYTOKINE PATTERNS INDUCED BY *ALTERNARIA ALTERNATA* IN MICE

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Introduction

Alternaria is a saprophytic fungus that is widespread in the environment. In this study, we found that BALB/c mice exposed to extract and spores of *Alternaria alternata* show rapid and highly elevated production of Th2 cytokines such as IL-4 and IL-13. Allergen exposure was also associated with pulmonary histopathological changes.

Materials and methods

Alternaria alternata were cultured and mold extract was prepared by freeze-defreeze method and sonication. One group of mice was sensitized by two intraperitoneal injections of *A. alternata* extract and then intranasally challenged with spores suspended in sterile normal saline solution. Another group of mice received only spores intranasally. Blood sampling and necropsies were performed at 1 and 72 h after spore inhalation. The sera were analyzed by ELISA to determine levels of IL-4 and IL-13 in the immediate response and late-phase reactions, respectively.

Results

Histopathological examination of the lungs showed inflammatory cell infiltrates including lymphocytes, macrophages, neutrophils and eosinophils. Airways in sensitized and nonsensitised mice showed mucus hypersecretion and epithelial cell hyperplasia and necrosis at 72 h. Challenged mice showed increased serum levels of IL-4 and IL-13. Inflammatory reactions and levels of Th2 cytokines were higher in sensitized mice than in the nonsensitized group.

Conclusion

Th2 cytokines play an important role in the response to allergen challenge, in addition to regulating local tissue modification.

THE INFLUENCE OF PUFA ON NUMBERS OF CUTANEOUS MAST CELLS AND EOSINOPHILS IN DOGS WITH ATOPIC DERMATITIS

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Introduction

Canine atopic dermatitis (AD) constitutes an important skin disease commonly seen in veterinary practice. Treatment is multifaceted and often involves polyunsaturated fatty acid (PUFA) supplementation. The aim of this double-blinded, randomized, placebo-controlled study was to assess the influence of PUFA on the inflammatory cell infiltration in the skin.

Materials and methods

28 dogs diagnosed with AD were assigned to one of three groups and supplemented for ten weeks with either one of two fatty acid products or a placebo. Skin biopsies were taken prior to and at the end of the trial. Clinical signs and pruritus were evaluated. The number of mast cells (MCs) and eosinophils (ELs) per mm^2 was quantified.

Results

There was no significant difference between cell counts before and after the study, nor when comparing groups amongst themselves. Positive correlations were observed between cell counts and pruritus and between cell counts and clinical signs.

Conclusions

The long survival time of inactive inflammatory cells and the relatively short study period may account for these findings. Since only MCs and ELs were evaluated, a decline in other cell populations involved in AD cannot be discounted. The observed positive correlations provide further evidence that PUFA influence mediators of effector cells.

118.

EHLERS-DANLOS SYNDROME IN A MALE YORKSHIRE TERRIER

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Introduction

Ehlers-Danlos syndrome (EDS, cutaneous asthenia) is a rare congenital connective tissue disease, involving the integumental, musculoskeletal, gastrointestinal, and cardiovascular systems. Defects include diminished collagen synthesis, intracellular collagen accumulation, improper collagen packing, pro-collagen structural mutations, and abnormal fibronectin formation. Clinical signs include skin hyperextensibility and laxity, skin and vessel fragility, easy or spontaneous lacerations, multiple wounds with poor bleeding, wound healing resulting in highly visible "cigarette paper" scars, and occasionally marked joint laxity and dislocation.

Material and methods

A 3-year-old male Yorkshire Terrier was born with trunk flaccidity and lack of primary ossification centers in the pelvic long bones. There was also absence of the third eyelid, nyctalopia, mesiclusion, and pelvic limbs bone rotation with tendency to fracture. Cutaneous abnormal laxity and hyperextensibility were observed. The skin extensibility index was 38%. The skin layer was thin and frequently injured, which caused small slight bleeding wounds. The hair was easily removed. A biopsy punch was taken to confirm EDS. Skin samples were taken from a few places on the trunk, fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with HE.

Results

Microscopic examination showed thinning and folding of the epidermis with hyperkeratosis. Collagen fibres were shorter than normal, fragmented, frayed, curly, and of uneven size. Wide interfibrillar spaces were present. There were no collagen fibres surrounding the hair follicles.

Conclusion

Abnormality of collagen fibres is characteristic for Ehlers-Danlos syndrome, which confirmed the clinical diagnosis.

ACCUMULATION OF EXTRACELLULAR MATRIX IN CHRONIC LESIONS OF CANINE DISTEMPER DEMYELINATING ENCEPHALITIS

119.

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Introduction

Canine distemper virus (CDV) infections are commonly associated with demyelinating lesions in the central nervous system of dogs. In demyelinating diseases, changes in the quality and quantity of the extracellular matrix (ECM) may contribute to demyelination and failure of myelin repair, especially in chronic lesions.

Materials and methods

Formalin-fixed, paraffin-embedded cerebellums from 15 dogs suffering from distemper leukoencephalitis and four healthy controls were examined. Light microscopic changes in the cerebellum of the diseased dogs were subdivided into groups of normal-appearing white matter as well as early and late CDV-induced lesions. Various histochemical stains and antibodies specific for the detection of ECM molecules were used and quantitatively analysed via morphometry.

Results

In CDV-infected dogs, a significantly increased amount of aggrecan was detected in early and late white matter lesions. In addition, a positive signal for collagens I and IV as well as fibronectin was significantly increased in late lesions. Conversely, the expression of phosphacan was significantly decreased in early, and especially in late lesions, compared to controls.

Conclusion

Severe alterations of the ECM occurred in CDV-induced demyelinating encephalitis. Considering the insufficiency of morphological regeneration in chronic distemper lesions, the accumulated ECM seemed to have predominately inhibitory properties upon regeneration.

120. LISSENCEPHALY WITH HYPOPLASIA OF THE CORPUS CALLOSUM AND CEREBELLUM IN A DOMESTIC CAT

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Introduction

An approximately two-year-old domestic cat was submitted for necropsy with a history of recurrent aggressive behaviour and sensory disturbances.

Materials and methods

Following necropsy and histopathological evaluation, neurofilament (NF) and glial fibrillary acidic protein (GFAP) were demonstrated immunohistochemically. PCR and subsequent sequencing of exon 2,3 and 4 of the TUBA1A gene were completed.

Results

Gross pathologic evaluation revealed a hypoplastic cranial cavity containing an overall small brain with only marginal formation of gyri and sulci in both cerebral hemispheres. The cerebellum was hypoplastic and the corpus callosum lacked midline unification. Histologically, areas of white matter were severely reduced. Abundant swollen disorderly axons labeled positively for NF. Using GFAP immunolabelling, nests of plump, activated astrocytes were demonstrated in the cortical white matter. Sequencing of exon 2 to 4 of the TUBA1A gene revealed no potential disease causing mutations.

Conclusion

This is the first report of lissencephaly in a domestic cat. Malformations of the corpus callosum combined with cerebellar hypoplasia are common in human beings but have not been described in the veterinary literature. Many of these malformations in human beings are related to mutations in exon 4 of the TUBA1A gene. Corresponding mutations were not detected in the present case. Other regions of the gene or other candidate genes such as the LISS gene may be involved.

