



**28th Meeting of the European
Society of Veterinary Pathology
and European College of
Veterinary Pathologists**

ESVP/ECVP2010

**8-11 September 2010
Belgrade, Serbia**

**Book of
Abstracts**

www.esvp2010.org

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PUBLISHER

Serbian Society of Veterinary Pathology

PRESS

Sava Centar, Belgrade, number of copies 300

ISBN: 978-86-913875-0-1

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Dear colleagues,

On behalf of the Organizing Committee it is my great pleasure to welcome you to the 28th ESVP/ECVP Meeting in Belgrade, Serbia.

This is the second joint congress of the European Society of Veterinary Pathology and the European College of Veterinary Pathologists that will take place together with European Division Meeting of the CL Davis DVM Foundation. The scientific program of the Meeting through keynote lectures and parallel sessions with oral and poster presentations will cover the subjects of Organ System Diseases, Oncology, Infectious Diseases, Wildlife diseases and Modern Methods in Pathology. The program also includes a Mystery Slide Session and a Workshop.

This Meeting will be a valuable opportunity to discuss the latest aspects and achievements in the field of veterinary and comparative pathology. Results of recent scientific research will be merged into keynote lectures and announced by invited speakers. All of you should feel encouraged to contribute to those keynote lectures with both oral and poster presentation as well as with your active participation. I hope this will enable fruitful discussions to take place and promote greater exchange of knowledge and experience.

This Meeting will also be an opportunity to explore the beautiful and vibrant city of Belgrade and enjoy Serbia's warm spirit of hospitality.

We promise to do our best to make your stay in Belgrade the most unforgettable scientific and social experience.

I hope the Meeting will be a benefit for all of us.

President of the Organizing Committee

Milijan Jovanović

KEYNOTE LECTURES

SKELETAL TUMOURS IN DOMESTIC ANIMALS – A CHALLENGE FOR THE DIAGNOSTIC PATHOLOGIST

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Skeletal tumours, particularly osteosarcomas, are relatively common in domestic animals, especially dogs, and often present a significant challenge to the diagnostic pathologist. Since osteosarcomas are highly malignant, an early and accurate diagnosis may be crucial to the survival of the patient, but differentiation from the variety of other neoplastic and non-neoplastic lesions of bones can be extremely difficult. The consequences of an incorrect diagnosis may include unnecessary amputation, inappropriate and expensive chemotherapy, or even euthanasia, but if a diagnosis of osteosarcoma is delayed or missed, metastatic spread of the primary lesion is highly likely. Litigation by disaffected clients is becoming increasingly common in such situations and is just as likely to target the pathologist as the surgeon. As a result, few biopsy specimens put more pressure on the diagnostic pathologist than one from a suspected osteosarcoma. Unfortunately, the importance of the decision that we must make on bone biopsies is not always matched by the quality of the specimen or the clinical information received.

This presentation will focus primarily on the histological and cytological diagnosis of osteosarcomas, including discussion of the value of imaging techniques, immunohistochemistry and special stains. A variety of other skeletal lesions that must be considered in the differential diagnosis of osteosarcoma will also be discussed.

Brief overview of osteosarcoma

Osteosarcoma accounts for over 80% of malignant bone tumours in dogs, and a slightly lower percentage in cats. In dogs, the predilection for medium-sized and large breeds is well recognised, as is the increased incidence in the metaphyses of long bones, especially the proximal humerus, distal radius, distal femur, distal and proximal tibia. But osteosarcomas also occur in bones of the axial skeleton (20-25%), including the mandible, maxilla, vertebrae, ribs, cranium and pelvis. The mean age of affected dogs is around 7-9 years, but the tumour also occurs in dogs as young as 1-2 years of age.

Most osteosarcomas arise in the medullary cavity, rapidly destroy metaphyseal bone, and penetrate the cortex, but seldom extend to involve joints. Periosteal new bone formation is often florid and must be penetrated when collecting biopsies for diagnosis. Grossly, the tumour varies from lytic to productive, or may contain multiple,

blood-filled cavities (telangiectatic form). Some osteosarcomas contain extensive areas of necrosis that are of no diagnostic value. Early metastasis to the lungs is a feature of osteosarcoma but metastasis to regional lymph nodes is uncommon (approximately 5% of cases).

In dogs, there is convincing evidence that osteosarcomas can be induced by bone infarcts, metallic implants used in fracture or cruciate ligament repair, or chronic inflammation; although it is important to recognise that the risk is small. In such cases the tumour may occur at atypical skeletal sites, such as distal humerus, proximal radius, or the mid-shaft of long bones. Similarly, infarct-associated osteosarcomas do not show the typical breed predilection; in fact miniature schnauzers, are notably over-represented. The mechanism is poorly understood but most likely involves chronic irritation and on-going stimulation of osteoblastic activity.

Predilection sites for osteosarcoma in cats are less well defined but, as in dogs, the appendicular skeleton is favoured. Implant-associated osteosarcomas are not reported in cats but I have seen fibrosarcoma, identical to cutaneous vaccine-associated sarcomas, in the distal femur of 2 young cats, possibly due to inadvertent injection of a vaccine, or other irritant material, into the bone.

Osteosarcomas are rare in domestic species other than dogs and cats, and published reports, often based on retrospective studies, are not always convincing.

Diagnosis of osteosarcoma

A definitive diagnosis of osteosarcoma is based on the demonstration of malignant mesenchymal cells producing osteoid and/or bone, but the cellular characteristics can vary markedly, as can the composition of the matrix. In some osteosarcomas the tumour cells are well differentiated and clearly recognisable as osteoblasts but in others they are highly anaplastic and cannot be distinguished from other anaplastic sarcomas in routinely stained sections. Osteoid produced by malignant osteoblasts may be sparse or abundant, but is typically disorganised and variably cellular. Formation of reactive bone in the endosteum and/or periosteum occurs in a variety of inflammatory and neoplastic diseases of bone, and must be differentiated from the tumour bone of an osteosarcoma. Trabeculae of reactive bone are typically lined by a single layer of well-differentiated osteoblasts and separated by connective tissue, while in osteosarcoma the spaces between tumour bone and osteoid deposits are generally filled with neoplastic cells. Mitoses are often abundant and abnormal.

The most significant limiting factor in making a definitive diagnosis of osteosarcoma in a live animal is the quality of the biopsy sample. Small core biopsies may not be representative of the lesion and, in my experience, often consist of nothing but reactive periosteal bone. It is crucial that surgeons examine radiographs prior to surgery and aim to include any lytic or productive areas in the medullary cavity. Even

when cellular areas are obtained, cell detail may have been reduced by exposure to acidic decalcifying agents. Not surprisingly, the chances of making a diagnosis are improved as the quality and quantity of biopsies from a lesion are increased.

Biopsies should be fixed in formalin as soon as possible after collection to avoid drying artefacts but I strongly encourage the preparation of smears for cytology prior to fixation. Gently rolling core biopsies on clean glass slides often yields excellent material for cytology, but it is important to keep unstained smears well away from formalin fumes or staining will be compromised. In tumours with little periosteal reaction it may be possible to obtain moderately to highly cellular preparations using fine needle aspiration (FNA) techniques. Osteosarcomas generally yield cells more readily than other sarcomas and the superior cell morphology of cytological preparations can be crucial in making a definitive diagnosis. In fact, examination of good quality cytological smears from an osteosarcoma will obviate the need for histology in some cases, especially when the clinical details, location of the lesion, and radiographic features are consistent with this diagnosis. The challenge is to differentiate reactive from malignant osteoblasts. In well-differentiated osteosarcomas this is extremely difficult if not impossible and histology is required in order to assess the architecture of the lesion, but in many cases the cellularity of the preparation and the pleomorphism of the osteoblasts justify a diagnosis of malignancy.

In cytological preparations, staining cells for alkaline phosphatase activity with nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate toluidine salt (NBT/BCIP) is useful in distinguishing osteosarcoma from other mesenchymal tumours. However, reactive osteoblasts will also stain positively so it is essential to ensure that the population of cells in question show convincing features of malignancy. Since the stain requires previously unstained slides it is useful to keep one or more slides unstained in cases where osteosarcoma is suspected. Immunoreactivity for the non-collagenous, bone specific proteins osteocalcin and osteonectin can be used as markers of osteogenic cells in histological sections, but will not distinguish between neoplastic and reactive cells. The sensitivity and specificity of these markers in animals has yet to be evaluated.

Value of imaging techniques

The value of radiography and/or CT imaging in the diagnosis of osteosarcoma should not be underestimated by pathologists and should be viewed in conjunction with the cytology or histology whenever possible. Such images generally yield more valuable information on skeletal lesions than gross examination and will usually be readily available, so why not make use of them? Surgeons should be encouraged to include digital radiographic images of skeletal lesions routinely with biopsies or cytological preparations from bone lesions, together with details of where the samples were collected from.

In radiographs, osteosarcomas vary from primarily lytic to highly productive, and possess characteristics of aggressive bone lesions, including: wide, poorly-defined margins between normal and abnormal bone, lack of sclerosis in the surrounding bone and an irregular, interrupted periosteal reaction. Cortical destruction and a periosteal reaction are consistent findings depending on the stage of the lesion. Periosteal new bone may progress from an early “brush” pattern to a coarse “sunburst” pattern characterised by radiating spicules of bone perpendicular to the cortical surface, but in many cases the periosteal reaction comprises an amorphous density reflecting the content of poorly mineralised woven bone. A characteristic triangular wedge of bone referred to as Codman’s triangle often merges with the cortex beneath the elevated periosteum at the periphery of the tumour. Unfortunately, these features are not specific for osteosarcoma and can also occur in other aggressive primary or secondary bone tumours, including chondrosarcoma, fibrosarcoma, and haemangiosarcoma, hence the need for confirmation using cytology and/or histology.

Benign skeletal lesions generally have well-defined margins with a short transition zone and may possess a sclerotic border, reflecting their slower growth. The cortex may be intact or expanded and thin, and the periosteal reaction, if present, will usually be more solid, smooth and continuous.

Alterations in the radiographic picture can be a useful aid in cases where a histological or cytological diagnosis of osteosarcoma is equivocal as these tumours often progress rapidly and repeat radiographs after 1 or 2 weeks may show dramatic changes. Such a suggestion to the surgeon must however be balanced against the increased risk of metastasis if the tumour is not removed immediately.

Other skeletal tumours and tumour-like lesions

The list of neoplastic and non-neoplastic skeletal lesions that could be confused with osteosarcoma is too long to discuss in detail here, but key differentials will be mentioned briefly and illustrated during my presentation.

Differentiation of osteosarcoma from other malignant tumours such as chondrosarcoma, fibrosarcoma and haemangiosarcoma is difficult, especially when these tumours occur at predilection sites of osteosarcoma and biopsy specimens are small. Matrix characteristics vary markedly between and within osteosarcomas and may include cartilage and fibrous tissue as well as osteoid. Furthermore, chondrosarcomas may include bone that has formed by endochondral ossification. In many cases it is impossible with the information and samples available to reliably distinguish between these tumours and the report to the surgeon or clinician should reflect this.

Plasma cell myeloma is associated with lytic lesions in bone in dogs and cats and may be confused with osteosarcoma, but, unlike osteosarcoma, the lesions are often multiple and have a typical punched out appearance. Vertebrae are most frequently

affected but lesions can also occur in other bones, including ribs and limb bones. FNAs from lytic bone lesions are often diagnostic in plasma cell myeloma, especially if the animal also has hyperproteinaemia and a monoclonal gammopathy.

Multilobular tumour of bone occurs primarily in the flat bones of the skull of dogs and may have a characteristic stippled pattern radiographically. Diagnostic confirmation is relatively straightforward if biopsies are large enough to demonstrate the characteristic lobular pattern histologically, but cytology is unlikely to be diagnostic. FNAs are usually poorly cellular and likely to contain predominantly well-differentiated mesenchymal cells that cannot be differentiated from reactive osteoblasts, fibroblasts or chondroblasts, but may provide useful information in tumours that have transformed into osteosarcomas or chondrosarcomas.

Histiocytic sarcomas of synovial origin may invade the adjacent bone causing cortical destruction and bone lysis but, unlike osteosarcoma, they sometimes involve bone on both sides of the joint. FNAs of such tumours are often highly cellular and can be diagnostic.

Other benign or tumour-like lesions that must be differentiated from osteosarcoma include: osteochondroma, ossifying fibroma, osteoma, fibrous dysplasia, aneurysmal bone cysts, intra-osseous epidermal inclusion cysts and exuberant fracture calluses. It is important to remember that pathological fractures will often occur at sites of benign or malignant bone tumours and cause confusion both radiographically and microscopically. Mycotic osteomyelitis can cause lytic lesions resembling osteosarcoma radiographically and is an important differential in some countries.

Conclusions

In spite of the many recent advances in diagnostic methods, confirmation or exclusion of osteosarcoma in biopsy samples or cytological preparations remains a significant challenge for veterinary pathologists. The quality of material submitted for examination is frequently a limiting factor, as is the accompanying clinical information, and pathologists should not feel compelled to make a definitive diagnosis if it cannot be adequately defended. Better use of cytology and radiographic images could be made by anatomical pathologist when interpreting skeletal lesions.

MURINE TERATOCARCINOMAS - TUMORS THAT OPENED THE ROAD TO EMBRYONIC STEM CELLS AND TRANSGENIC MICE

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Teratomas are benign tumors composed of somatic tissues derived from all three primordial germ layers: ectoderm, mesoderm and endoderm. The malignant equivalents of these benign tumors are called teratocarcinomas. Like teratomas, teratocarcinomas are composed of differentiated somatic tissue, but in addition they contain undifferentiated malignant stem cells, called embryonal carcinoma cells (ECC). Teratocarcinomas may also contain extraembryonic tissues, such as yolk sac or placental derivatives. Most teratomas and teratocarcinomas originate from germ cell in the male or female gonads, but may also occur in extragonadal sites. These tumors occur in humans as well as in several laboratory animals, but at a relatively low rate.