POSTER PRESENTATIONS

Session 13 A – Infetious Diseases/Naturally-Occurring Diseases

121. PATHOLOGY AND EPIDEMIOLOGY OF VIRAL HAEMORRHAGIC SEPTICAEMIA OUTBREAKS IN POLAND

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Introduction

Nineteen outbreaks of viral haemorrhagic septicaemia (VHS) were recorded in Poland in 2008.

Materials and methods

In VHSV-infected fish, the most common symptoms were petechiae, congestion, and darkening of the skin. In some cases, petechiae were also present in the gills and dorsal muscle. Blood vessel dilation within the intestinal wall was also frequently observed. Mortality usually approached 80%. Phylogenetic analysis of 18 Polish VHS G-gene sequences has been performed based on the procedure described by K. Einer-Jensen *et al.* (J. Gen. Virol. 2004). The analysed sequences originated from VHSV isolates collected from different farms in Poland where VHS outbreaks were diagnosed. Six of these isolates were obtained from three farms (two isolates from each farm at one year intervals), which had been diagnosed with the disease repeatedly in two subsequent years.

Results

The results indicate that some of the isolates are identical with others, despite the distance between farms from which the viruses were isolated. Some of the VHSV isolates only differed by a few nucleotides, based on the comparison of the G-gene sequence. The results suggest that at least some of the virus variants may have arisen from the same source.

ASSOCIATION OF BOVINE PAPILLOMAVIRUS TYPE 2 (BPV-2) AND URINARY BLADDER TUMOURS IN CATTLE FROM THE AZORES ARCHIPELAGO

122.

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Introduction

Urinary bladder tumours in cattle are caused by chronic ingestion of bracken fern and BPV-2 infection. BPV-2 is associated with naturally-occurring urothelial bladder tumours in cattle from some countries, but has not been investigated in Portugal, despite the endemic state of urinary bladder tumours in the the Azores.

Materials and methods

A total of 42 cows, (3.5–12 years old) were investigated. All cows were bred in São Miguel Island. At the slaugherhouse, samples of the urinary bladder were collected and fixed in 10% neutral-buffered formalin, routinely processed for histological examination and immunolabelled for the detection of E5 oncoprotein. PCR using primers specific for BPV-2 DNA was performed in paraffin sections.

Results

A 28% detection rate of BPV-2 DNA was present in different lesions of the urinary bladder. Samples positive for BPV-2 DNA also expressed E5 viral oncoprotein.

Conclusion

This is the first report of BPV-2 detection in urinary bladder tumours associated with CEH in cattle from the Azores archipelago.

123. EXPERIMENTAL HEPATITIS-E INFECTION IN PIGS

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Introduction

Hepatitis E virus (HEV) is the causative agent of a waterborne, enterically-transmitted, acute and self-limiting hepatitis, affecting mostly adult humans with low mortality. It is assumed that domestic pigs and wildboars represent the reservoir host for HEV worldwide.

Materials and methods

Five post-weaning pigs were infected with an isolate recovered from a male immunosuppressed patient chronically infected with HEV. One to five weeks post-infection, HEV-infected animals as well as the two mock-infected control animals were euthanised, necropsied and examined histologically. Fresh organ samples were taken for RNA extraction and RT-PCR.

Results

Macroscopically, no alterations were observed in the liver or any other organs. Histopathological examination revealed a mild to moderate lympho-histiocytic hepatitis and pericholecystitis. In two out of five infected animals, HEV RNA could be detected in the liver, bile, mesenteric lymph node and musculus psoas major. Multiple alignment was performed and this revealed a nucleotide identity between the virus isolated from the human patient and that recovered from the infected pigs of 90.8% and 96.0%, respectively. On the amino-acid level, both porcine isolates exhibited 100% identity with the original human strain.

Conclusion

HEV replicated successfully in pigs. An important role of the domestic pig as a reservoir host for human infection can be assumed.

RANAVIRUS INFECTION OUTBREAK IN THE SALAMANDER (*HYNOBIUS NEBULOSUS*) IN JAPAN

124.

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Introduction

Ranaviruses are important pathogens that are devastating amphibian populations worldwide. We discovered the first ranavirus infection (RCV-JP) in Japan in a mass die-off of *Rana catesbeiana* larvae in 2008. This examination confirmed ranavirus infection in *H. nebulosus*.

Materials and methods

The outbreak occurred in a protected colony of 80 *H. nebulosus*, which had been in captivity for over one year. The animals began to die 15 days after the introduction of some newly collected animals, and the entire colony was annihilated by the 20th day. The dead animals were examined using pathological and molecular biological methods.

Results

The animals died regardless of age. Macroscopically, immediate signs of morbidity were confined to skin ulcers. Histologic examination showed extensive glomerular necrosis with renal tubular hyaline droplet degeneration and various degrees of hepatic cell degeneration and necrosis. Cytoplasmic ranavirus-like particles that were icosahedral with a diameter of about 120 nm were detected within interstitial cells of the kidneys by electron microscopy. In addition, a PCR technique with a pair of M153 and M154 primers successfully amplified a ranavirus-specific gene encoding the major capsid protein (MCP). The partial MCP DNA sequence analyses revealed that the present ranavirus from *H. nebulosus* differed from frog virus 3 and RCV-JP.

Conclusion

This is the first report of ranavirus infection in salamanders in Japan.

125. FLOGOSIS/ZOONOSIS OF THE CENTRAL NERVOUS SYSTEM IN VETERINARY PATHOLOGY: A RETROSPECTIVE STUDY

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Introduction
Meningoencephalitis in veterinary medicine is frequently associated with zoonotic infections.

Materials and methods
Between 1997 and 2009, 523 brains of domestic and wildlife animals with inflammatory lesions were examined by means of histopathological, immunohistochemical, microbiological, and serological methods, and PCR.

Results
Sixty-one cases were attributed to specific biological agents (CDV, FeLV, FIP, FIV, CAEV, Visna, *Herpesvirus* spp, BDV, FCMV, *Borrelia* spp., *Campylobacter* spp., *Clostridium* spp., *Histophylus somni*, *Mannheimia haemolytica*, *Pseudomonas* spp, *Staphylococcus aureus*, *Streptococcus* spp., *Aspergillus* spp.) and 194 cases were zoonoses (*Clostridium tetani*, *Escherichia coli*, *Listeria monocytogenes*, *Encephalitozoon cuniculi*, *Neospora* spp., *Toxoplasma gondii*, Coenurus). The aetiology of 268 cases (primarily non-suppurative encephalitis) was not determined.

Conclusion
Determination of the aetiology of non-suppurative encephalitis remains difficult, although modern approaches may improve the diagnostic success. Further studies are needed to understand the immunopathological mechanisms underlying many of these processes.

MORPHOLOGICAL CHARACTERISTICS OF ALVEOLAR ECHINOCOCCOSIS IN DOGS

126.

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Introduction

Echinococcus multilocularis is a tapeworm with zoonotic potential and is common in the northern hemisphere. Naturally, foxes and other canids are its definitive hosts and rodents act as intermediate hosts. Additionally, many other mammalian species, including dogs and human beings, can be infected as intermediate hosts. In the intermediate host the liver is most often affected with cystic and infiltrative, tumour-like lesions.

Materials and methods

Lesions in 10 dogs with naturally acquired alveolar echinococcosis were characterised using morphological and immunohistochemical methods.

Results

Metacestode tissue was surrounded by prominent, fibrous connective tissue intermixed with inflammatory cells consisting of varying numbers of epithelioid macrophages, Langhans- and foreign body-type giant cells, T-lymphocytes, B-lymphocytes, plasma cells, as well as neutrophils and/or eosinophils. Many of the metacestodes contained no protoscolices and thus were sterile.

Conclusions

Alveolar echinococcosis is an emerging differential diagnosis for proliferative lesions of the liver. Similar to the situation in human beings, a high proportion of metacestodes in dogs are sterile.

127. PCV2 ASSOCIATED MYOCARDITIS WITH NECROSIS IN A FEEDER PIG

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Introduction

Porcine circovirus type 2 (PCV2) is associated with a variety of disease syndromes in pigs, including post-weaning multisystemic and wasting syndrome (PMWS). Microscopic lesions in PMWS are depletion and histiocytic infiltrations in lymphoid tissues. PMWS usually occurs in wasted pigs, 7–12 weeks of age. Cardiovascular lesions associated with PCV2 are reported in mummified and stillborn piglets and in piglets 4–7 weeks of age without typical PMWS lesions.

Materials and methods

A feeder pig, 20 weeks of age, was investigated grossly and by histology and immunohistochemistry for PCV2.

Results

Clinical signs were wasting, dyspnoea, and cardiac insufficiency. Hydrothorax and dilation of the right atrium were visible on gross examination.

Microscopic studies showed interstitial nephritis, bronchointerstitial pneumonia, severe depletion and necrosis of lymphoid tissues with large quantities of PCV2 antigen. Mild lymphoplasmacytic infiltrates and multifocal necrosis were present in the myocardium. PCV2 antigen was present in the necrotic areas and in macrophages and endothelial cells in the heart vessels.

Discussion

Myocarditis and myocardial necrosis in a feeder pig severely infected with PCV2 is a unique finding. The occurrence of PCV2 antigen in endothelial cells and associated leucocytes is suggestive of an underlying vasculitis in the course of PMWS.

COMPARISON OF IMMUNOHISTOCHEMISTRY AND *IN SITU* HYBRIDISATION FOR PCV2 DETECTION IN WEAKLY POSITIVE TISSUE SECTIONS

128.

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Introduction

The aim of the study was to develop an immunohistochemical (IHC) method for PCV2 identification and to compare this method with *in situ* hybridisation (ISH).

Materials and methods

An avidin-biotin-complex immunoperoxidase method was developed for PCV2 identification. Twenty-nine samples of lymph nodes and intestines from pigs, previously analysed by ISH, were tested in IHC for the presence of PCV2. All of the samples were positive (+) or weakly positive (+/-) in ISH.

Results

Seventeen samples showed more intense reaction in IHC compared to ISH. Nine samples yielded similar results with both methods and three samples that were weakly positive in ISH were negative in IHC.

Conclusions

This small-scale study shows that IHC can be used for PCV2 detection in tissue as well as ISH. The discrepancies in the findings may be related to possible failure of the DNA probe due to the absence of complementarity, degradation of viral nucleic acid after long-term formalin fixation, and differences in the quality of the tissue sections. Similar results were obtained in other studies comparing these two techniques.

129. MIXED INFECTIONS WITH *CHLAMYDIA* AND PORCINE EPIDEMIC DIARRHOEA VIRUS – A PERSISTENCE MODEL *IN VITRO* AND *IN VIVO*

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Introduction

Chlamydia and porcine epidemic diarrhoea virus (PEDV) occur worldwide and can cause diarrhoea in pigs. Mixed infections with Chlamydia and PEDV may result in generation of persistent chlamydial infections. To test this hypothesis an *in vitro* model of dual infection with cell culture adapted PEDV and *Chlamydophila abortus* or *Chlamydophila pecorum* in Vero cells was established.

Materials and methods

Infected cultures were investigated by immunofluorescence (IF), transmission electron microscopy (TEM) and re-infection experiments.

Results

By IF, Chlamydia monoinfected cells showed normal inclusions after 39hpi. Dual infections with *Chlamydophila abortus* revealed three types of inclusions: small inclusions consisting of aberrant bodies (ABs), medium-sized inclusions consisting of ABs and reticulate bodies, and normal inclusions. Dual infection with *Chlamydophila pecorum* resulted in the exclusive production of aberrant inclusions. TEM examinations of mixed infections revealed enlarged chlamydial inclusions containing reticulate-like, pleomorphic ABs, up to 2 µm in diameter and no re-differentiation into EBs. In re-infection experiments, co-infected cells produced fewer EBs than monoinfected cells. Differences between co-infection with *Chlamydophila abortus* and *Chlamydophila pecorum* were observed similar to the IF and TEM results.

Conclusion

Our results demonstrated that PEDV-co-infection alters the chlamydial developmental cycle similarly to other inducers of chlamydial persistence. Chlamydial persistence was more prominent in co-infection with *Chlamydophila pecorum* than with *Chlamydophila abortus* indicating species-specific differences.

CD43 EXPRESSION IN CANINE MALIGNANT MAMMARY TUMOURS

130.

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Introduction

Canine mammary tumours affect mainly older bitches and comprise approximately 25–50% of all their tumours, 40–50% being malignant. CD43 is overexpressed in several human cancers and contributes to carcinoma progression. Altered CD43 expression is linked to a poorer prognosis. CD43 expression has not been previously described in canine mammary tumours (CMT).

Objective

To characterize CD43 expression in CMT and to evaluate its relationship with clinicopathological features such as tumour histological type, mode of growth, tumour grading, lymph node metastasis and distant metastasis.

Methods

Forty paraffin CMT sections were examined for CD43 immunostaining patterns. CMTU27, CMM115, CMM26 carcinoma cell lines and CMA07 adenoma cell line were cultured in standard conditions. Western blots were performed. Protein extracts were analysed by standard SDS-PAGE, and blotted with anti-CD43 monoclonal antibody.

Results

All tumours had CD43 immunostaining. In normal adjacent mammary gland tissue, CD43 was detected in the cell membranes. In the carcinomas, CD43 expression was detected both in the cytoplasm and membrane, in most cases. A decreased membrane expression of CD43 (< 25% membrane staining) seemed to be associated with distant metastases. All studied cell lines expressed CD43 although its expression was slightly higher in the CMA07 adenoma cell line.

Conclusions

CD43 seems to be constitutively expressed in mammary gland cell membranes. Decreased CD43 expression in the membrane was associated with tumour progression. Our findings indicate, for the first time in CMT, that the CD43 pattern of expression may be an important prognostic marker in these tumours.

131. CANINE PHEOCHROMOCYTOMA, AMYLOID ARTERIOPATHY AND ARTERIOLOSCLEROSIS. COINCIDENCE OR DIRECT ASSOCIATION?

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Introduction
The concurrent presence of malignant pheochromocytoma, amyloid arteriopathy, and arteriolosclerosis in a dog raises the question of coincidence or direct association.

Materials and methods
Gross and histological examination including immunohistology and electron microscopy on renal and tumour tissue were performed on an adult male Bull Terrier.

Results
A malignant pheochromocytoma, severe diffuse glomerular and arterial secondary amyloidosis, mild glomerular sclerosis and arteriolar fibrosis in the heart, liver, and kidneys, left concentric cardiac hypertrophy and secondary renal hyperparathyroidism were diagnosed.