Modern research of teratoid tumors began with the work of L.C. Stevens of Jackson Laboratory, Bar Harbor Maine, who reported in 1954 that 1% of all adult males of the 129 strain mice have teratomas of the testis. L.C. Stevens subsequently increased the incidence of spontaneous testicular teratomas by cross breeding the original 129 strain mice with other strains. In these experiments he also discovered malignant teratoid tumors which he called teratocarcinomas. Upon retransplantation of teratocarcinomas it was possible to generate pure stem cell tumors composed exclusively of embryonal carcinoma cells, and other malignant tumors as yolk sac carcinoma. Stevens and other scientists, including our group, subsequently discovered that teratocarcinomas can be produced from early mouse embryos transplanted to extrauterine sites. These experiments proved that ECC represent malignant equivalents of early embryonic ectodermal cells and cells forming the inner cell mass in the mouse blastocyst. ECC were isolated *in vitro* and propagated as permanent cell lines. Furthermore it was shown that they resemble human ECC, which could also be passaged *in vitro* as permanent cell lines. The developmental equivalence between the ECC and early embryonic cells of the inner cell mass of the mouse blastocyst led several scientific groups to isolate permanent cell lines from the mouse inner cell mass. These cells called embryonic stem cells (ESC) showed remarkable similarity to malignant ECC derived from teratocarcinomas. Both ECC and ESC could be injected into the mouse blastocysts whereupon the ECC or ESC would integrate with other cells of the blastocyst to produce chimeric mice composed of the normal cells of the blastocyst and ECC or ESC respectively. These experiments led to a pro-

duction of transgenic mice and also stimulated the production of ESC from human embryos as well as embryos of many laboratory and domestic animals. Finally it was shown that cells equivalent to ESC can be produced from somatic cells enriched by not more than four crucial genes, which transformed these terminally differentiated cells into induced pluripotent embryonic cells. Taken all together these experiments performed over a period of 60 years illustrate how the study of a rare mouse tumor led to a major change in our perception of the embryonic cells, their pluripotency and developmental potential and the entire process of cellular differentiation. These experimental studies also showed that embryonic and neoplastic development are closely related one to another, and that by studying one process we could learn a lot about the other and vice versa. The relevance of animals studies for the understanding of the human condition was reconfirmed.

NEW VIRAL DISEASES IN WILDLIFE

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There is increasing interest in viral diseases of wildlife, not only in Europe, but worldwide, and veterinary pathologists have an important role to play in this field. The main reason for this increased interest is the recognition of the role that wildlife plays as a potential reservoir for emerging viral diseases in humans, such as West Nile fever and influenza: 75% of these are zoonotic, with the majority originating from wild animals. However, both domestic and wild animal populations also may suffer from viral diseases, e.g., classical swine fever and phocine distemper, that have a reservoir in wildlife.

Veterinary pathologists have an important role to play both in the first discovery of an emerging viral disease and in its surveillance. The etiology of diseases like SARS, West Nile fever, and dolphin morbillivirus infection was determined thanks in part to the involvement of veterinary pathologists. Veterinary pathologists also are in the front line of passive surveillance of viral diseases in wildlife, which are often first recognized in a new area based on unusual morbidity or mortality in wildlife.

Therefore, it is important for veterinary pathologists to: understand the context in which emergence of viral diseases is occurring; realize the important role that they can play in discovery and surveillance of viral diseases of wildlife; and to be updated about the pathology of the most significant viral diseases found recently in wildlife. These are the areas that I propose to cover in this talk.

TOXICOLOGIC PATHOLOGY: ENTER THE ZEBRAFISH

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The first acute toxicity studies using fish were undertaken in 1863. Following World War II, the use of fish models increased with the widespread introduction of environmentally persistent pesticides. During the 1970's-1990's, the use and refinement of fish as efficacy and toxicological test species gained momentum and since the early 21st century, there has been an explosion in the number of publications referencing the use of fish, particularly the zebrafish species, in toxicological testing.

Zebrafish lend themselves particularly well for use in acute and chronic toxicity testing through a variety of advantageous characteristics, including their small size (1-1.5 inches long), replicable/reliable breeding and maintenance conditions and low relative cost per animal.

Zebrafish have been successfully used in the area of developmental toxicology, namely the investigation of teratogenesis. For example, the administration of ethanol, to zebrafish embryos, is associated with cranio-facial abnormalities and aberrations in learning behaviours, features analogous to Foetal Alcohol Syndrome in humans. Such applications are well suited given the high degree of conservation between zebrafish and human developmental processes and the fact that zebrafish embryos can be cultured to a late stage of organogenesis. Furthermore, the presence of optical clarity allows for direct visualization of developmental mutagenesis and the opportunity for whole-mount larval antibody staining techniques.

Cardiotoxicity, in particular QT prolongation leading to "Torsades de pointes", a serious ventricular tachycardia, has been responsible for the withdrawal of several major pharmaceuticals over recent years. The zebrafish is well placed in pre-clinical screening to detect potential cardiotoxins, including those that may lead to prolonged QT interval in man. Notably, the zebrafish *Ether-a-go-go* Related Gene (ERG) shares 99% amino acid sequence conservation in the pore-forming region when compared to human ERG. Assessment of heart rate, rhythmicity, contractility and circulation at 48 hours post fertilization (hpf) can be enhanced with the use of heart fluorescence. Bradycardia, decreased contractility and slow circulation in zebrafish has been associated with arrhythmia, cardiomyopathy and QT prolongation in man, proving this species as a reliable predictor of clinical cardiotoxicity.

Neurotoxicity of both pharmaceuticals and agrochemicals is still a major cause of concern and current testing methods using *in-vivo* mammalian models have notable limitations and are often inherently subjective. Zebrafish models are well placed to study the effects of potential neurotoxins. The zebrafish nervous system shares some notable similarities to that of the mammal, for example, the well-characterized susceptibility of mammalian dopaminergic neuronal sensitivity to Parkinsonian-like toxicity induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Furthermore, zebrafish, given their temporal transparency and small size, are well placed for both *in-vivo* behavioural and morphological screens. Further scientific investigation can be complemented by the use of Glial Fibrillary Acidic Protein detection (a protein highly conserved between zebrafish and mammals) and the TUNEL technique for the detection of neuronal apoptosis. More recent work has focused on the representation of inner ear mammalian hair cells by "neuromasts", present within zebrafish, in the detection of potential ototoxicity.

Experimental hepatotoxicity screens in zebrafish have demonstrated several similarities to those obtained in conventional preclinical toxicity species, for example, the presence and upregulation of a CYP3A orthologue (which is responsible for up to 50% of drug metabolism in man). Indeed, for the metabolism of some compounds, the zebrafish liver may be a more reliable model of human hepatic drug interaction than the rodent, as exemplified by the ability of rifampicin to upregulate CYP3A in human and zebrafish, but not rat, hepatocytes. The zebrafish liver is functional by 72hpf and lends itself to high-throughput screening of potentially hepatotoxic pharmaceuticals at comparably lower financial and, arguably, ethical costs when compared to the testing of such compounds in rodents, dogs and primates.

Perhaps one of the best explored areas regarding fish models of toxicopathology remains the field of endocrine disruption. Overwhelming evidence, published since the 1970's suggests that our water bodies receive quantities of synthetic chemicals (such as contraceptive effluent) sufficient to perturb the reproductive balance within native fish species, with subsequent consequences to man. Teleost fish act as sensitive indicator species. Chemical androgenic/estrogenic agonism and antagonism, along with aromatase inhibition, can lead to primary effects upon gonadal development and maturation, in addition to secondary perturbation of reproductive duct formation. Endpoints assessed as evidence for endocrine disruption include the measurement of secondary sex characteristics, histopathological examination of the reproductive organs and measures of reproductive fecundity – e.g: spawning, fertilization and sperm quality. Peripheral measurement of vitellogenin, spiggin and sex steroid concentrations act as confirmatory biomarkers.

Sequencing of the zebrafish genome was completed by the Sanger centre by 2005 and multiple DNA microarrays are now available for genetic expression profiling, along

with the establishment of several transgenic lines. In addition, morpholino antisense oligonucleotide insertion and zinc finger nuclease techniques have been successfully used in gene knock-out experiments. For example, genetic knockouts can be compared to wild-type zebrafish treated with enzyme inhibitors, to elucidate off-target effects of an administered chemical.

Zebrafish have a relatively low incidence (1%) of spontaneous neoplasia; seminoma of the testes, hepatocellular and pancreatic adenoma being notable examples. Carcinogenicity testing of chemicals in small fish models was initiated following observations of environmental, chemically-induced, tumors in wild fish, for example, the finding of hepatic megalocytosis and neoplasia in English sole from the Puget Sound, due to the presence of polycyclic aromatic hydrocarbons in the water sediment and the finding of hepatic and exocrine pancreatic neoplasia in mummichog from a creosote-contaminated site in the Elizabeth River, Virginia. A comprehensive study undertaken at Oregon State University assessed the carcinogenetic effects of exposing embryos, fry and juvenile fish to a panel of carcinogens, for up to 9 months. The greatest diversity of neoplastic morphology was observed in the younger cohorts. The liver was identified as a primary target, irrespective of stage of exposure. Other studies have refined the technique of transgenic manipulation to increase the consistency of potential models, for example overexpression of *mycn* as a predisposition to the development of neuroblastoma.

The use of zebrafish in toxicological research is now far-reaching. Work has recently expanded into such diverse areas as assessing biomaterial nanotoxicity and investigating the reinforcing properties of drug abuse.

To summarize, interest and use of the zebrafish in both toxicology and pathology, along with allied disciplines, has increased dramatically over the last decade. The species offers notable advantages over the traditional use of mice, rats, dogs and non-human primates. However, as with any model species, there are still limitations and potential caveats. For example, the strain and source of stock fish is often omitted in the scientific literature, precluding the establishment of a common control population. Furthermore, microsporidiosis (*Pseudoloma neurophilia*) and Piscine mycobacteriosis remain problematic infectious diseases within experimental colonies. Skewed sex ratios amongst zebrafish colonies remain a worldwide problem, often via variable genetic and environmental causes.

Finally, few toxicology studies to date have incorporated rigorous light/electron microscopic evaluation in zebrafish and there remains a dearth of experience regarding this species, amongst the community of veterinary pathologists. This presents as a significant limitation in the promotion of the zebrafish as a preclinical and environmental test species, but conversely offers an exciting opportunity to those interested in this field.

Recommended Articles

In-Vivo Drug Discovery in the Zebrafish: Zon, L.I. and Peterson, R.T. Nature Reviews (2005) Vol.4. 35-44

1. Issues Related to the Use of Fish Models in Toxicologic Pathology: McHugh Law, J. *Tox. Path.* Vol. 31 (supp.) (2003) pp49-52
2. Molecular Biomarkers of Endocrine Disruption in Small Model Fish: Scholz, S. and Mayer, I., *Mol. And Cell. Endo.* (2008) 293: 57-70
3. The State of the Art of the Zebrafish Model for Toxicology and Toxicologic Pathology Research – Advantages and Current Limitations: Spitsbergen, J.M. and Kent, M.L. *Tox. Path.* Vol. 31 (supp.) (2003) pp62-87
4. Zebrafish: A Predictive Model for Assessing Drug-Induced Toxicity: McGrath, P. and Li, Chun-Qi., *Drug Dis. Today.* (2008) Vol. 13, 9/10
5. Zebrafish as a Correlative and Predictive Model for Assessing Biomaterial Nanotoxicity: Fako, V.E. and Furgeson, D.Y., *Adv. Drug Delivery Revs.* (2009) 61: 478-486
6. The Zebrafish as a Model System for Assessing the Reinforcing Properties of Drugs of Abuse: Ninkovic, J. And Bally-Cuif, L., *Methods* (2006) 39: 262-274
7. Zebrafish as a Model Vertebrate for Investigating Chemical Toxicity: Hill, A., Teroka, H., Heideman, W. and Peterson, R.E., *Tox. Sci.* (2005) 86(1), 6-19
8. Zebrafish Assays for Drug Toxicity Screening: Rubinstein, A., *Toxico.* (2006) 2(2): 231-240

**ORAL PRESENTATIONS
SESSION 2A - ONCOLOGY**

HISTOMORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF BOVINE PERIPHERAL NERVE SHEATH TUMOURS

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Introduction

Bovine peripheral nerve sheath tumours (PNSTs) are some of the most frequently found neoplasms at routine meat inspections. Despite this, the knowledge on their genesis is limited and inconsistency exists regarding their nomenclature and sub-classification. The aim of present study was to describe and classify bovine PNSTs and to evaluate new immuno-diagnostic markers for the lesion.

Material and methods

From the archives at the Department of Veterinary Disease Biology, University of Copenhagen, 71 samples from 43 cows were retrieved. Following histomorphological examination, the slides were immunolabelled with antibodies against S100, neurofilament and 2',3'-cyclic nucleotide-3'phosphohydrolase (CNPase).

Results

Positive immunostaining was seen in 67 (94%) and 69 (97%) samples labelled with antibodies against CNPase and S100, respectively. Given the histomorphological and immunohistochemical characteristics, the tumour tissues were divided into four different patterns: 1) An Antoni A pattern, 2) an Antoni B pattern, 3) a fascicular pattern, and 4) a neurofibromatous pattern. In the majority of the slides more than one pattern was recognized.

Conclusion

In bovine PNSTs, the schwannomatous and neurofibromatous tissues often coexist and it is therefore irrelevant to sub-classify these tumours. Antibodies against S100 and CNPase are valuable diagnostics markers for bovine PNSTs.

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PROTEINS IN THE PRIMARY TUMORS OF METASTASIZING CANINE MAMMARY TUMORS USING 2D-DIGE AND MASS SPECTROMETRY

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Introduction

Mammary gland tumors are a major health problem in women and female dogs. In both species metastasis of the primary tumor to distant organs is the most common cause of tumor-related death. Nevertheless, the molecular mechanisms of tumor metastasis are far from being understood and it is still unknown why some human and canine carcinomas metastasize and others do not.

Material and Methods

Canine normal mammary gland (n=6), adenoma (n=6), lymph node-negative carcinomas (n=6) and lymph node-positive carcinomas (n=6) were compared using 2D-DIGE and MALDI-TOF mass spectrometry to identify metastasis-associated protein expression patterns.