Discussion
Hypercalcaemia, genetic factors, increased pituitary hormones, autonomic nervous stimulation or diet are thought to contribute to the development of pheochromocytomas. In rats, they are associated with chronic progressive glomerulopathy. Functional pheochromocytomas are rare and lead to hypertension due to catecholamine secretion. Secondary amyloidosis is seen with chronic inflammatory or neoplastic processes due to high and continuous levels of serum amyloid A, an acute phase reactant, which possibly plays a role in carcinogenesis or tumour metastasis. Arteriolosclerosis is often associated with systemic hypertension. The possible association between the different conditions will be presented.

IMMUNE-DEPLETION RELATED TO BOVINE VIRAL DIARRHOEA VIRUS IN A HEIFER WITH NATURALLY OCCURRING MUCOSAL DISEASE

132.

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Introduction

Bovine viral diarrhoea virus (BVDV) induces mucosal disease (MD) in young persistently infected cattle. MD affects the gastrointestinal (GI) tract and lymphoid tissues; however the pathogenesis of lymphoid lesions is still scarcely understood.

Materials and methods

Samples of different parts of the gastrointestinal tract, lymph nodes, and spleen from nine naturally MD-infected heifers were collected during necropsy, processed for histology and immunostained with BVDV, CD3, CD79a, MAC387, MHCII, and HSP72 (heat shock protein 72) antibodies.

Results

All the animals had macroscopic and histological lesions (mucosal ulcers, necrosis, vasculitis and intestinal crypt herniation characteristic for MD. Lymphoid organs had follicular centre necrosis and apoptosis, with macrophage infiltration. Immunohistochemically, BVDV antigen stained only GI epithelial cells. HSP72 stained both mucosal epithelial cells and macrophages; macrophages were also MAC387 and MHCII-positive. Rare residual CD79a B-lymphocytes, surrounded by a few CD3 T-lymphocytes were detectable within follicular centres.

Discussion

The presence of BVDV antigen with restriction to the epithelium and the lack of reaction of lymphoid cells to BVDV antigen suggested that immune-depletion was mediated by pro-inflammatory cytokines up-regulation, as previously reported in pigs infected with classical swine fever.

133. DENTAL CARIES AND CARIES-RELATED PERIODONTAL DISEASE IN MICE WITH TYPE 2 DIABETES

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Introduction

Diabetic complications have been investigated in C57BL/KsJ-db/db mice, but few reports relate to dental caries or periodontal disease. The purpose of this study was to identify if dental caries and periodontal disease is directly induced by diabetic conditions.

Materials and methods

A total of 38 mice of diabetic db/db and non-diabetic db/+ strain, aged 50 weeks, were used.

Results

Mild dental caries with small foci of necrotic dentin on the crown surface were present in the molar teeth. Severe lesions in more advanced stages consisted of partial defect of crown, cleft formation throughout the entire crown and abscess formation with widespread necrosis in the pulp. Dental caries was detected in 30 of 120 teeth in 17/20 diabetic db/db mice and in four of 108 teeth in 4/18 non-diabetic db/+ mice. The lesions were more severe in db/db mice than in db/+ mice. Periodontal disease including gingivitis, proliferation of the mucosal epithelium, inflammatory cell infiltration, and alveolar bone resorption occurred in close association with severe dental caries in the diabetic db/db mice.

Conclusion

Spontaneous diabetic conditions play a role in the development of dental caries in db/db mice.

NCL (NEURONAL CEROID-LIPOFUSCINOSES) IN SHEEP

134.

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Introduction

Although this storage disease may be widespread in several organs, it is principally affects neurons of the cerebral cortex, retina, and cerebellar Purkinje system. There is cellular loss and atrophy, dementia, blindness, and ataxia in some severe cases.

Materials and methods

123 ruminants with neurological signs were examined histopathologically. The brain was fixed in 10% neutral-buffered-formalin and embedded in paraffin. 5 μm thick sections were stained with HE and Oil-Red-O (ORO).

Results and Discussion

In four sheep, the storage granules were pale brown-red with HE staining and bright red by ORO. Some parts of the cerebral cortex had mild neuronal atrophy and gliosis. Storage granules accumulated in the form of a cap around the nucleus. The pathogenesis of the disease is unclear but it is assumed that genetic or environmental factors may be involved.

135. IGH INCIDENCE OF *HELCOCOCCUS OVIS* IN BOVINE VALVULAR ENDOCARDITIS: PATHOLOGICAL AND BACTERIOLOGICAL RESULTS

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Introduction

Helcococcus ovis is a catalase-negative, facultatively anaerobic, Gram-positive, and pyridoxal-dependent coccus. Based on a few case reports, it appears to be aetiologically involved in infections of different mammalian hosts and organ systems.

Materials and methods

The investigations were carried out on 54 bovine hearts with lesions of valvular endocarditis. Bacteriology, histopathology, and fluorescence *in situ* hybridisation (FISH) were performed according to standard protocols.

Results

All 54 hearts had lesions characteristic for chronic valvular endocarditis. *H. ovis* was isolated from 17 bovine hearts (31%). The 16 cows and one bull originated from 16 different farms located throughout four federal states in Eastern and Northern Germany. Histologically, *H. ovis*-induced valvular lesions were characterised by large aggregates of Gram-positive cocci accumulating within the fibrinous layer of the valvular vegetations. In addition to Gram-staining, *H. ovis* was also specifically detected by FISH within the thrombi.

Discussion

In our study, *H. ovis* (31%) represented the second most common bacterial isolate after *Arcanobacterium pyogenes* (63%) and was recovered in pure culture from the affected hearts. This indicates that *H. ovis* is an emerging pathogen in bovine valvular endocarditis. For the future, more attention should be paid to this bacterium in veterinary medicine.

PATHOMORPHOLOGY OF THE LIVER IN DOGS WITH CLINICAL SIGNS OF PORTOSYSTEMIC SHUNTS

136.

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Introduction

Congenital portosystemic shunts (PSSs) are vascular anomalies, usually of single vessels, which directly connect the portal venous system with the systemic venous circulation. Characteristic clinical signs of PSSs in dogs are hepatic encephalopathy, vomiting, diarrhoea, polyuria, polydypsia, sometimes dysuria, and urolithiasis. Non-specific signs include apathy, anorexia, weight loss, and retarded growth in young animals. The degree of histopathological changes varies depending on the site of the shunt vessel and the liver lobe. The aim of this study was to characterise the histopathological lesions in the liver of dogs with clinical signs of PSSs.

Material and methods

Twenty surgical liver biopsies from dogs (aged 4–36 months; 11 males, 9 females; different breeds) were fixed and stained routinely with HE, Masson's, Perls's, and prolonged Ziehl-Neelsen staining were also performed.

Results

Intrahepatic PSS was diagnosed in one case and extrahepatic PSSs were diagnosed and confirmed during laparotomy in 18 cases. In 14 cases, regressive changes of hepatocytes; arteriolar and biliary proliferation; lipo- (6) and pigment granulomas (7); and nodular hypertrophy (3) were noted. Fibrosis and hypertrophy of vascular walls were observed in four cases. One dog showed lesions of hepatic microvascular dysplasia.