Results

For 32 proteins significant changes in protein expression (fold change > 1.5, $p < 0.05$) were found in the primary tumors of lymph node-positive carcinomas when compared with normal mammary gland, adenomas and lymph node-negative carcinomas.

Conclusion

Several new potential markers of metastatic spread were identified in canine mammary tumors. Interestingly, a significant number of these have been described as prognostic markers in human (breast) cancer before. Further investigations are now necessary to test whether these markers are of prognostic value for canine mammary carcinomas and whether their expression is directly involved in canine mammary carcinogenesis or represent solely a secondary reactive phenotype.

MAXILLOFACIAL ALVEOLAR RHABDOMYOSARCOMA IN A JUVENILE DOG

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Introduction

Most round-cell tumours can be accurately diagnosed cytologically. However, other tumours also sometimes exhibit round-cell tumour-like morphology. This report describes the unique cytological and histological features of a maxillofacial mass in a juvenile dog.

Materials and Methods

A maxillofacial mass in a 15-month-old castrated male dog with a history of intermittent epistaxis and sneezing was examined. Computed tomography revealed invasion of the orbit and nasal cavity and lytic destruction of the right maxilla. A punch biopsy of the mass and its impression smears were obtained and routinely subjected to pathological examinations.

Results

Numerous round cells (diameter, 5–25 μm), resembling lymphoid cells, with moderate amounts of clear-to-pale blue cytoplasm were observed. Mitotic figures were frequently observed. Malignant round-cell neoplasia was diagnosed by cytological examinations. Histological examinations revealed diffusely arranged small atypical round cells with a sparse fibrovascular stroma. Immunohistochemical analysis revealed negative staining for CD antigens, cytokeratin, melan-A, chromogranin A, α -smooth-muscle actin, and myoglobin whereas positive staining for vimentin, desmin, myogenin, and MyoD1. Thus, solid-pattern alveolar rhabdomyosarcoma was diagnosed.

Conclusions

Alveolar rhabdomyosarcoma should be considered in the differential diagnosis of tumours in juvenile dogs, especially on observation of round undifferentiated cells.

BAG3 IS OVEREXPRESSED AND COIMMUNOPRECIPITATES WITH BOVINE PAPILLOMAVIRUS TYPE 2 (BPV-2) E5 PROTEIN IN UROTHELIAL TUMORS OF THE URINARY BLADDER IN CATTLE

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Introduction

Bovine papillomavirus type 2 (BPV-2) is actually believed to be the most important infectious agent involved in bladder cancer of cattle. BAG3 plays a role in Ca²⁺ mobilization and protein kinase C activation via phospholipase C-gamma. Furthermore, it appears to sustain cell survival in some cancer cells via the NF- κ B pathway and regulates motility and adhesion of epithelial cancer cells suggesting a crucial role to the invasive and metastatic phenotype of cancers.

Materials and Methods

Twenty-five urothelial tumors were collected at public slaughterhouses in the south of Italy. All the animals were grazed in lands rich in bracken fern and clinically suffered from chronic hematuria. In all of the urinary bladders were detected neoplastic lesions diagnosed following morphologic parameters as recently reported (Roperto et al., 2010).

Results

BAG3 is overexpressed in urothelial tumors of cattle. It colocalizes and coimmunoprecipitates with BVP-2 E5 which allow us to suggest that BAG3 interacts with the viral oncoprotein and may represent an another partner involved in transforming mechanism(s) in papillomavirus-associated cancer cells.

Discussion

The potential role of Bag 3 in urothelial tumors of the urinary bladder of cattle is discussed.

References

Roperto et al. Review of Bovine Urothelial Tumors and Tumor-like lesions of the urinary bladder, J. Comp. Pathol. 142, 95-108, 2010

MECHANISM OF EQUINE LIPOMA STRANGULATION

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Introduction

Lipoma-related intestinal strangulation of the equine small intestine is a common cause of colic in the horse. The pathogenesis is poorly understood. The Glass Horse Equine Colic CD is a teaching tool that shows animations of normal anatomy and 28 colic conditions. The CD's animation shows that lipomas tie themselves into a half hitch knot, and the intestine slips into the loop formed and undergoes venous infarction.

Materials and Methods

To test the hypothesis that lipomas knot themselves to cause strangulation, five strangulated lipomas were undone. The site of the compromised intestines, length of the lipoma stalk and the presence of knots or rents in the mesentery were recorded.

Results

No knots or holes in the mesentery were found. All lipomas had short (5-7cm) stalks originating 4-7 cm from the attachment of the mesentery to the intestine. The diameter of the lipomas (4-7cm) often made tying a knot after undoing the strangulation difficult. In all cases, the greater omentum was involved.

Conclusion

The omentum pulls on lipomas and creates the loop through which the intestines pass. Pressure of the swollen intestines lock lipomas in position, and omental tension is not needed to maintain the strangulation.

ORAL PRESENTATIONS
SESSION 2B - INFECTION DISEASES

ANTI-INFLAMMATORY EFFECTS OF BUTYRICICOCCCUS PULLICAECORUM, A NORMAL INHABITANT OF THE CHICKEN INTESTINAL TRACT

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Introduction

An increasing number of data support the idea that protection against inflammation in the normal intestinal tract is a dynamic process mediated by “beneficial” microorganisms. We recently isolated *Butyricoccus (B.) pullicaecorum*, a strictly anaerobic, gram-positive, coccoid organism, from the caeca of a broiler chicken. This new species produces high amounts of butyrate in vitro. The purpose of the present study was to evaluate the anti-inflammatory effects in a rat colitis model.

Materials and methods

Ten rats were orally inoculated every day with 10×10^9 *B. pullicaecorum*. On day 7, the rats were administered TNBS intrarectally. Two days later, the rats were euthanized. There were also 10 positive and 10 negative control rats. The Wallace score was used for evaluation of gross lesions. This score was converted into a protection score.

Results

B. pullicaecorum gave a protection score of approximately 70%. The ratio of ulcer length / colon length was reduced from 15.8% in the positive controls to <4% in the treated group. Myeloperoxidase activity was reduced from 1.66 to 1.28. Histologic lesion score was reduced from 15.2 to 8.2.

Conclusion

These results show that *B. pullicaecorum* can protect against colitis in a TNBS rat model.

BVD TYPE 2, CLOSELY RELATED TO THE NORTH AMERICAN HYPERVIRULENT VIRUSES, IN BELGIUM

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Introduction

Severe acute bovine viral diarrhoea virus type 2 (BVDV-2) was first identified in Belgium in 1991 and is periodically isolated since that time but so far, has not been associated with severe epidemics.

Material and methods

Case 1, a cow with uterine and intestinal hemorrhages, died shortly after initial examination. Case 2 was a 1.5 years old Belgian Blue bull with a one day history of teeth grinding, anorexia, hypothermia, tenesmus and recumbency and watery faeces with fresh blood.

Results

Necropsy of case 1 revealed edema and hemorrhages of the uterine wall. In case 2, enteritis, colitis and proctitis were found and two small erosions were visible on the dental pad, where the incisors have contact with the gums. Uterus of case 1 and blood samples of case 2 respectively were positive for BVDV-2. PCRsequences were aligned with other BVDV-2 sequences. Both viruses clustered with the North American BVDV-2, responsible for hemorrhagic syndrome and severe outbreaks.

Conclusion

Even if no severe epidemic was recorded in Belgium, two isolates closely related to strains from severe outbreaks in North America were associated with the hemorrhagic case. This is the first isolation and characterisation of such virus in Europe.

BRAIN INFECTION FOLLOWING EXPERIMENTAL STAPHYLOCOCCUS AUREUS SEPSIS IN PIGS

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Introduction

Sepsis is a major problem in humans and both the incidence and mortality is increasing. Multiple microabscesses can be found in the brain of septic patients. *Staphylococcus aureus* is one of the most common causes of sepsis and brain abscesses. *S. aureus* is also a frequent cause of spontaneous porcine pyemia including endocarditis and associated brain lesions. We present a porcine model of haematogenous *S. aureus* induced brain infection.

Materials and Methods

Twelve pigs received an intravenous injection of *S. aureus* of 10^8 CFU/kg body weight once at 0h or twice at 0h and 12h. Four pigs were kept as controls. The pigs were euthanized in groups of four at either 6, 12, 24 or 48 h post infection. The brain was collected from all the animals and examined histologically.

Results

All the inoculated pigs developed sepsis and 7 out of 12 animals had microabscesses in the prosencephalon. The brain lesions showed a temporal progression.

Conclusion

The brain lesions in the experimental infected pigs were similar to the brain lesions observed in pigs with spontaneous endocarditis and those observed in humans with septic encephalopathy respectively. This proves the porcine model as valid.

ANAPLASMA PHAGOCYTOPHILUM- SITES OF PERSISTENT INFECTION IN SHEEP

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Introduction

Anaplasma phagocytophilum (AP) is an intracellular rickettsial organism and is the causative agent of tick borne fever (TBF) in ruminants. AP is transmitted by the tick *Ixodes ricinus* and infection causes pyrexia, thrombocytopenia, and immune suppression. After infection, the organism causes bacteraemia followed by prolonged persistence in tissues with possible subsequent bouts of bacteraemia. In acute infection, the organism has been demonstrated in alveolar macrophages, Kupffer cells, and other tissue macrophages. Sites of persistence have yet to be established.

Materials and Methods

12 naïve cross-bred lambs were infected intravenously with a TBF variant of AP and surveyed for an interval between three weeks and 12 months. A range of tissues was assessed by light microscopy including immunohistology and DNA-ISH, by transmission electron microscopy and by quantitative PCR for AP DNA.

Results

Lung and liver were identified as sites of persistent infection in experimentally infected sheep. Pulmonary intravascular macrophages and hepatic Kupffer cells were shown to bear the agent.

Conclusions

Our results demonstrate AP persists in specific tissue macrophages in constant contact with the blood. These have been shown to also be the primary site of AP infection, prior to bacteraemia.

OF MAN AND PIGS: NEW INSIGHTS INTO THE PATHOGENESIS OF CLOSTRIDIUM PERFRINGENS TYPE C ENTERITIS

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Introduction

Clostridium perfringens type C causes fatal necrotizing enteritis in animals and humans. The main virulence factor of pathogenic strains is beta-toxin (CPB), a member of the beta-barrel pore forming toxin family. Previous immunohistochemical studies showed CPB binding at thrombotic vessels in intestinal lesions. To investigate whether endothelial cells are specifically targeted by beta toxin, we established a novel in vitro system using primary porcine and human endothelial cells and recombinant CPB (rCPB).

Material and methods

Primary porcine and human endothelial cells were incubated with different concentrations of clostridial culture supernatants and rCPB expressed in *E. coli*. Cellular effects were monitored using live cell imaging, immunofluorescence, cell viability assays, FACS analyses and western blot analyses of membrane fractions.

Results

Both, culture supernatants and rCPB, induced marked cytopathic and cytotoxic effects, with a rapid disruption of the actin cytoskeleton, cell border retraction and cell shrinkage followed by cell death. These effects led to a disintegration of the cell monolayer and were inhibited by neutralization with monoclonal antibodies against CPB.

Conclusions

Our results provide the first indication of how CPB may mediate the disruption of the endothelial barrier leading to vascular thrombosis and ischemic tissue necrosis in *Clostridium perfringens* type C enteritis.

**ORAL PRESENTATIONS
SESSION 4A - MODERN METHODS
IN PATHOLOGY**

RNAI MEDIATED INHIBITION OF XIAP COMBINED WITH SYSTEMIC MESENCHYMAL STEM CELL-MEDIATED DELIVERY OF STRAIL INHIBITS METASTATIC GROWTH OF PANCREATIC CARCINOMA CELLS.

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Introduction

Metastasis is a common complication of oncological disease where conventional treatments show limited benefits. Combining the apoptosis-inducing activity of TRAIL with the ability of mesenchymal stem cells (MSCs) to infiltrate tumors and lymphatic tissues, we targeted primary tumors and metastasis in a human pancreatic cancer mouse model. Furthermore, we silenced XIAP by RNAi inside the tumor cells for apoptosis sensitisation and anti-metastatic effects.

Material and Methods

We generated MSCs expressing a soluble wild-type TRAIL (MSC.sTRAIL) containing an isoleucine zipper for higher activity and the signal peptide from the human alpha-1 antitrypsin gene for optimal secretion. Their effect on the human pancreatic carcinoma cells Panc-1 and PancTu1 was studied in vitro. In addition xenograft models were treated with MSC.sTRAIL.

Results

Panc-1 and PancTu1 cell cultures, which are normally TRAIL resistant, were slightly sensitive to MSC.sTRAIL. Additionally, knocking-down XIAP by RNAi in the tumor cells increased apoptosis. Xenografts showed partial responses to MSC.sTRAIL, whereas tumors with silenced XIAP went into remission and showed low rate metastasis, compared with animals treated with control MSCs.

Conclusion

Combined approach using systemic MSC-mediated delivery of sTRAIL together with inhibition of XIAP inside pancreatic carcinoma cells reduced metastatic growth.

OPTIONS AND LIMITS FOR THE DETECTION OF BACTERIA IN THE MARE'S UTERUS BY IN-SITU HYBRIDISATION

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Introduction

While in human medicine bacterial biofilms are noted for a common cause of persistent infections, little is known about their existence in the genital tract of the mare. In this context, the use of in-situ hybridisation as diagnostic tool was assessed.

Materials and Methods

Endometrial biopsies were obtained from 96 thoroughbred mares showing genital infection with *Streptococcus* spp. that were detected by cultural examination of uterine swabs. The biopsies were processed for conventional H.-E.-staining and for in-situ hybridisation (ISH) with digoxigenin-labeled probes.

Results

Histologically, 33 mares showed an endometritis varying in degree and character. By means of ISH *Streptococcus* spp. were detected on the luminal epithelium and in the superficial secretory ducts of the uterine glands in 6 of these mares. In 9 more biopsies *Streptococcus* spp. were found without histological signs of endometritis.

Conclusion

Although cultural examination of uterine swabs provides more comprehensive results, by means of ISH the spatial organisation of bacteria in the endometrium can be characterised. In this study no evidence for the formation of biofilms by *Streptococcus* spp. could be found, but nevertheless they may possibly contribute to endometritis in some cases. Further examinations on genital pathogenic germs (e.g. *E. coli*, *Pasteurella* spp.) are planned.