Conclusions

In dogs with clinical signs suggesting PSSs liver biopsy can be helpful in diagnosis, prognosis, and treatment.

137. INTRAOCULAR LYMPHOMA: IMMUNOPHENOTYPE AND PROGNOSIS

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Introduction

Ocular disease may be the first indication of lymphoma, but little is known of the outcome of intraocular lymphoma in veterinary patients.

Materials and methods

Thirteen feline and two canine cases of ocular or orbital lymphoma from the University of Melbourne archives for the years 2000–2009 were retrieved. Sections were cut for immunohistochemistry using CD3 for T cells and Pax 5 for B cells. Referring clinicians and owners were contacted for follow up information.

Results

Most tumours were B cell tumours, with variable numbers of normal T cells in association. Only one patient had a diagnosis of lymphoma prior to ocular presentation. Average survival time was about 3 months, but ranged from 1 day to 8 or 9 months. Necropsy follow up was not available for any patient; the major reasons for euthanasia were renal tumours (3), CNS disease (2), and general ill health (2). Three recently diagnosed cats were still alive and apparently well at the time of writing.

Conclusion

Ocular and adnexal lymphomata are commonly aggressive B cell tumours and prognosis for these patients is very poor.

POSTER PRESENTATIONS

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138. DEMODICOSIS IN A SWEDISH ROE DEER (*CAPREOLUS CAPREOLUS*)

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Introduction

Hair follicle parasites of Demodex species are rare in cervids, and have previously not been reported in Sweden as a cause of alopecia in Roe deer (*Capreolus capreolus*).

Materials and methods

As part of the Swedish passive surveillance of wildlife diseases, a fresh skin sample and gross photograph from a hunted male Roe deer shot in the south-east of Sweden in September 2008 were submitted for investigation. The deer had patchy alopecia especially along the neck. The skin was scraped for parasitological examination, and samples were fixed in formalin for histopathology (HE and PAS stains).

Results

Grossly, the skin was alopecic and hyperpigmented. In both direct microscopy and in histopathologic sections, hair follicle parasites consistent with *Demodex* sp. were identified. Affected follicles showing follicular hyperkeratosis and hair shaft fragmentation were accompanied by mild dermatitis.

Conclusion

The alopecia could be attributed to the presence of Demodex parasites. This is the first known report of demodicosis in Roe deer in Sweden, and one of very few reports overall of the disease in cervids.

ENCEPHALOMALACIA AND INTRAHEPATIC CHOLANGIOCELLULAR CARCINOMA IN A BUDGERIGAR (*MELOPSITTACUS UNDULATUS*)

139.

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Introduction

This is believed to be the first report of encephalomalacia associated with intrahepatic bile duct carcinoma in an adult budgerigar.

Materials and methods

An adult male budgerigar was presented for necropsy in May 2009 with a history of ataxia and star gazing of several days duration. At necropsy, the liver was pale and enlarged and two pale areas were detected in the cortex of the cerebrum. Representative samples from all organs were fixed in formalin and stained with HE.

Results

Histological examination of the liver showed numerous epithelial lined ducts with an associated dense fibrous stroma. Based on morphological criteria, the tumour was classified as a low-grade bile duct carcinoma. In the cerebrum, spongiform change in the gray matter was accompanied by status spongiosus in the white matter. Perivascular and perineuronal spaces were dilated.

Conclusion

A case of encephalomalacia and intrahepatic bile duct carcinoma in an adult male budgerigar is reported. Vitamin E deficiency may have contributed to the development of encephalomalacia in this bird.

140. *PSEUDOMONAS AERUGINOSA* INDUCED LESIONS IN NON-VENOMOUS SNAKES

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Introduction

Pseudomonas aeruginosa infection of captive snakes is considered opportunistic, meaning that it usually induces disease in a compromised individual due to immune suppression, cool environment, malnutrition or viral infection.

Materials and methods

Two young snakes (a 5-month-old male Python molurus bivittatus and an 18-month-old male *Boa constrictor*) were submitted for necropsy. Deaths occurred 3 days after onset of clinical signs that included restless movements, anorexia and changes of skin colors into dark nuances. The liver, kidneys, heart, small intestine and lungs were sampled for bacteriological, cytological and histological investigations.

Results

The most relevant lesions observed on gross investigation were necrotizing enteritis, fatty liver and discrete areas of acute edema. Cytological and histological findings revealed necrotic pneumonia and pulmonary edema, acute tubular necrosis with hyaline droplet degeneration of nephrocytes and diffuse hepatic lipidosis.

Discussion and Conclusions

No evidence of paramyxovirus infection or inclusion body disease (IBD) was detected. Hyperplasia and hypertrophy of septal and faveolar pulmonary epithelial cells (specific for paramyxovirus infection) and oxyphilic or amphophilic inclusions (indicative of IBD) were absent. Poor condition and fatty liver may have created a suitable microenvironment for opportunistic Pseudomonas infection.

A CASE OF MUSCULAR SARCOCYSTOSIS IN HORSFIELD'S TORTOISE (*TESTUDO HORSFIELDII*)

141.

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Introduction

Sarcocystosis (sarcosporidiosis) is a parasitic disease caused by protozoan of the genus Sarcocystis. For complete maturation the parasite requires two hosts. Horsfield's tortoises (*Testudo horsfieldii*) are intermediary hosts that get infected by consuming plants contaminated with feces of carnivores.

Materials and methods

A 15-year-old male Horsfield's tortoise (*Testudo horsfieldii*) had mobility problems for 8 months and limb swelling appeared. Gradually the tortoise stopped crawling and developed muscle atrophy. Treatment proved ineffectual and, because the animal appeared to be in continuous pain, the decision was taken that it be euthanised. A necropsy was completed and muscle samples from the front and hind limbs were fixed in 10% formalin, embedded in paraffin and stained with HE.

Results and conclusion

The necropsy revealed poor condition and swelling of the hind limbs. The limb muscles were limp and pale pink with significantly decreased fiber diameter. Infrequent crescent-shaped sarcocysts, approximately 1mm in diameter, were aligned along the muscle fibers. Histological studies showed sarcocysts inside muscle fibers and small blood vessels, leucocytic infiltrates, and muscle atrophy and degeneration.

142. *YERSINIA ENTEROCOLITICA* INFECTION IN A VERVET MONKEY (*CERCOPITHICUS AETHIOPS*) IN IRAN

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Introduction

Yersinia enterocolitica (*Y. enterocolitica*) is one of the major species of the genus Yersinia and is widely distributed in nature in aquatic and animal reservoirs. It is primarily an enteric pathogen but, under defined host conditions, has a strong tendency for extra intestinal spread. Swine serve as a major reservoir for human pathogenic strains.

Materials and methods

A male vervet monkey (*Cercopithecus aethiops*), in good body condition and housed in quarantine, died after a short illness. The most outstanding clinical signs were depression, anorexia, diarrhea and dehydration. Multiple liver abscesses and enlargement of the spleen with pinpoint white spots were the most prominent macroscopic features. Samples of different organs were collected and referred for further examination to the Departments of Bacteriology, Parasitology and Pathology.

Results

Bacteriological examination of different organs yielded *Y. enterocolitica*. Lesions of acute hepatitis, splenitis and enteritis were evident on histological examination.