BUTYRATE ENHANCES GALT DEVELOPMENT IN THE EARLY POST-HATCH PERIOD IN BROILERS

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Introduction

The gut-associated lymphoid tissue (GALT) is of major importance in the defense against pathogens. In chickens, GALT development and functional maturation occurs during the first 2 weeks of life. During this period, increasing numbers of lymphocytes appear in the mucosa. Simultaneously, lymphocyte functions mature, lowering the risk of disease. Butyrate is known to alter enterocyte chemokine expression, thereby influencing leukocyte recruitment in the intestine. This study was undertaken to assess the effect of butyrate on gut histomorphology and GALT development in chicks.

Materials and methods

Chicks receiving butyrate (Ovocrack®) were compared with control-fed chicks. At 14 days of age, zootechnical parameters were determined and ileum histomorphology was assessed.

Results

Butyrate improved weight gain and feed conversion ratio, and increased villus length. In addition, more mucosal lymphocytes were present. Other intestinal histomorphological features (crypt depth, villus fusion, muscularis thickness, crypt epithelial cell proliferation) were not significantly different.

Conclusion

Adding butyrate to the feed of young broilers not only results in better zootechnical parameters and increased villus lengths but also induces a more densely populated GALT at the age of 14 days. The latter may correspond to a more rapid maturation of mucosal immune responses.

PATHOLOGICAL EFFECTS OF QUINAZOLINONES ON THE STOMACH AND INTESTINE OF NEWBORN BALB/C MICE

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Introduction

Quinazolinones are heterocyclic components with various biological activities. In this survey, the teratogenic effects of two new synthetic derivatives of Quinazolinone including 4(3H) quinazolinone-2-propyl-2-phenyl ethyl (QPPE) and 4(3H)quinazolinone-2-ethyl-2-phenyl ethyl (QEPE) were performed on stomach and intestine of newborn Balb/C mice as an animal model.

Materials and Methods

100 pregnant Balb/C mice were randomly divided into 4 groups of control, sham and experimental 1&2 which received IP, 10 ml/kg distilled water (control group), 10 ml/kg methyl cellulose 0.05% (sham group) and 100 mg/kg QPPE, QEPE (experimental groups 1 & 2). After euthanasia, newborn stomach and intestine were removed. Tissue samples were fixed in neutral buffered 10% formalin and stained with H&E.

Results

Histopathological examination indicated an reduction in lengths of the villi and depths of the crypts of proximal portion in newborn Balb/C mice intestine of the experimental groups 1 & 2 which treated with (QPPE),(QEPE). The newborn mice in experimental groups had developed gastritis, hyperemia and decrease in thickness of stomach mucosal layer.

Conclusions

Investigation of the effects of Quinazolinones on fetus because of their consumptions during pregnancy can be useful to detect congenital abnormalities due to teratogens. This study showed that Quinazolinones could be the cause of pathological changes in stomach and intestine of newborn Balb/C mice.

PORCINE CIRCOVIRUS TYPE 2 (PCV2) MORPHOGENESIS IN A CLONE DERIVED FROM THE L35 LYMPHOBLASTOID CELL LINE

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Introduction

PCV2 is the essential infectious agent of postweaning multisystemic wasting syndrome (PMWS), one of the most important swine diseases. Although several studies have described different biological properties of the virus, some aspects of its replication cycle including ultrastructural alterations remain unknown. The aim of the present study was to describe for the first time a complete morphogenesis study of PCV2 in a clone of the L35 cell line at the ultrastructural level using electron microscopy techniques.

Materials and methods

Cells were infected with PCV2, MOI of 10, and examined at 0, 6, 12, 24, 48, 60 and 72 hours post infection.

Results and discussion

PCV2 was internalized by endocytosis after which the virus aggregated in intracytoplasmic inclusion bodies (ICIs). Later, PCV2 was closely associated with mitochondria, completing a first cytoplasmic phase. The virus entered the nucleus for replication, and virus assembly and encapsidation occurred with the participation of the nuclear membrane. Immature virions left the nucleus and formed ICIs in a second cytoplasmic phase. The results suggest that at the end of the replication cycle (between 24-48 hours), PCV2 was released by two different ways; either by budding of mature virion clusters or by lysis of apoptotic/death cells.

Conclusion

The L35 derived clone represents a suitable in vitro model for PCV2 morphogenesis studies and characterization of PCV2 replication cycle.

ORAL PRESENTATIONS
SESSION 4B - INFECTIOUS DISEASES

THE ROLE OF ANTIGEN PRESENTING CELLS AND T LYMPHOCYTES IN THE TONSIL OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS-INFECTED PIGS

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Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is an economically important disease in swine production. PRRS virus modulates the host immune response but the mechanisms involved remain still unclear. The aim of this study was to evaluate the changes in the subpopulations of antigen presenting cells and T lymphocytes in tonsils of PRRS virus infected pigs.

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. Tonsil samples were collected and fixed in 10 % buffered neutral formalin and in Bouin solution. Antibodies against PRRS virus, SWC3, S-100, HLA-DR and CD3 antigens were used in the immunohistochemistry study.

Results

Viral antigen was mainly expressed in the cytoplasm of macrophages and, secondly, in interdigitating dendritic cells, with a peak at 7 dpi. The different cell subpopulations showed an undulating kinetic, being observed only a mild enhancement in macrophages and follicular dendritic cell counts at 7dpi and 10dpi, respectively, and a decrease in the expression of Major Histocompatibility Complex and T lymphocytes at 3dpi.

Conclusion

PRRS virus may cause an ineffective antigen presenting cells and T lymphocytes activation.

This study was supported financially by the Spanish Ministry of Science and Innovation (AGL2009-12438).

ROLE OF CYTOKINES AS MEDIATORS OF THE LOCAL IMMUNE RESPONSE IN LUNG AND TONSIL OF PIGS INOCULATED WITH A EUROPEAN PRRSV FIELD ISOLATE

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Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is a significant economic burden. An erratic immune response to infection is characteristic of this syndrome. The aim of this research was to study the correlation between the expression of PRRSV antigen and the expression of cytokines in lung and tonsil of PRRSV-inoculated pigs.

Materials and Methods

Twenty eight, five-week-old pigs were randomly distributed in batches of four, inoculated with PRRSV field isolate 2982 and killed at 3, 7, 10, 14, 17, 21 and 24 days post-inoculation (dpi). Four other pigs, were used as controls, inoculated with sterile medium and killed at the end of the study (24dpi). Samples from the lung and tonsil were fixed in 10% buffered formaldehyde and in Bouin.

Results

Viral antigen and all the cytokines studied were expressed mainly by macrophages in lung and tonsil. All antigens displayed a higher and earlier expression in the tonsils than in the lungs, always following a similar trend in both tissues. The expression of IL-10 was correlated in both organs with the expression of PRRSV antigen.

Conclusion

PRRSV may modulate the immune response by the expression of IL-10, which might induce lower levels of other cytokines implied in viral clearance.

NOVEL GROUP A ROTAVIRUS G8 P[1] AS PRIMARY CAUSE OF AN OVINE DIARRHEIC SYNDROME OUTBREAK IN WEANED LAMBS

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Introduction

Ovine group A rotavirus (OVgAR) is a primary cause of ovine diarrheic syndrome (ODS) mainly in neonates. An outbreak of ODS was reported in 40-60 days-old Rasa Aragonesa lambs, with a morbidity of 95% (192/203) and a mortality of 17% (35/203).

Methods

Twenty-five lambs were divided following clinical affectation (healthy [n=5], mild [n=10], moderate [n=4] and severe [n=6]). Rectal faeces were analyzed by culture (aerobic and anaerobic bacteria, and fungi), zinc sulfate flotation test and one-step OVgAR immunochromatography. Besides, five severe NDS-affected lambs were necropsied (n=30) and OVgAR immunohistochemistry performed. Necropsied-lambs faeces were used for serotyping and genotyping of isolated *Escherichia coli* strains (LT, STa, STb, K88, P987, K99, F41, VT1, VT2, eae and F17), and for genotyping detected OVRgA strains (VP4, VP6 and VP7 genes).

Results

A new OVgAR G8 [P1] strain was sequenced. Among all potential studied causes of ODS, only OVgAR detection (14/30) was significantly correlated with clinico-pathologic findings (ANOVA $p=0.026$). Concurrently detected pathogens, not statistically associated to ODS, were *Campilobacter* spp (n=7), *C. parvum* (n=4), *Coccidium* spp (n=4), *C. perfringens* (n=3), *P. mirabilis* (n=3) and *E. coli* eae+ (5 positive/ 8 cultured strain).

Conclusion

A new OVgAR strain was apparently the major cause of ODS in 40-60 days-old lambs and, therefore, it should be considered within the ODS differential diagnosis.

INFLUENCE OF CANINE DISTEMPER VIRUS INFECTION ON THE CORTACTIN EXPRESSION AND DISTRIBUTION IN CANINE HISTIOCYTIC SARCOMA CELLS IN VITRO

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Introduction

Cortactin is a cytoskeletal protein that is commonly overexpressed in cancer. Infection of canine histiocytic sarcoma cells (DH82 cells) with the Onderstepoort (Ond) strain of Canine Distemper Virus (CDV) leads to morphological and functional modifications suggesting a less malignant biological behaviour. The aim of this study was to evaluate the potential underlying mechanism by investigating cortactin expression as a critical mediator of tumor cell migration and invasion.

Materials and methods

For immunofluorescence and immunoelectron microscopy, non-infected and persistently CDV infected DH82 cells were incubated with an anti-cortactin (H-191) antibody. The cellular morphology including cytoplasmic processes and the cortactin distribution were analyzed.

Results

100% of both cell types displayed cortactin expression. However, persistently CDV infected DH82 cells showed only a diffuse expression of cortactin in the cytoplasm (82%) or an additional accentuation in the cell membrane (12%). In contrast, non-infected cells exhibited a diffuse cytoplasmic reaction only (32%) or an increased expression in cell membrane and cytoplasmic projections (62%).

Conclusion

These preliminary data indicate that a CDV-mediated reduced cortactin expression in tumor cell processes may contribute to reduced cellular migration and may therefore be associated with a less malignant behaviour of canine histiocytic neoplasms.

PYOGRANULOMATOUS PLEUROPNEUMONIA AND MEDIASTINITIS IN FERRETS ASSOCIATED WITH CHRYSEOMONAS-LIKE BACTERIA

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Introduction

We present a new infectious disease of ferrets.

Material and Methods

In 2009, three, 1-2 years old ferrets from different sources, presented an acute episode of dyspnea, lethargy, hyperthermia, anemia, hyperproteinemia and leukocytosis with neutrophilia. Pleural exudate was obtained by thoracocentesis. Cytology revealed severe purulent inflammation with clusters of rod-shape to serpentine, 5-6 µm length microorganisms with clear halo. Medical treatment with antibiotics and oxygen supplementation was ineffective and the animals died.

Results

Two ferrets were necropsied and pyothorax, mediastinal lymph nodes lymphadenopathy and multiple, 1-2 mm, white nodules were observed in the lung parenchyma. Histopathology showed multifocal necrotizing-pyogranulomatous or suppurative bronchopneumonia, pleuritis and mediastinitis with intralesional, similar microorganisms. In situ hybridization for *Pneumocystis*, Ziehl-Neelsen, Gram, periodic acid-Schiff, and immunohistochemistry for distemper, coronavirus and influenza antigen were negative in all cases. Microbiology of pleural exudate from one ferret yielded a pure culture of Gram negative bacteria phenotypically identified as *Chryseomonas luteola*. By electron microscopy these organisms corresponded to 2-3 µm length bacteria with a thick electrolucent halo. Molecular identification is in progress.

Conclusions

These serpentine-bacilar bacteria are the cause of a fatal respiratory infectious disease of ferrets.

ORAL PRESENTATIONS
SESSION 6A - WILDLIFE DISEASES

PATHOLOGY OF PANDEMIC (H1N1) 2009 INFLUENZA VIRUS INFECTION IN MACAQUES, FERRETS AND CATS

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Introduction

A new influenza A virus of the subtype H1N1 (pH1N1), which first emerged in Mexico at the beginning of 2009, is the first pandemic human influenza virus of the 21st century. Previous influenza pandemics caused variable mortality with development of severe pneumonia. However, the ability of pH1N1 to cause pneumonia was poorly understood.

Materials and Methods

Macaques, ferrets and cats were inoculated with pH1N1 and seasonal H1N1 virus (sH1N1). The ability of both viruses to cause pneumonia was compared by clinical, virological, and pathological analyses.

Results

pH1N1 caused pneumonia in all animals that was more severe than that of sH1N1. In pathology, the percentage of affected lung parenchyma in pH1N1 was higher than that in sH1N1, showing diffuse alveolar damage with more flooding of the alveolar lumina and more inflammatory infiltrates. Diffuse alveolar damage was associated with higher loads of pH1N1 in the lower respiratory tract.

Conclusions

Disease associated with pH1N1 in macaques, ferrets and cats, resembles that in humans with a similar pattern of infection. These studies show that the pneumonia of pH1N1 is more severe than that of sH1N1. Therefore, the pH1N1 is intrinsically more pathogenic for humans than sH1N1.

CHLAMYDIAL PREVALENCE IN ALPINE IBEX (CAPRA I. IBEX) FROM SWITZERLAND

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Introduction

Chlamydomphila abortus is the most common infectious abortigenic agent in small ruminants in Switzerland. In contrast, the knowledge about Chlamydiae in wild ruminants is scarce. Therefore, we investigated the prevalence of Chlamydia in Alpine ibex in Switzerland.

Materials and Methods

In total, 626 sera, 704 eye swabs, 84 organ samples and 51 fecal samples were investigated. Eye swabs, organs and fecal samples were examined by a Chlamydiaceae-specific real-time PCR followed by ArrayTube (AT) Microarray method for chlamydial species determination. Serum samples were tested by two commercial ELISA kits specific for *C. abortus*.

Results

Of 626 serum samples investigated, 617 animals were tested negative, whereas nine sera (1.4%) reacted positive in one of the two tests. The eye swabs of 405 ibex were negative for Chlamydiaceae by real-time PCR. By AT Microarray, *C. pecorum* was identified in two animals, *C. pneumoniae* was detected in one animal and in four animals, a mixed infection with *C. abortus* and *C. pecorum* was found. Organs and fecal samples were all negative by real-time PCR analysis.

Conclusion

We conclude that *C. abortus* is not common in the Swiss ibex population. To our knowledge, this is the first description of *C. pneumoniae* in ibex.

DETECTION OF BLUETONGUE VIRUS-1 AND BTV-8 RNA IN EXPERIMENTALLY INFECTED RED DEER

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Introduction

An experimental infection with Bluetongue virus serotype 1 (BTV-1) and BTV-8 was carried out in red deer (*Cervus elaphus*). The results of BTV RNA detection in skin biopsies and post-mortem sampled tissues are presented.

Material and methods

Four red deer were inoculated with BTV-1; four with BTV-8; and three acted as controls. Skin biopsies obtained at 14 and 50 days post infection (dpi); mucosal swabs collected at 50 dpi; and 13 selected post-mortem samples at 98-122 dpi were analyzed by RT-PCR. Skin biopsies and post-mortem tissues were also analyzed by histopathology. Selected spleen, tonsil and mesenteric lymph node samples were further investigated by immunohistochemistry using two monoclonal antibodies and two staining methods.

Results

Five out of six skin biopsies at 14 dpi were RT-PCR positive, but those taken at 50 dpi yielded negative results. All swabs were RT-PCR negative. Spleen post-mortem samples were the most consistently RT-PCR positive. No significant macroscopic and no microscopic Bluetongue-compatible lesions were found. Immunohistochemistry revealed no stain with the protocols applied.

Discussion

Detection of BTV RNA in skin at 14 dpi, but not later, suggests that transmission of the virus to *Culicoides* vectors may be more efficient during the peak of the viraemia. The consistent detection of BTV RNA in spleen suggests that this organ is a good target for Bluetongue surveillance in hunter-harvested wild deer.

RESTRICTED INVOLVEMENT OF THE CENTRAL NERVOUS SYSTEM IS A CONSISTENT FEATURE OF POST-EPIDEMIC CHRONIC DOLPHIN MORBILLIVIRUS INFECTION IN MEDITERRANEAN STRIPED DOLPHIN (*STENELLA COERULEOALBA*)

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In 1990 an epizootic of dolphin morbillivirus (DMV) was observed in the Mediterranean striped dolphin (*Stenella coeruleoalba*). Between 1991 - 1994 five dolphins from Catalonia showed a DMV infection confined to the brain. No new cases were observed until 2007 when the epizooty reappeared at a milder scale.

From July 2007 to April 2010 samples of 93 striped dolphins were studied by means of H/E, DMV immunohistochemistry or RT-PCR for DMV.

DMV immunopositivity was found in 22 dolphins, restricted to the CNS. Dolphins showed non-suppurative meningoencephalitis with inclusion bodies. A neuropathological study was performed in three cases. Lesions localized mainly in thalamus, basal nuclei and cerebral cortex. Distribution of DMV immunolabelling was similar. Viral-RNA was found in 3 dolphins, only in the brain; the results confirmed the exclusive nervous localization. Vero-cells were inoculated with one brain homogenate but did not show cytopathic effect. RT-PCR of the inoculated cells was negative.

This study shows that after the 2007 epizootic, as in 1990, several striped dolphins have shown a DMV infection restricted to the CNS. Our hypothesis is that such cases are latent chronic, CNS-restricted infections originated in the epizootic outbreak, re-activated during the ensuing years. These cases are a consistent DMV post-epizootic feature.

INTRAMUSCULAR BCG VACCINATION REDUCES SIGNIFICANTLY THE PATHOLOGY INDUCED BY MYCOBACTERIUM BOVIS IN BADGERS (MELES MELES)

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Introduction

Badgers are a wildlife reservoir for *Mycobacterium bovis* in Great Britain and therefore constitute a potential source of infection in cattle.

Material and methods

3 groups of captive badgers were used. 2 groups were vaccinated by IM route with a Low Dose (3 x 10⁵ CFU) and a High Dose (HD) (3-5 x 10⁶ CFU) respectively of BCG. The third group remained unvaccinated. Animals were challenged by intra-bronchial delivery of 10³-10⁴ CFU of *Mycobacterium bovis*. 12 weeks post challenge, all badgers were examined at post-mortem.

Results

Lesions were typically multifocal to coalescent tuberculous granulomas within the lung lobes and the draining lymph nodes. The severity for macro and microscopic lesions was scored. The main differences between the groups were a significantly lower severity of gross and histopathological lesions in the lung and the draining thoracic lymph nodes (left/right bronchial and anterior/posterior mediastinal) in the HD BCG group compared with the unvaccinated. In addition, the higher average score of the granulomatous lesions for all tissues and the presence of more collagen in the unvaccinated control group, together with a higher number of AFBs, are indicative of more severe/advanced lesions in this group in comparison with the HD BCG.

Conclusion

Parenteral BCG vaccination reduced significantly the pathology induced by experimental infection with *Mycobacterium bovis* in badgers.

ORAL PRESENTATIONS
SESSION 6B - ORGAN PATHOLOGY

EVALUATION OF MUCOSAL P-GLYCOPROTEIN EXPRESSION IN THE SMALL INTESTINE OF DOGS DIAGNOSED WITH LPE.

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Introduction

Inflammatory Bowel Disease (IBD) is an idiopathic chronic inflammation of stomach, small and/or large intestine. In dogs, lymphocytic-plasmocytic enteritis (LPE) is one of the chronic diseases of the small intestine included among IBD. P-glycoprotein (P-gp) is a membrane bound drug efflux pump involved in transport of many drugs prescribed in LPE. Changes in intestinal P-gp expression may explain important interindividual differences in the bioavailability and response to treatment. In this study, we evaluated mucosal P-gp expression in the small intestine in dogs with LPE compared to disease free animals.

Materials and Methods

Formalin fixed endoscopic intestinal biopsies of 78 dogs, histopathologically diagnosed with LPE, were immunolabelled using the antibodies C494 and C219. Endoscopic biopsies of 8 healthy beagles were used as controls.

Results

None of the controls showed mucosal P-gp expression in the duodenum and all showed consistent P-gp labelling at the colon mucosa. 20% of dogs with LPE showed mucosal P-gp expression in duodenum, jejunum and/or ileum with localized immunoreactivity at the tips of the villi while some showed reduced expression in the colon.

Conclusion

Mucosal P-gp expression is increased in the small intestine of some dogs with LPE. This might affect oral bioavailability of drugs used in LPE.

IMMUNOHISTOCHEMICAL STUDY OF CONGENITAL HEPATIC FIBROSIS IN ABORTED CALVES

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Introduction

Congenital hepatic fibrosis is a developmental disorder that belongs to the family of hepatic ductal plate malformation and is characterized histologically by a variable degree of periportal fibrosis and irregularly shaped proliferating bile ducts.

Materials and methods

We found 5 aborted calves with liver lesions compatible with congenital hepatic fibrosis between 2007 and 2010. Tissue samples were fixed in 10 % neutral buffered formalin and stained with hematoxylin and eosin and van Gieson stains. Liver samples were also used for immunohistochemical analysis by antisera specific for cytokeratin, vimentin, α - smooth muscle actin, GFAP and chromogranin.

Results

Histologically, the liver lesions included widespread fibrosis and linkage of periportal areas to the central vein region and to other periportal areas via connective tissue which stained strongly with van Gieson stains. Immunohistochemical investigation revealed fusiform cells and hyperplastic fibers in the portal area, around the central vein and in the sinusoids that showed positive responses to vimentin and α - smooth muscle actin.

Conclusions

The cells that reacted positively to anti- vimentin and anti α - smooth muscle actin antibodies displayed the features of myofibroblasts, thereby suggesting their participation in the process of fibrogenesis in congenital hepatic fibrosis.

LESION DISTRIBUTION IN CEREBRAL CORTEX AFTER DIFFERENT DURATIONS OF NO-FLOW IN A PIG CARDIAC ARREST MODEL

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Introduction

Neurological deficiencies following successful resuscitation after cardiac arrest (CA) are a major problem in human medicine. In selectively vulnerable brain regions neuronal degeneration takes place over days, thus suggesting a therapeutic window for neuroprotective strategies.

Materials and Methods

35 female Large White pigs (35-45 kg) underwent experimental CA for 0, 7, 10 or 13 minutes. In the brains of all animals that survived until the final endpoint (72 h post CA) frontal, parietal, temporal, occipital and insular cortex were examined on paraffin-embedded sections in terms of type and extent of lesions.

Results

Significant differences were found in all cortex regions between the ischaemic groups (7- and 10-min groups) and the control group (0-min group). No animals of the 13-min group were successfully resuscitated. The main findings were hypereosinophilic neuronal degeneration and oedema in layers III and V. In some animals from the 10-min group, necrotic neurons were evident in all cortical layers, and many neurons in layer III were missing.

Conclusion

The neuronal lesions in the cerebral cortex in this pig model phenocopy the known patterns of neuronal defects that are found in humans and other animal models. It shall be used in future research to test neuroprotective strategies.

BACTERIAL GASTRITIS IN HUMANS AND ANIMALS – TWO DIFFERENT MORPHOTYPES IN CARNIVOROUS AND IN OMNIVOROUS MAMMALS

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Introduction

Helicobacter pylori caused gastritis is well known in human medicine. In routine gastro-pathological examinations, a Helicobacter-like organism (HLO) is always associated with medium- or high-degree gastric inflammation, both in adults and in children. Histological properties of human gastric inflammation described by Sydney-classification of gastritis, we compared with the samples of gastric mucosa of dogs, foxes and pigs.

Materials and Methods

For routine pathological diagnostics of gastritis we distinguished two morphological type of Helicobacter, a short Helicobacter-like organism (HLO), and long multi-coined, morphologically designated as Gastrospirillum-like organism (GLO). Type of gastric inflammation was staging according to the Sydney-classification of gastritis.

Results

In humans, both types, HLO and GLO are pathogenic, caused active chronic gastritis. In dogs and foxes infection rate is very high, over 80 percent, with GLO morphotype. In contrast, histological signs of gastritis are very rare and not in correlation with GLO infection. In pigs we found infection rate of 50% with HLO morphotype only and associated with gastritis, from mild superficially to chronic follicular and atrophic.

Conclusion

In humans and pigs there are very similar histological picture of gastritis caused with short HLO and infection is always associated with gastritis. In carnivorous mammals GLO rate of infection is very high, but in the most cases without any sign of gastritis.

ENZOOTIC NASAL TUMOUR OF GOATS IN GREECE

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Introduction

The enzootic nasal tumour (ENT) of goats arises from secretory epithelial cells of the ethmoid turbinate and follows infection by ENTV-2, an exogenous retrovirus. The geographical distribution of the disease is believed to be wide but has not been studied comprehensively.

Materials and Methods

Intranasal tumours of goats that were included in the study were retrieved from the archives or were recruited as part of an ongoing prospective study. The tumours were examined grossly and microscopically and a selected number of tumour samples was analysed using PCR and sequencing.

Results

54 intranasal tumours were examined, the first of which was initially examined in 1984. Although no metastases were observed, a small number of cases was locally aggressive. Histologically, most cases were low grade adenocarcinomas. The cases originated from four geographical regions in Greece (Thrace, Macedonia, Thessaly, Central Greece). PCR detected ENTV-2 proviral DNA within the tumours examined. Sequencing and phylogenetic analysis demonstrated clustering of these isolates with the ENTV-2 prototypes in a branch distinct from ENTV-1, JSRV and SERV representatives.

Conclusions

ENT of goats is present and appears to have widespread geographical distribution in Greece; further examination and monitoring of the disease status is warranted.

ORAL PRESENTATIONS
SESSION 8A - CLINICAL PATHOLOGY

INFLUENCING ORAL PREDNISOLONE ABSORPTION IN BEAGLE DOGS BY MODULATION OF P-GLYCOPROTEIN EXPRESSION IN THE INTESTINAL TRACT.

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Introduction

The drug efflux pump P-glycoprotein (P-gp) is important in limiting oral bioavailability. Prednisolone, a known P-gp substrate in humans, is frequently used in small animal practice. In this study we evaluated the impact of P-gp modulation on oral prednisolone therapy in dogs, using rifampicin as inducer and ketoconazole as inhibitor.

Materials and Methods

Prednisolone was administered orally alone or after pretreatment with rifampicin or ketoconazole. Endoscopic biopsies of duodenum and colon of six beagles in a cross-over design were immunohistochemically evaluated for P-gp with the antibodies C494 and C219. Blood samples were taken at predetermined time points for analysis of plasma prednisolone concentration using LC-MS/MS.

Results

Oral rifampicin treatment resulted in induction of P-gp expression in the duodenum of 2/6 and 5/6 dogs with C494 and C219 respectively. Ketoconazole treatment resulted in complete loss of P-gp expression in the colon of 2/6 and 5/6 dogs with C494 and C219 respectively. The plasma prednisolone concentration was significantly reduced after rifampicin treatment ($p < 0.05$) while ketoconazole resulted in a slightly higher plasma concentration, although not statistically significant.

Conclusion

This proof-of-concept study documents the feasibility of modulation of intestinal P-gp expression with rifampicin and ketoconazole in dogs, which might influence oral bioavailability.

GENETIC MARKERS FOR THE DETECTION OF CIRCULATING TUMOR CELL IN DOGS WITH METASTATIC MAMMARY TUMORS

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Introduction

The diagnosis of canine mammary tumors is currently based on histological examination but discrepancies between the diagnosis and prediction of distant metastasis exist. Detection of circulating tumor cells (CTC) has a proven predictive value for human breast cancer. We aimed at indentifying genetic markers to detect CTCs in the peripheral blood of female dogs with metastatic mammary carcinomas.

Materials and Methods

A total of 108 canine gene products were tested as potential markers of CTC using RT-PCR. Peripheral blood of healthy female dogs (n=10), metastatic mammary carcinomas (n=10) and 2 canine mammary carcinoma cell lines were tested for expression of these genes. Sensitivity of the marker gene assays was determined using serial dilutions of tumor cells in peripheral blood of healthy dogs.