Conclusion

This is the first report of fatal Yersiniosis in a vervet monkey in Iran. Transmissible pathogenic and opportunistic zoonotic enteric bacteria, such as *Y. enterocolitica*, comprise a recognized occupational health hazard and those working with non-human primates in exotic animal quarantine stations must take adequate precautions.

BORNA DISEASE IN AN ADULT ALPACA STALLION (*LAMA PACOS*)

143.

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Introduction

Borna disease (BD) is an endemic, sporadically occurring, usually fatal disorder caused by the highly neurotropic Borna disease virus (BDV). Clinical BD has only been recognised in the German speaking part of Central Europe predominantly in equidae and ovine.

Materials and methods

A 2-year-old male alpaca with a history of stretching, convulsions and depressed libido was submitted for necropsy. Routine histology as well as immunohistochemistry, *in situ* hybridization and PCR for BDV were performed. Serology from the flock which the animal originated from was also available.

Results

Histologically, the animal exhibited a typical non-purulent meningoencephalitis with mononuclear perivascular cuffs throughout the central nervous system, especially in the hypothalamic region. BDV infection was confirmed by detection of viral proteins, RNA and PCR.

Conclusion

In alpacas with neurological disorders, BD must be considered as a possible differential diagnosis.

144.

GROSS AND HISTOPATHOLOGIC LESIONS OF THE INTEGUMENTARY SYSTEM IN DROMEDARY CAMELS

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Introduction

Although camels are important sources of meat, milk, wool and hides and are widely used as a multipurpose local animal in the arid and semiarid deserts, they have not received adequate attention so far as their skin diseases are concerned.

Materials and methods

Gross and histopathologic lesions of the integumentary system were studied in 103 camels (*Camelus dromedarius*) slaughtered in the Yazd Provine of Iran. The gross appearance of lesions, particularly in cross section, were studied comparatively. Multiple samples about 1 cm in diameter were fixed in 10% neutral-buffered formalin, processed routinely, sectioned at 5 μm, stained with haematoxylin and eosin (HE) and examined histologically.

Results

Gross lesions consisted of focal-multifocal skin thickening with or without hair loss and crust formation, ulcerative lesions associated with tick infestation, and nodules or masses on the epidermis or in the subcutis. Histopathological characteristics allowed categorization of lesions with regard to frequency and included granulomatous dermatitis (19%), dermatophilosis (15%), purulent dermatitis (13%), eosinophilic dermatitis (11%), abscesses (8%), onchocercosis (6%), papillomatosis (4%), fibropapilloma (4%), subcutaneous lipoma (2%), melanocytoma (2%) and sebaceous gland hyperplasia (1%). Tick infestation (*Hyalomma* spp.) was observed in about 65% of studied camels.

Conclusion

The most prevalent skin lesions of camels were tick-induced dermatitis, granulomatous dermatitis, dermatophilosis and onchocercosis.

DETECTION OF AVIAN BORNAVIRUS IN PARROTS WITH PROVENTRICULAR DILATION DISEASE (PDD) BY *IN SITU* HYBRIDIZATION

145.

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Introduction

Avian bornavirus (ABV) is an enveloped, single- and negative-stranded, non-segmented RNA virus and the suspected etiological agent of proventricular dilatation disease (PDD) in psittacine birds (parrots). This virus has been found in the USA, Israel, Germany, Austria and Switzerland. Six genotypes have been identified so far. PDD is a fatal disease characterized by lymphoplasmacytic infiltration of the central, peripheral and autonomic nervous system. It primarily affects ganglia and nerves supplying the musculature of the digestive tract causing atrophy of the smooth muscles of the esophagus, crop, proventriculus, ventriculus and small intestine. PDD cases present with gastrointestinal tract dysfunction (dysphagia, regurgitation, presence of undigested food in feces) and/or neurologic symptoms (ataxia, abnormal gait). The detection of ABV is achieved by histological and immunohistochemical approaches and/or PCR-based techniques.

Results

In this study we report on naturally occurring cases of PDD in Switzerland with evidence of ABV infection, and demonstrate lesions and detection of ABV genomic and messenger RNA in paraffin-embedded tissues by *in situ* hybridization using digoxigenin-labelled sense and antisense RNA probes.

146. AA AMYLOIDOSIS IN CAPTIVE BEIRA ANTELOPES (*DORCATRAGUS MEGALOTIS*) LINKED TO MYCOPLASMAL PLEUROPNEUMONIA

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Introduction

During 2006 and 2007, a group of captive Beira antelopes kept at the Al Wabra Wildlife Preservation (Qatar) suffered from a severe respiratory epidemic characterized by a high mortality rate.

Materials and methods

A total of 48 Beira antelopes died within two years from a population of 58 alive at the beginning of 2006 with a mortality of 32.8% and 46.2% in 2006 and 2007, respectively. Morbidity in 2007 reached 100%. All animals that died were investigated by post mortem and samples for histopathology and bacteriology were taken.

Results

Lesions consisted of moderate to severe fibrinous pleuropneumonia with mucoid airway obstruction. Bacteriology demonstrated the presence of *Mycoplasma ovipneumoniae* in 6 animals whereas *Mycoplasma* species were detected by a generic PCR in 22 further animals. AA amyloid was observed in 24 antelopes and was mostly located in spleen and liver, whereas kidney, lymph nodes and gut were only slightly affected.

Conclusion

AA (systemic) amyloidosis is described for the first time in Beira antelopes following the inflammatory stimulus induced by respiratory infections linked to *Mycoplasma* spp.

CAPYBARA FASCIOLIASIS IN BRAZIL

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Introduction

Capybara (*Hydrochoerus hydrochaeris*), a wild, South American, semi-aquatic mammal, is the largest rodent in the world. These animals can be a reservoir of *F. hepatica* due to the introduction of domestic animals into their habitat.

Materials and methods

Two capybaras were found dead in the city's municipal park (Curitiba, Brazil) and were submitted for necropsy on the same day. Liver samples were fixed in 10% buffered formalin, processed histologically, sectioned and stained by haematoxylin-eosin (HE).

Results

Several flukes were found in the largest bile ducts. Gross hepatic lesions of both animals consisted of fibrous perihepatitis, irregular scars in the hepatic surface, and thickening of the bile ducts and gallbladder. Microscopically, the lesions comprised multifocal necrosis with cellular debris, surrounded by mild to moderate lymphoplasmocytic reaction and a small number of eosinophils. Multifocal granulomas surrounded by neutrophils, eosinophils, lymphoplasmocytic cells and fibrous tissue were present. In some areas, severe bile duct hyperplasia accompanied by a mild lymphoplasmocytic infiltration and diffuse fibrosis could be observed.

Discussion and conclusion

These findings are consistent with trematode infection. They indicate that capybara can be a wild host, and that this species should be included in fascioliasis control programs.

148. PATHOLOGICAL FINDINGS IN AN ASIAN ELEPHANT (*ELEPHAS MAXIMUS*)

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Introduction

The dominant pathologies in captive elephants are represented by tuberculosis, Herpesvirus infection, cystic endometrial hyperplasia, viral encephalomyocarditis, salmonellosis, rabies, tetanus, degenerative joint disease, pododermatitis and bone fracture.

Materials and methods

Gross and microscopic lesions in a 48-year-old captive female Asian elephant are presented. A necropsy together with cytological, histological, bacteriological and parasitological investigations was performed.