Results

5 candidate marker genes were identified that were present in metastatic carcinomas but not in blood from healthy dogs. Furthermore, RT-PCR assays were sensitive enough to detect up to 1 tumor cell in 10⁷ peripheral blood leukocytes.

Conclusions

Several potential genetic markers for CTC detection in dogs were identified. These will now be applied on blood samples from dogs with or without mammary tumors to correlate their presence with prognostic factors in the primary tumors and the course of the disease following long term clinical follow-up.

**ORAL PRESENTATIONS
SESSION 8B - MODERN METHODS
IN PATHOLOGY**

TIME-DEPENDING MORPHOLOGICAL ALTERATIONS CAUSED BY CRIMPING OF ARTIFICIAL HEART VALVES

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Introduction

Transcatheter aortic valve implantation is used to treat elderly high risk patients with aortic stenosis. The application by catheter requires temporary crimping of the valves to the diameter of the catheter's lumina. This study aimed to investigate time-dependent biochemical, histological and ultrastructural effects of the crimping procedure in a rat model.

Material and Methods

Standard transcatheter valves (Sapien™, Edwards Lifesciences, Irvine, USA) were crimped for different duration (uncrimped, 1 hour, 1 day, 1 month) and implanted subcutaneously in 15 Sprague-Dawley rats for 12 weeks. Histological (H&E, van Kossa, Picrosirius red) and electron microscopical examinations were performed. Grade of calcification was measured by frozen dry mass.

Results

Leaflet morphology differed significantly from no fragmentation (uncrimped control group) to highly fragmented collagen fibers (one month crimped). However, degree of calcification was not significantly different among the groups.

Conclusion

Transcatheter valve crimping does not necessarily affect leaflet calcification which is a well known complication in transplanted valves in man. However, the presence of structural changes of the leaflets which were caused by crimping may have clinical significance. Duration of crimping should be as short as possible and too tight crimping to small diameters should be avoided.

THE ROLE OF MMPS, TIMPS AND TGF- β ISOFORMS IN THE PATHOLOGY OF FELINE MYOCARDIAL FIBROSIS

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Introduction

Myocardial fibrosis in cats has not been described in detail until now.

Materials and Methods

We examined 40 feline hearts with myocardial fibrosis in comparison to pathologically normal hearts of 25 cats. The hearts were investigated clinically, grossly, histologically (H-E, picosirius red) and immunohistochemically (MMP-2, -9, -14, TIMP-2, -3, TGF- β 1, - β 2, - β 3).

Results

Hearts with extensive myocardial fibrosis were found to show hypomotile and hyperechoic areas in the ventricular wall when assessed by echocardiography. In the normal ventricular myocardium, cardiomyocytes expressed MMP-2, -9, -14, TIMP-2 and TGF- β 2. Fibroblasts only expressed TIMP-2.

In extensive myocardial fibrosis, a significantly increase in the expression of MMP-2, TIMP-3, and TGF- β 2 in the cardiomyocytes was shown but TIMP-2 expression was decreased. In several cases, TIMP-3 expression by fibroblasts was detected.

Conclusions

From the pathological point of view it seemed likely that several cases, classified by echocardiography as UCM are cases of extensive cardiac fibrosis. These findings suggest a complex pathogenesis of fibrotic remodelling of the feline myocardium. It appeared that the cardiomyocytes are markedly involved in the process of myocardial remodelling. Renal diseases with elevated angiotensin II, aldosterone and endothelin-1 values may contribute to this pathogenesis.

SPONTANEOUS LESIONS IN THE CENTRAL NERVOUS OF AGING SYRIAN HAMSTERS

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Introduction

Syrian golden hamsters (*Mesocricetus auratus*) represent increasingly used laboratory animals for toxicological studies. Despite the need for an adequate knowledge of spontaneously occurring lesions, studies investigating the background pathology of different organ systems in hamsters are lacking. The aim of this study was to investigate spontaneous age-dependent lesions in the central nervous system to increase the current level of available background data for this species.

Material and Methods

Multiple brain and spinal cord transversal sections of 520 hamsters aging 28 days, 13 weeks, 6 months, 1 year, or 2 years were investigated using histological, immunohistochemical, and electron microscopical methods.

Results

19 male and 37 female animals died ahead of schedule. Few of these cases were caused by pituitary tumors and meningoencephalitis. Animals reaching the end of the experimental schedule showed mineralization and neuropil vacuolization as the most prominent lesions increasing with age. In addition, mast cells and the deposition of lipofuscin, lipopigment, and melanin were detected amongst other changes as incidental lesions. Amyloid deposition, senile plaques, and neurofibrillary tangles were absent.

Conclusion

The presented data may help to interpret toxicological studies using Syrian golden hamsters and provide crucial informations for a better understanding of age-related changes for comparative investigations.

MORPHOLOGICAL CHARACTERIZATION OF TRAUMATIC SPINAL CORD INJURY CAUSED BY INTERVERTEBRAL DISC DISEASE IN DOGS

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Introduction

Traumatic spinal cord injury represents one of the most common neurologic diseases in dogs, and is frequently attributed to intervertebral disc disease. Novel therapeutic approaches including cell transplantation require detailed knowledge of the pathomorphological changes following the initial insult. Thus, the aim of the study was to investigate the spatio-temporal course of degenerative processes in canine spinal cord tissue affected by naturally occurring trauma.

Materials and Methods

Spinal cord samples of 20 dogs with intervertebral disc protrusion or extrusion and 10 control dogs were evaluated histologically using various immunohistochemical markers.

Results

Traumatic spinal cord injury caused by intervertebral disc disease was characterized by degenerative changes including necrosis, occurrence of gitter cells and spheroid formation, occasionally accompanied by a mild inflammatory response. Protrusions or a short disease course usually caused mild lesions, confined to the epicenter of the injury site, whereas severe or long-lasting insults led to pronounced lesions spanning up to 15 cm of the spinal cord.

Conclusions

There is considerable variation in the morphology and extent of spinal cord lesions in dogs depending on both the severity of the initial insult and the post-traumatic time period. These morphological data together with the results of future molecular investigations will provide the essential base for innovative therapeutic intervention.

EARLY EFFECTS OF 3 DIFFERENT DIETS ON THE INTESTINAL MORPHOLOGY AND PERFORMANCE IN CALVES

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Introduction

The calf rearing industry is continuously searching to improve milk replacers. At present, there is experience in replacing milk fat. Based on earlier experiments however, the industry is reluctant to replace the milk proteins. The purpose of the present study was to evaluate the effect of partial replacement of milk protein by vegetable protein.

Materials and methods

Fourteen-weeks-old Holstein calves were given 3 different diets during 12-13 days. Group C (n=10) received cow milk. Group S (n=10) was fed with a standard milk replacer and group V (n=10) with a modified milk replacer containing 8% vegetable protein. Subsequently, necropsy was performed and small and large intestinal samples were collected. Villus length and width, crypt depth and muscularis thickness were measured.

Results

At the end of the trial group V had a tendency of lower body weight than S. In addition V had a significantly decreased villus length compared to C and S. Villi were significantly wider in V at the level of proximal and central jejunum as well as ileum. Crypts were significantly deeper in V compared with S and C. Tunica muscularis thickness increased linearly from C over S to V.

Conclusion

Vegetable protein had a negative effect on small intestinal morphology and performance.

**POSTER SEMINAR TOUR
SESSION 1A - ONCOLOGY**

1. THE LARGE OSSIFIED PARAPROSTATIC PSEUDOCYST WITH OSTEOMA IN DOG

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Introduction

Paraprostatic pseudocysts develop predominantly in older, large breed dogs. Their wall is usually of compressed fibrous tissue and the outer lining is mesothelium. The mineralisation and neoplasia are rare. They are congenital or acquisition but their actual origin is obscure.

Material and Method:

A 10-year-old sexually intact male German Shepherd with unilateral abdominal distension and urinary tract obstruction. Ultrasound examination showed the bladder enlargement and spherical mass laterally to the bladder at the right side. The dog was euthanised. Necropsy examination was performed and tissue samples were taken and HE stained.

Results

Necropsy showed a cyst approx. 14 cm in diameter, attached to the prostate with well vascularised and ossified wall, connecting with post-prostatic urethra by duct. The cyst was full of pale yellow fluid with cauliflower-like masses. Enlargement of the prostate and the bladder distension were noticed. Histopathologically, the pseudocyst was lined with fibrous tissue and mesothelium outside, with osseous metaplasia and osteoma producing neoplastic masses. Cauliflower-like masses were made by osteoma and fibrous tissue with focall calcification and yellow pigment concentration. Benign hypertrophy, necrosis of epithelial cells, stroma proliferation and calcification were observed in the prostate.

Conclusion

A paraprostatic pseudocyst with osseous metaplasia, osteoma and secondary opening to the urethra was diagnosed.

PREDICTIVE VALUE OF TOPOISOMERASE-II ALPHA EXPRESSION IN MAMMARY GLAND TUMORS IN FEMALE DOGS

2.

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Introduction

The research objective was to evaluate topoisomerase-II alpha (TOP-2A) expression in mammary gland tumors in female dogs as a potential predictive indicator of response to a topoisomerase inhibitor therapy.

Material and Methods

Research material comprised 33 surgically removed specimens from malignant mammary gland tumors in female dogs, typed according to WHO histological classification. TOP-2A expression was measured by immunohistochemical technique and estimated using computer-assisted image analysis to calculate the index – a positive cell percentage in 1000 tumor cells.

Results

TOP-2A positive reaction was observed in 20 (60,6%) tumors. In 8 tumors, TOP-2A expression was found in less than 5% cells, while in 10 tumors - medium level expression (5-20%) was noted. In only two cases, the expression involved >20% cells. The studies did not show any relationship between a histological type and TOP-2A expression intensity.

Conclusion

The present researches have indicated that mammary gland tumors in female dogs are characterized by low level TOP-2A expression that predicts low efficacy of chemotherapy with topoisomerase-II inhibitors use.

3. OVARIAN MIXED GERM CELL-SEX CORD STROMAL TUMOR IN A DOG

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Setsunan University, Japan

Introduction

Mixed germ cell-sex cords stromal tumor has rarely been reported as a testicular tumor in the dogs, but was not classified as an ovarian tumor in 2nd series of WHO classification in domestic animals. We encountered an ovarian mass diagnosed as canine ovarian mixed germ cell-sex cord stromal tumor by histopathological and immunohistochemical characteristics.

Materials and Methods

A mass sized about 4 cm in diameter of right ovary was surgically resected from a mixed-breed dog aged 11 years.

Results

The mass was soft and the surface was smooth and colored milky white with solid homogeneous cut surface. Histologically, the tumor mass consisted of cellular nests surrounded by connective tissue stroma and took an appearance similar to granulosa-theca cell tumor, but nuclear size were varied among the cells. Large round cells with abundant cytoplasm similar to germ cells were mingled with smaller cells in the nest. Immunohistochemically, large round cells were positive for one of the germ cell markers, PLAP (placental alkaline phosphatase) and negative for granulosa cell markers such as WT-1 and vimentin, whereas other cells were positive for these markers.

Conclusion

Morphological and immunohistochemical characters of this tumor were suggestive of ovarian mixed germ cell-sex cord stromal tumor.

STUDY OF POLY(ADP-RIBOSE) POLYMERASES 1 AND 2 4. (PARP-1 AND PARP-2) IN INDUCED LYMPHOMAS IN-P21 CIP1/WAF1 AND P-27 KIP2 MICE.

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p21cip1/waf1 and p27kip1 are able to arresting the cell cycle at G1 phase. There is evidence that p21 modulates apoptosis and p27 deficiency is related to thymic hyperplasia and T lymphocyte proliferation. Poly (ADP-ribose) polimerase (PARP) is one of the first mechanisms that participates in DNA repair after irradiation. Our aim is to analyze the effect of p21 and p27 proteins on apoptosis using a double-deficient mouse model. 24 mice with 4 different genotypes, were γ -irradiated to induce thymic lymphomas. Immunohistochemistry was performed using: anti-Pax1, anti-CD3, anti-PARP 1, anti-PARP 2, caspase-3 active and Ki67 antibodies. Lymphomas diagnosed was: (p21+/+p27+/+) n=7; (p21+/+p27-/-) n=3; (p21-/-p27+/+) n=4; (p21-/-p27-/-) n=3. In wild-type and p27-null mice all the tumors were T-cell lymphomas while in p21-null and double-deficient mice the majority (75%) were B-cell lymphomas. We observed strong apoptosis (caspase-3 active) in the thymus of wild-type and p21-null mice, whereas p27-null mice and double KO were more resistant to it. This result was correlated with the level of immune-expression of cleaved PARP-1 in lymphocytes. PARP-2 detection was practically absent in all the tumors analyzed. No differences were observed in the mitotic (ki67) indexes among groups.

Conclusion

PARP-1, but no PARP-2, plays an essential role in the apoptosis observed in induced lymphomas in p21-null mice and in lower proportion in p27-null mice and double-KO mice.

5. ESTABLISHMENT AND CHARACTERIZATION OF NEW CELL LINES DERIVED FROM CANINE HEMANGIOSARCOMAS

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Introduction

Recently, we created canine hemangiosarcoma (HSA) xenograft models by using nude mice (Kodama, et al., BMC Cancer, 2009). In this study, we established and characterized the canine HSA xenograft tumour cell lines.

Materials and methods

Tissues of 4 canine HSA xenograft tumours were minced and enzymatically digested. The cells were cultured in M199 medium (10% fetal calf serum [FCS]) and passaged over 60 times. Uptake of Dil-acetylated low-density lipoprotein (Dil-LDL) by these cells was evaluated. The expressions of endothelial markers, angiogenic growth factors, and their receptors and angiogenic homeobox genes were investigated by RT-PCR with canine-specific primer sets and immunocytochemical analysis.

Results

We established 6 canine HSA cell lines. RT-PCR and immunocytochemical analyses revealed the expression of von Willebrand factor and/or CD31. Further, all the cells exhibited active uptake of dil-acetylated LDL. RT-PCR and immunocytochemical analyses revealed the expressions of VEGF-A, bFGF, flt-1, flk-1, FGFR-1, HoxA9, HoxB3, HoxB7, HoxD3, Pbx1, and Meis1.

Conclusions

All the cells expressed endothelial markers, angiogenic growth factors and their receptors and angiogenic homeobox genes similar to that observed in the original xenograft tumour tissues. These cell lines may be useful to study malignant progression of the canine HSA.

NECROSIS OF SKELETAL MUSCLE DUE TO ADENOCARCINOMA IN CAT - CASE REPORT

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Introduction

Coagulation necrosis of skeletal muscle due to embolisation of an adenocarinoma in animals is sporadic.

Material and Methods

Macroscopic examination was carried out of a 14-year-old female European cat, body weight 3.6 kg, euthanized during diagnostic laparotomy. For several years the cat had received medroxyprogesterone acetate (Provera Vet, 5 mg, Pfizer Poland), 1 tab/wk. The X-rays revealed a mass in the stomach region. Specimens of tumours, alimentary tract and muscles of abdominal wall were taken for microscopic examination (HE staining).

Results

The macroscopic examination revealed a tumour on mesentery (6 x 4 x 3 cm), touching the stomach, spleen and pancreas. The serous membranes of alimentary tract, urinary bladder and mesentery were covered with numerous straw-coloured small, firm nodules. On the parietal peritoneum, the capsule of the liver and the spleen there were numerous deposits similar to fibrin. The microscopic examination of the tumour and nodules revealed well- and medium-differentiated adenocarcinoma. The cells were columnar, with foamy cytoplasm. Large secretory vacuoles were in some of them. The nuclei were situated parabasal, and sometimes chaotically. Numerous mitotic figures and aniso- and macrocariosis were observed. There was secretion in the follicles. Parietal peritoneum revealed fibrosis with neoplastic infiltration. Within the muscles of the abdominal wall there were areas of coagulative necrosis. That lesion is a consequence of peripheral arterial embolisation.

Discussion

Such muscular lesions in animals are usually associated with underlying cardiac diseases, whereas in humans with malignant tumours, mainly lung cancers.

7. A PRIMARY POORLY DIFFERENTIATED SARCOMA IN A FELINE LIVER

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Introduction

This is a case of a 9 year old DSH cat in a good physical condition that died suddenly at his owner's house, and was brought to a private vet clinic. It was delivered immediately after a determination of death for post mortem evaluation and diagnosis. The clinical assessment of the private vet was a suspicion of an intoxication, because the rapid nature of the deterioration in the cat condition, and a history of changing food management by the cat's owners a day before the death.

Method

Gross pathology was performed according to normal regulations. The internal organs from the thoracic and abdominal cavities were sampled and immersed in buffered 10% formaldehyde solution. Histologic sections were cut, and were routinely stained with hematoxylin and eosin. Other stains and staining methods included CD 34 for embryonic haemopoietic cells and factor VIII staining to identify endothelial cells. Vimentin and cytokeratin were also used in order to differentiate between the mesenchymal and epithelial components of the tumor.

Results

Post Mortem: Within the abdominal cavity there was about one liter of free blood. Adjacent to the left ventral liver lobe, a large, solitary mass of 15 cm in diameter was found. This mass had a very irregular structure on cross section with many small cavities that were filled with blood (Figure 1). Both kidneys had an irregular cortex and were swollen. No further lesions were found in the rest of the abdominal organs. Histology: Histologically, within the liver there was a large process that was markedly infiltrative, densely cellular, poor demarcated and unencapsulated. The cells were spindle shaped and poorly differentiated and arranged in bundles and streams. They had a fine eosinophilic granular cytoplasm with a large elongated, finely stippled nuclei with 1-2 nucleoli. Mitotic activity in this neoplastic lesion was high (1-2 40X per HPF). Next to the neoplastic cells there are many round cells that resembled premature hematopoietic cells that varied in shape and size, with round to oval, vacuolated nuclei (anisocytosis, anisokaryosis) and few megakaryocytes. Inside the process there are many congested blood vessels with swollen endothelium, and there was an abundant amount of extravascular hemorrhage and large vascular spaces, frequently filled with blood and occasionally associated with foci of necrotic hepatocytes in the adjacent parenchyma. There was also a marked edema in the adjacent liver parenchyma. Immunohistochemistry is still under investigation.

Conclusion

Undifferentiated liver sarcoma, also known as undifferentiated embryonal sarcoma of the Liver, is a rare, highly malignant neoplasm which mostly affects the pediatric population, although a few cases have been reported in adults. It accounts for about 13% of pediatric hepatic malignancies.

CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF AORTIC BODY CHEMODECTOMA IN A COW

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Introduction

Chemoreceptor tissue is present at several sites in the body such as carotid body and aortic bodies but in animals tumors often arise from the aortic bodies, especially in dogs. Aortic body chemodectoma is rare in the bovine, with only five cases reported previously worldwide mostly without immunohistochemical diagnosis.

Materials and Methods

A 3-year-old Holstein cow with a history of severe dyspnea, pale mucous membranes, severe arrhythmia and tachycardia was referred to slaughterhouse by the referring veterinarian due to poor prognosis. Tissue samples from the tumor mass were fixed in 10% natural buffered formalin, routinely processed, and stained with hematoxylin and eosin and Masson's trichrome and also immunohistochemistry was done with primary monoclonal antibodies specific for S-100 protein (S1/61/69), neuron specific enolase (NSE; 5E2) and chromogranin A (CrA).

Results

After slaughter, a large, firm, white, multilobulated mass about 5 cm in diameter was located at the base of the heart that extended to the right atrium and obliterated partially the right atrial lumen. Microscopically, Tumor cells were aligned along and around the capillaries and were discrete, cuboidal to polyhedral cells with little cytoplasm arranged in distinct packets surrounded by thin fibrous septa. The nuclei were round to oval and were placed centrally in the cells. Randomly scattered round shaped capillaries similar to fat cells were seen. Immunoreactivity for S100 protein was positive multifocally, mostly in the peripherally located sustentacular cells, and for NSE and CrA very weak positive.

Conclusion

On the basis of the clinicopathological and immunohistochemical findings, the tumor was diagnosed as aortic body chemodectoma. Although the etiology of this tumor is unknown, it has been suggested that a genetic predisposition aggravated by chronic hypoxia may account for the higher risk to develop aortic body tumors. The case of the present study was living in a high altitude environment and hence, chronic hypoxia was probably involved in the pathogenesis of the tumor.

9. SOFT TISSUE SARCOMA IN AN ALPACA (VICUGNA PACOS): PATHOLOGICAL FINDINGS

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Introduction

South American camelids have gained popularity in the UK over recent years. A 6-year-old, male Huacaya alpaca presented with a four day history of lameness. A pedunculated mass was excised from the right hind metatarsal region and submitted for histopathological evaluation.

Materials and methods

A 3x3, 5x2 cm, ovoid, pedunculated, multilobular, white/grey, firm skin mass was sectioned, processed and examined with a routine HE staining method. Additional Alcian Blue, Masson's Trichrome, Van Gieson, Reticulin and PAS histochemical stains were performed. Immunohistochemistry for vimentin, desmin, S-100, von Willebrand factor and CD31 was also performed.

Results

Histopathology showed a completely excised mass composed of neoplastic cells arranged in whorls with fibrillar eosinophilic material (stained positive with Alcian Blue) and loose collagen bundles (clearly identified using Masson's Trichrome). Neoplastic cells were strongly positive for vimentin and negative for the other antibodies tested.

Conclusions

Based on our observations and according to the literature the mass is consistent with a soft tissue sarcoma (myxosarcoma), low grade. Soft tissue sarcomas are fairly uncommon in alpacas but complete surgical excision should be curative. To our best of our knowledge, this is the first report of a myxosarcoma in an alpaca.

IMMUNOHISTOCHEMISTRY FOR MARKERS OF PROLIFERATION AND APOPTOSIS AND ANTI-APOPTOTIC BCL-2 FAMILY MEMBERS IN CANINE LYMPHOMA

10.

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Introduction

Proliferation and apoptosis are deregulated in cancer. We study their association with each other and with selected anti-apoptotic proteins in canine lymphoma.

Materials and Methods

Tissue arrays containing immunophenotyped and classified archival canine lymphoma samples (93 cases) were evaluated immunohistochemically. The numbers of tumour cells positive for Ki-67, cleaved caspase-3 (cC3) and cleaved lamin A (cLA), as well as the labelling intensity of tumour cells for anti-apoptotic Bcl-2 family proteins Mcl-1 and Bcl-x were determined. Correlations between these parameters were explored.

Results

Overall, tumour cell counts for cC3 and cLA, Ki-67 and cC3, as well as Ki-67 and cLA were highly positively correlated. Cell counts for these markers were significantly larger in B-cell than T-cell tumours. B-cell tumours expressed Mcl-1, but not Bcl-x, at significantly higher levels than T-cell tumours. Expression of Mcl-1 was highly positively correlated with cell counts for cC3, cLA and Ki-67. Expression of Bcl-x was associated the same way with cell counts for cC3 and cLA, while it was highly negatively correlated with cell counts for Ki-67.

Conclusion

Study results indicate an association between proliferation, apoptosis and the expression of anti-apoptotic Bcl-2 family proteins Mcl-1 and Bcl-x in canine lymphoma, with differences between the latter.

11. ENDOGLIN MRNA EXPRESSION AND ENDOTHELIAL CELL PROLIFERATION IN BENIGN MAMMARY GLAND TUMORS IN DOGS

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Introduction

Tumor angiogenesis is essential for tumor growth. It has been demonstrated that endoglin is highly expressed in activated endothelial cells and is suggested as a useful angiogenic marker. The purpose of this study is evaluating endoglin mRNA as an angiogenic marker in canine mammary tumors.

Materials and Methods

Expression of vWF, endoglin, and VEGF164 mRNAs were evaluated by RT-PCR and/or real-time RT-PCR in normal mammary tissues, benign mammary gland tumors and their adjacent non-neoplastic tissues. Localization of VEGF, PCNA and vWF was examined by immunohistochemistry or immunofluorescence.

Results

The percentage of endoglin mRNA expression was significantly higher in tumor tissues than adjacent non-neoplastic tissues. The proliferation activity of endothelial cells and the percentage of anti-VEGF positive microvessels were highest in the tumor tissues and lowest in the normal ones. Both proliferation activity and number of VEGF-positive microvessel were significantly higher in endoglin mRNA positive tissues than those in negative tissues. The level of VEGF164 mRNA in the tissues was more closely correlated with that of endoglin mRNA than vWF mRNA.

Conclusions

Endoglin gene expression may be a better angiogenic marker than that of vWF mRNA.

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Introduction

Skin tumors are divided into mesenchymal, epithelial and melanocytic tumors according with the World Health Organization's (WHO) classification. Prevalence of dogs' skin tumors is variable between different authors and year of report. Epidemiology of skin tumor was not been investigated in Latvia. Aim of study is estimate dogs' skin tumors according with WHO classification.

Materials and methods

Collecting 40 skin operation tissues from 40 dogs. Tissues were received at 2008 and 2009 for diagnostic purposes. Its were fixed in 10% formalin, processed embedded in paraffin, sectioned at 4 μ m thickness and stained with Haematoxylin and Eosin.

Results

Dominant skin tumors origin was epithelial with 55.9 %, less tumors of mesenchymal origin were 41.2 % and of melanocytic origin 2.9 % of all skin tumors and tumor-like lesion in dogs. The common skin tumors and tumor-like lesions were fibroma, epidermal cyst, mast cells tumor and sweat gland hyperplasia. Less common tumors were trichoepitelioma, calcinosis circumscripta, fibrosarcoma, hamartoma, hepatoid gland adenoma, papilloma and other.

Conclusion

estimation of dogs' skin tumors is relatively common with Sharif's study (2006), but contradictory with Willemse's (1991), Goldschmidt's and Shofer's (1992) study.

P120 CATENIN EXPRESSION IN CANINE MALIGNANT MAMMARY TUMOURS

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Introduction

The E-cadherin/catenin complex promotes cell-to-cell adhesion and plays a crucial role in the maintenance of a differentiated epithelial phenotype. Perturbation in the expression or function of this complex results in loss of intercellular adhesion, invasion and metastasis. In canine mammary tumours, there are few studies available on adhesion molecules, mostly regarding E-cadherin expression. The present study aimed to investigate the expression of p120 catenin in canine mammary tumours.

Material and methods

p120 catenin expression was evaluated in forty nine canine malignant mammary tumours, by using an immunohistochemical approach. Immunoreactivity was scored according to the subcellular localisation of p120ctn in normal or reduced membrane, cytoplasm and negative expression.

Results

Adjacent normal or hyperplastic canine mammary gland showed a strong epithelial p120 catenin expression, located at the cell membrane. In contrast, most neoplastic tissues showed a reduction 31/49 (63,2%) or complete absence 8/49 (16,3%) of membrane p120 catenin expression. A normal membrane expression was observed in six cases.

Conclusion

We observed that an abnormal expression of p120 catenin is a common phenomenon in canine mammary tumours, suggesting that this catenin may play a role in tumour progression.

DETERMINATION OF BOVINE LEUKEMIA VIRUS GP51 14. EXPRESSION AND CYTOKINE PRODUCTION IN BOVINE DENDRITIC CELLS

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Introduction

Dendritic cells (DCs) are most potent antigen presenting cells (APCs) with 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 DCs generated from the blood, spleen, bone marrow and lymph nodes with the use of microbeads magnetically labelled with CD14. Flow cytometry was used for determination of DCs immunophenotype and gp51 expression in natural and experimental infection with BLV. Cytokine concentrations in culture fluids were determined by ELISA

Results

In dendritic cells infected with BLV we observed very high percentage of determinants: CD11a, CD11b, CD11c and MHC-II class and very high expression of IL-6, IL-10. IL-12p40 and IL-12p70.in culture fluids. The presence of gp51 protein was detected in DCs one week after experimental infection.

Conclusion

Infection with BLV caused increase of cytokines expression and changes in the percentage of surface molecules on dendritic cells. The presence of gp51 was detected in DCs much earlier than in other cells.