Results

The most important gross lesions were myocardial degeneration, focal calcification and valvular erosions, subendocardial and subepicardial hemorrhages, multiple hydatid cysts in liver and lungs, pulmonary atelectasis and compensatory emphysema, peliosis hepatis, cystic endometrial hyperplasia and presence of a macerated foetus. The diameter of hydatid cysts ranged from 20–30 cm, with numerous hydatid daughter cysts. Histopathological findings comprised myocardial lipofuscinosis, arteriolosclerosis, focal myocardial fibrosis, hepatic, pancreatic and renal fibrosis, adrenal cortical hyperplasia and pancreatic nodular hyperplasia. Bacteriological investigation for specific pathogenic bacteria was negative.

Conclusions

Vascular injury and chronic age-related interstitial fibrosis inducing cardio-pulmonary failure, are considered to be causative of death.

PATHOLOGY OF THE KIDNEYS AND SERUM CONCENTRATION OF CREATININE, UREA AND PROTEIN IN EUROPEAN BISON

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Introduction

The aim of this work was pathological evaluation of the kidney and correlation with serum concentrations of creatinine, urea and protein in European bison.

Material and methods

45 European bison aged 3 months-20 years old of both sexes were examined post mortem. Kidney specimens were collected for histopathological examination. Specimens were stained with HE, AB-PAS, Masson-Trichrome and von Kossa. Blood samples were collected from all examined bison for biochemical tests.

Results

Post mortem kidney analysis showed focal inflammation and/or cysts in 16 cases. Most renal cysts were single and small. Histopathological examination showed focal proliferative or membranoproliferative glomerulonephritis and chronic interstitial nephritis in 29 cases. Deposition of calcium salts in 6 cases was also observed. In one case, numerous granulomas with focal calcification were observed. The analysis of biochemical parameters of blood from bison with renal lesions showed increased levels of urea and decreases or increases in protein.

Conclusion

Focal nephritis and simultaneously increased levels of biochemical parameters were observed. The case with granulomas was probably caused by parasites.

150. MULTIPLE RESPIRATORY INFECTIONS IN SAKER AND GYRFALCONS (*FALCO CHERRUG*, *FALCO RUSTICOLUS*) INCLUDING CRYPTOSPORIDIA AND A NOVEL SPECIES OF *MYCOPLASMA*

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Introduction

A group of captive Saker falcons (*Falco cherrug*) and Gyrfalcons (*Falco rusticolus*) developed signs of respiratory disease including dyspnoea, respiratory sounds, swollen eyelids, and, in some cases, death.

Materials and methods

Three dead falcons were necropsied and samples for bacteriology were taken. Infectious agents were characterised serologically, by immunohistochemistry (IHC), *in situ* hybridisation (ISH), and polymerase chain reaction (PCR) with subsequent nucleotide sequencing. A conjunctival swab from a fourth bird was examined with PCR.

Results

Escherichia coli and *Proteus* spp. were isolated from the conjunctiva, nasal cavity, lungs, and air sacs and were associated with fibrino-purulent to necrotising inflammation. In the lungs, *Mycoplasma buteonis* and *Mycoplasma aquilae* spp. nov. were identified. Samples from the trachea had a mixed cell infiltration and epithelial hypertrophy and hyperplasia containing numerous protozoa (*Cryptosporidium baileyi*).

Conclusion

These are the first confirmed cases of infection with *M. aquilae* spp. nov. and one of a few reports of *C. baileyi* respiratory infection in falcons.

BOID INCLUSION BODY DISEASE IN CAPTIVE BOAS (*BOA CONSTRICTOR*) IN AUSTRIA

151.

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Introduction

Boid inclusion body disease (BIBD) is a well-known disease of giant snakes but of unknown aetiology. Infected snakes present predominantly with anorexia, central nervous disturbances, and immunosuppression. Diagnosis can only be achieved by histopathological examination.

Materials and methods

Forty-seven Pythoninae and 37 Boinae were submitted for necropsy and HE-stained slides were examined histologically.

Results

The species *Boa constrictor* had an exceptionally high prevalence (20 snakes, 71%) of eosinophilic cytoplasmic inclusions typical for BIBD, while only two pythons (4%) were positive histologically. Inclusions were especially numerous in hepatocytes, exocrine pancreas, neurons in brain, and in the retina. Numerous secondary diseases can lead to symptoms and pathological findings that could mask the underlying BIBD.

Conclusion

In captive giant snakes submitted for necropsy in Austria, *Boa constrictor* had a notably high prevalence for BIBD, often associated with secondary diseases. Eleven Burmese pythons (*Python molurus*) were all negative in this study, although BIBD has previously been well documented in this species.

152. HAEMOCHROMATOSIS IN THE BLACK RHINOCEROS (*DICEROS BICORNIS MICHAELI*), AQUIRED OR CONGENITAL?

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Introduction

Haemosiderosis in the captive African black rhinoceros (*Diceros bicornis*) is relatively common although the pathogenesis remains obscure.

Materials and methods

Four African black rhinos aged between 23 and 39 years from the Zürich Zoo were admitted to necropsy due to poor body condition, old age, or recumbency.

Results

From one animal a total of 17 blood samples were taken for biochemistry. Serum iron was high compared to values of free-ranging animals, mean transferrin saturation – 90% (normal 28%), and mean ferritin – 6046 ng/mL (normal 133ng/mL). Macroscopically, the animals were almost cachectic with several decubitus skin ulcers overlying prominent bone structures. In one animal, the small intestine was diffusely blackened. The liver was friable and red to dark brown. Histologically, the animals had heavy haemosiderin deposits in macrophages and parenchymal cells of the spleen, liver, bone marrow, and lungs. The liver had extensive haemosiderin deposition in Kupffer cells, hepatocytes, and biliary epithelium, and there was moderate bile duct proliferation but only minimal fibrosis. Aside from haemosiderin deposits, the bone marrow was hypocellular. In one animal, massive phagocytosed deposits in the lamina propria and villus tips of the small intestine were seen.

Discussion

The distibution of histological lesions together with the clinical data is indicative of an enteric origin of excess iron, rather than recurring haemolytic anaemia or hereditary haemochromatosis.

MURAL ENDOCARDITIS IN BRITISH ALPACAS (*VICUGNA PACOS*)

153.

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Introduction

Bacterial endocarditis in farm animals is well recognised with lesions usually centred on the atrioventricular valves. In contrast, right-sided mural thrombotic endocarditis appears to be the common location for this lesion in alpacas in the United States (Firshman *et al.* 2008).

Materials and methods

To investigate the character and pathogenesis of endocarditis in alpacas, gross and histopathological examinations were performed on 12 alpacas aged between 9 months and 8.5 years from eight British herds.

Results

Subacute to chronic mural endocarditis predominantly involving the right ventricle was present in nine alpacas. In the remaining three alpacas, lesions were predominantly seen in the left ventricle. Histopathology comprised variable amounts of necrotic debris and mineralisation, loss of endocardial cells, fibrosis, haemosiderin-laden macrophages, and, in some cases, multinucleated cells. In seven alpacas, adult *Fasciola hepatica* organisms and/or their eggs were detected. Most of the other alpacas had lesions suggestive of fascioliasis. There were no consistent bacteriological findings.

Conclusion

Endocarditis commonly affects the mural endocardium in British alpacas, with valvular involvement probably occurring late in lesion development. Chronic fascioliasis may predispose alpacas to the condition by favouring opportunistic bacterial infections in association with endocardial damage caused by toxaemia due to liver damage.

154. TUBERCULOSIS IN ALPACA (*LAMA PACOS*): PATHOLOGICAL FINDINGS

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Introduction

Tuberculosis has been thoroughly studied in ruminants; however, the information on tuberculosis in camelids is still very limited.

Clinical case

We report the diagnosis of tuberculosis due to *Mycobacterium* spp. infection in a 7-year-old female alpaca from a commercial herd with 32 camelids in Southern Spain. Clinical signs including dyspnea, fever, depression, lethargy, anorexia, and weight loss continued for two weeks and antibiotic therapy was unsuccessful. A repeated intradermal herd test using bovine and avian tuberculins yielded negative results. The animal was euthanised because of its poor condition.

Results

Macroscopic findings consisted of multifocal caseous lesions of different size in mediastinal and mesenteric lymph nodes, lung, trachea, liver, and spleen. Well-delimited tuberculous granulomas were observed in the lymph nodes, liver and spleen together with more diffuse tuberculous granulomas in the lung and trachea. Numerous acid-fast bacteria morphologically similar to Mycobacterium spp. were identified in Ziehl-Neelsen-stained smears.

Conclusions

A case of *Mycobacterium* spp. infection in an adult alpaca is reported. The results of culture and spogliotype studies are ongoing and will be presented during the meeting.

MYSTERY SLIDE SESSION ON DERMATOPATHOLOGY

Sponsored by International Society of Veterinary Dermatopathology

CASE 1
Submitted by E.A. Mauldin

Domestic short-haired cat, 16-year-old, MN.
One week following an upper respiratory tract infection, the cat developed erosions and ulcers on nasal planum and adjacent muzzle with serous to hemorrhagic crusts. Initial mild improvement was seen with a topical antifungal/antibiotic/steroid lotion, but lesions continued to progress. No improvement with oral cyclosporin.

CASE 2 (09-0233)
Submitted by E.J. Walder

Labrador retriever, 4-year-old, MN.
The dog has had nasal depigmentation and crusting since 6 months of age. The dog had been maintained on 20 mg prednisone daily for 3 years; the skin disorder worsened after discontinuation of steroids for 3 weeks prior to biopsy. The primary clinical differential diagnosis was cutaneous lupus.

CASE 3
Submitted by V.K. Affolter, R. Burns

Dutch Warmblood, 14-year-old, mare.
The horse presented for a mass within the left cheek (3.5 × 3.5 × 1.5 cm subcutaneous

mass extending into the skin, with multifocal ulcers). The mass was previously excised and diagnosed as trichoblastoma. Lesion was somewhat painful to touch.

CASE 4
Submitted by V.K. Affolter, D.J. Gasper

Chinchilla, adult, female, 644 g.
The chinchilla, along with approximately 100 others, was confiscated by animal control from a private individual suspected of hoarding.

At presentation, the chinchilla was bright, alert, responsive, and in thin body condition. The right forepaw was missing the second digit, and a 4 × 1 cm degloving injury was present on the right lateral tarsus. The wounds were clipped, cleaned, and over the next 60 h the chinchilla received subcutaneous fluid therapy, subcutaneous injections of enrofloxacin, oral meloxicam, and topical wound treatment with silver sulfadiazine. 24 h after admission, the chinchilla became anorexic and the caudal ventral skin was noted to be very erythematous. 48 h after admission, scant feces and continuation of ventral erythema were observed and metoclopromide was added to the treatment regimen. 60 h after admission the chinchilla was found dead.

On post-mortem exam, the right paw was swollen, and the second digit was missing. The skin of the third digit was degloved, and the remaining digits and pads were adherent to each other and coated by a crust. The skin of the medial right pelvic limb was abraded with multifocal erosions and ulcers from mid-tibia to metatarsus.

CASE 5
Submitted by J. Nimmo

Foxhound, 8-year-old.
An 8-year-old Foxhound developed swellings over toes and joints, which rapidly extended up distal limbs and progressed to dermal thickenings and ulcers over entire body. Treatment with corticosteroids resulted in 70% reduction of lesions after a week.

CASE 6
Submitted by EA Mauldin

Domestic short-haired cat, 2-year-old, FS.
The cat was evaluated of otitis and growing nodular masses in both ear canals. The le-

sions developed over a period of one month and were unresponsive to multiple courses of antifungal/antibiotic/steroid otic preparations as well as systemic antibiotics (enrofloxacin, amoxicillin/clavulanic acid). The mass in the left ear completely obstructed the ear canal, and the cat subsequently developed a large abscess in the periauricular soft tissue. Following bilateral total ear canal ablation, a small nodule of similar tissue recurred on the left pinna.

CASE 7
Submitted by Chiara Brachelente

European shorthaired cat, adult (stray).
The cat presented with multifocal to coalescing small and firm nodules, varying in size, with alopecic and partially eroded or ulcerated surface with a bilateral, non-symmetrical distribution above the eyes. Some isolated nodules were also present around the lips and on the dorsal aspect of the neck. The cat had head and neck pruritus. The cat was treated with selamectin and prednisone (4mg/day for 10 days followed by 2 mg/day for 10 days). The pruritus disappeared and the lesions were softer and no longer showed ulcerations. When the prednisolone was discontinued, the pruritus recurred in two weeks.

CASE 8
Submitted by V.K. Affolter, P. Rowland

Golden retriever, 8-year-old, MN.
The dog was evaluated for nonpruritic, erythema and crusting on the feet and axilla with thickening of the skin. The lesions remained fairly stable with little response to corticosteroids. Biopsy "A" was taken six months prior to "B".

CASE 9
Submitted by V.K. Affolter, T.L. Gross, K. Coyner

Cairn terrier, 2.5-year-old, female spayed.
Presented for a one-year history of numerous pigmented crusty papules, which are dispersed over the trunk and face. There is also hypotrichosis on the dorsal muzzle, medial to both eyes. Skin scrapings revealed a few cocci and a few yeast organisms. The bacterial and yeast infections cleared with systemic antibiotic and fluconazole treatment.

However, the crusty pigmented lesions remained. Dermatophyte cultures were negative. There was minimal improvement with subsequent treatment with cyclosporine and pentoxifylline for 6 weeks.

CASE 10
Submitted by V.K. Affolter, P.F. Moore

Bernese mountain dog, 5-year-old, MN.
Two dermal masses were removed 9 months before and diagnosed as a histiocytic inflammation. A new dermal mass on the right forelimb was removed 4 months later. Upon histologic evaluation a histiocytic sarcoma was diagnosed. An additional mass was found on the left forelimb 2nd digit. In addition to the skin mass, a pulmonary mass was identified on radiographs. Abdominal ultrasound was normal. Removed skin nodule was sent in for histopathologic evaluation.

CASE 11
Submitted by E.A. Mauldin

German shorthaired pointer, 4.5-year-old, M.
The dog had a four-year history of progressive alopecia, erythema and scaling which started on the face and was soon followed by trunk, scrotum and extremities. Erosions and ulcers followed onset of scaling and hair loss. The dog also had a hunched stance, shifting leg lameness and mild lymphopenia.

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