15. HEMANGIOSARCOMA OF THE THIRD EYELID IN A HORSE.

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Introduction

Spontaneous tumors of blood vessel endothelial cells have commonly been described in the dog, less frequently in the cat and horse. Hemangiosarcoma is a malignant tumor of the endothelial cells and can be classified as either capillary or cavernous type, depending on the size of the vascular channels.

Materials and methods

A 12-year-old Belgian Warmblood mare was referred to the equine clinic of the Faculty of Veterinary Medicine for a mass on the right third eyelid. The horse had a recurrent red-tinged ocular discharge for several months. After ophthalmologic examination, the third eyelid was surgically excised and submitted for histopathologic examination. Endothelial cell origin was demonstrated using antibodies against von Willebrand factor. Ki67 immunohistochemistry was performed to estimate the proliferative capacity.

Results

The histopathologic examination revealed a proliferation of blood-filled vascular spaces lined by a single layer of flattened cells, which were immunoreactive for von Willebrand Factor. A cellular proliferation index of 8 % was determined by Ki67 immunostaining.

Conclusion

Using a similar proliferation index as in a recent dog study the tumor was identified as a cavernous low-grade hemangiosarcoma of the third eyelid, which is a uncommon localization. No complications were reported 12 months after surgery.

**POSTER SEMINAR TOUR
SESSION 1B - ONCOLOGY**

16. IMMUNOPHENOTYPIC CHARACTERIZATION OF CANINE HISTIOCYTIC SARCOMA CELLS WITH AND WITHOUT CANINE DISTEMPER VIRUS INFECTION

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Introduction

Adhesion to basement membranes and extracellular matrix represents one of the main steps resulting in an invasive phenotype of tumor cells. Oncolytic viruses are believed to be able to interfere with these processes efficiently resulting in a less malignant phenotype. The aim of this study was to investigate whether infection with the Onderstepoort strain of canine distemper virus (CDV-Ond) of canine histiocytic sarcoma cells (DH82 cells) modulates the expression of adhesion and signalling molecules that could interfere with their invasive phenotype.

Materials and Methods

FACS analysis was performed for cell surface phenotyping of non-infected and persistently CDV-Ond infected DH82 cells using antibodies against CD1c, CD11a, CD11b, CD11c, CD14, CD18, CD44, CD45, CD80, CD86, ICAM-1, MHCI and II. CDV-Ond infection was assessed by using an anti-CDV nucleoprotein antibody. Migration of cells was determined by using a transwell system.

Results

CD11a, CD11b, CD11c, CD14 and CD80 were up-regulated in CDV-Ond DH82 cells. Interestingly low MHC II expression was found in both cell types. CDV-Ond infected cells displayed a significantly reduced migration.

Conclusions

The up-regulation of adhesion molecules following CDV-Ond infection of DH82 cells is suspected to alter the adhesion / anti-adhesion duality required for tumor cell motility resulting in reduced migration.

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Introduction

Pylomatricoma is a benign follicular tumor showing only matrical differentiation. This tumor was previously referred to as the calcifying epithelioma of Malherbe. This case describes a pilomatricoma a 14 –year-old, female, terrier dog.

Materials and Methods

General examination was normal. Macroscopically cutaneous mass was circumscribed and not encapsulated and the nodule was localised in the dermis of the right thoracal skin region which has connection of the epidermis. No radiologic changes were found in thoracal and abdominal region. The oval shaped, tumoral mass was completely resected. Tumoral mass was investigated histochemically and immunohistochemically.

Results

The diameter of this nodule was 2.1x2.6 cm with ulcerated skin. The cut surface of the nodule was firm and grey-white. Histologically, the nodule consisted of basaloid epithelial cells, with shadow cells, multinucleated giant cell and also bone trabeculae. The shadow cells had a central clear area showing remnants of the cell nucleus. Immunohistochemically, epithelial component were positive for pancytokeratine (AE1/AE3).

Conclusion

Microscopically and immunohistochemically, the tumor was classified as ossifying pilomatricoma.

18. MORPHOLOGICAL DESCRIPTION OF THREE RARE CANINE INTRAOCULAR TUMOURS

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Introduction

Tumours of glial and of neuroepithelial origin (except for iridociliary adenomas) are rare intraocular neoplasms in dogs. In the present study morphological and immunohistochemical features of three intraocular neoplasms are presented and categorised according the current WHO classification of domestic animals and humans.

Material and Methods

Case 1: The eye of a Golden Retriever presented a 0.5 cm large mass adherent to the optic disc. Case 2: A Labrador Retriever showed a 1.5 x 1 x 0.5 cm wide neoplasm expanding the ciliary body. Case 3: Within the eye of a German shepherd a 0.6 cm in diameter, retinal mass was found near-by the ora serrata.

Results

Pathohistologically, tumour No. 1 consisted of densely arranged polygonal cells forming few rosette-like structures with intraocular spreading. Neoplasm No. 2 was composed of polygonal and spindle cells embedded in expanded chondroid areas. The retina was focally expanded by neoplasm No. 3 mainly composed of spindle cells. Various Several immunohistochemical markers (Vimentin, GFAP, S-100, neurofilament) were applied.

Discussion

On base of the pathohistological features a medulloepithelioma was diagnosed in case 1, a benign teratoid medulloepithelioma in case 2 and a glioma (astrocytoma) in case 3.

A CASE OF PRIMARY INTESTINAL OSTEOSARCOMA IN A DOG

19.

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Introduction

Extraskelatal osteosarcomas are generally extremely rare tumors in the dog. They have been reported in various organs. The paper presents a case of osteosarcoma originating from the wall of the small intestine.

Materials and Methods

Anemic and anorectic 9-year-old female Fila Brasileiro, 55 kg of weight, with the signs of 3 week lasting melena was euthanized and necropsied. Two separated tumorous masses proliferating through the jejunal wall, pancreas, mesenteric lymph nodes and liver were sampled and fixed. Tissue blocks were routinely processed and stained for histopathology and immunohistochemistry.

Results

Gross examination revealed pale tissues and organs and hemorrhagic content of small and large intestine. In the cranial jejunal segment, local tumorous swelling with muscular layer atrophy, partly drawn inside the intestinal lumen was found. The other tumorous mass located 20 cm caudally and protruding on serosal surface of jejunum was observed. Microscopically, both tumorous lesions were characterized by proliferation of polygonal to spindle cells with formation of focal areas of osteoid and numerous foci of chondroid tissue. Immunohistochemically, the diagnose of extraskelatal chondroblastic osteosarcoma was confirmed. Additionally, nodular hyperplasia of exocrinne pancreas, chronic non-specific reactive hepatitis and chronic eosinophilic enteritis was observed.

Conclusion

Our case confirms the extreme rarity of extraskelatal osteosarcoma reported in dogs, in this case no skeletal lesions were observed.

20. ESTROGEN RECEPTOR MODULATORS IN THE TREATMENT OF SMOOTH MUSCLE TUMOURS OF THE GENITAL TRACT OF THE BITCH: THE MODEL OF THE OVARIECTOMYZED RAT

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Introduction

The model of the ovariectomized (OVX) rat allows exploring the role of ER isoforms α and β on the action of estrogens. Oestrogen receptor (ER) modulators may influence growth of smooth muscle tumours of the genital tract of the bitch because 56.3% express (ER).

Materials and Methods

Two-weeks-OVX adult Wistar rats were injected with 0.2 ml oil, 25 μ g estradiol benzoate (EB), 1.5 mg propylpirazole-triol (PPT), 1.5 mg diarylpropionitrile (DPN), 3.0 mg tamoxifen (TX), 1.0 mg raloxifene (RX), 0.5 mg RU58668 and 0.25 mg ICI182780. Uterus samples were taken at necropsy for immunohistochemistry. Progesterone receptor (PR) expression and proliferation were determined in myometrium cells using PR10A9 and MIB-1 monoclonal antibodies, respectively.

Results

PR expression was induced by EB (48.4%), PPT (43.7%), DPN (27.8%), TX (33.6%) and RX (2.4%). Proliferation was seen in EB- (3.3%), PPT- (4.8%), DPN- (1.3%) and TX- (1.9%) treated rats. A positive correlation was observed between PR expression and proliferation.

Conclusion

1. Estrogens exert their effects on PR expression and proliferation through activation of ER α and β isoforms.
2. TX has agonistic effects.
3. RX, RU58668 and ICI182780 have no effects on PR expression and proliferation.

Financial support

P07-CVI-2559 (CICE-Junta de Andalucía) y BFU2008-0048 (DGICYT).

PROLIFERATION, APOPTOSIS AND PROGESTERONE RECEPTOR EXPRESSION IN CANINE MAMMARY CARCINOMA WITH NEOADJUVANT AGLEPRISTONE 21.

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Introduction

Progesterone receptor (PR) antagonist aglepristone (RU534) has been used successfully for pregnancy termination, pyometra and therapy of vaginal tumours in bitches and for therapy of mammary fibroadenomatous hyperplasia in queens. These conditions share the expression of PR. This work analyses the effects of RU534 in the proliferation (PI) and apoptosis indexes (AI) of canine mammary carcinomas.

Materials and methods

Twenty-seven non-spayed bitches with mammary carcinomas were treated with RU534 (n=22) (two doses of 20 mg/kg) or placebo (n=5). Tumour samples were taken before (day 1) and after (day 15) treatment for immunohistochemistry. PR expression, PI and AI were determined using PR10A9 and MIB-1 monoclonal antibodies and cleaved Lamin A polyclonal antibody, respectively.

Results

Differential expression of PR between day 1 (60%) and day 15 (38.5% became negative) was observed in treated tumours exclusively. A significant decrease of PI after treatment was observed in tumours expressing PR on day 1. Furthermore, the AI was significantly lower in PR-positive tumours on day 15 when compared to PR-positive control tumours.

Conclusions

RU534 administration is associated with a decrease of both PI and AI of PR-positive carcinomas. While the former effect seems to be associated with the down-regulation of PR, the latter appears to be independent of the level of PR expression.

Acknowledgements: P07-CVI-2559 (CICE- Junta de Andalucía)

22. CONGENITAL CUTANEOUS PAPILOMATOSIS IN A PIG

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Introduction

Multiple yellowish white cauliflower-like tumors in the skin of head and back in a 3-month-old pig were examined in this study. These lesions had developed before weaning period and no significant clinical abnormalities had been noted by the owner except for the cutaneous lesions.

Materials and Methods

Four tumors were investigated by light microscopy, immunohistochemistry (IHC) and polymerase chain reaction (PCR), especially for papilloma virus (PV) infection.

Results

Histologically, the cutaneous neoplasms were characterized by papillary outgrowth of fibro-vascular tissue covered by thick epidermis. The epidermis showed moderately laminated hyperkeratosis. The cytoplasm of keratinocytes was occasionally vacuolated but did not lade any inclusion bodies. Hyperplastic and rete peg-like extension of epidermis were partly observed associating with the proliferation of capillaries, infiltration of mononuclear cells and proliferation of fibroblasts in the dermal stroma. By IHC and PCR, there were no evidences of papilloma virus infection.

Conclusion

The papillomatosis of the present case is not quite likely to be caused by papilloma virus infection, but it might be a hamartomatous lesion.

A SHORT REVIEW OF CANINE TUMORS DIAGNOSED AT DEPARTMENT OF VETERINARY PATHOLOGY, ZAGREB, CROATIA, DURING YEARS 2006-2009

23.

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Introduction

The significance of monitoring of animal tumors incidence is indisputable. Taking that in account, we analysed data about canine tumors occurrence in Croatia, and presented certain aspects of it.

Materials and methods

Data about canine tumors diagnosed at our institution (either submitted from live animal or obtained at necropsy) were retrospectively analysed for years 2006-2009. A WHO classification was used at designating tumor type.

Results

There was a total of 1627 tumors, of which 962 (59,1%) were malignant, and 576 (35,4%) benign, the rest of 89 (5,5%) were treated as not specified. Most tumors originated from the skin or subcutis (747; 45,9%) and mammary gland (352; 21,6%). Most frequent individual tumor type was mammary tubulopapillary carcinoma (119; 7,31%), followed by squamous cell carcinoma (89; 5,47%) and mast cell tumor (81; 4,98%). Most common tumor types in particular age groups were as follows: 0-1,99 and 2-3,99 years - canine cutaneous histiocytoma (17 cases - 27,87% and 13 cases - 16,25%), 4-5,99 and 6-7,99 years - mast cell tumor (16 - 12,9% and 17 - 8,09%), 8-9,99, 10-11,99, 12-13,99 years and 14 years and up - mammary tubulopapillary carcinoma (27 - 8,65%; 29 - 8,36%; 32 - 11,98%; 14 - 8,75%, respectively).

Conclusion

Overall frequencies of dominant tumor types show figures comparable to other investigations.

24. URETERAL TRANSITIONAL CELL CARCINOMA WITH INTRA-ABDOMINAL AND DISTANT METASTASES IN TWO HORSES

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Introduction

Ureteral transitional cell carcinoma (TCC) or urothelial carcinoma has been reported in dogs but not in horses. In humans, TCC of the renal pelvis and ureter is well documented and classified into non-invasive and invasive (T1-T4) types. T3 and T4 invade through the ureteral wall, and often do not bulge into the lumen, thus leaving a functionally intact ureter. In human, dogs and cattle, UP-3 immunolabelling can be used to specifically identify transitional epithelium.

Case history

Two warmblood horses (20 (1) and 6 (2)-years-old) were euthanized based on clinical evidence of an abdominal malignancy. At necropsy, in both cases, a 15-20cm diameter mass was unilaterally surrounding and coalescing the ureter. Further, multiple pale tan, 5-10cm diameter masses were encircling the abdominal aorta. Lung (1,2) and liver (2) metastases were evident.

Materials and methods

Samples from neoplastic tissues were taken for histopathology and immunohistochemistry for pancytokeratin, HMW-cytokeratin and UP-3.

Results

Tumor histopathological characteristics were typical for TCC. Tumor cells were diffusely reactive to pancytokeratin and HMW-cytokeratin. UP-3 immunolabelling was not successful in horse urothelium.

Conclusion

According to the human classification system, these horse ureteral TCCs can be graded as an invasive T3 type. UP-3 immunolabelling was not useful in these horses.

IMMUNOHISTOCHEMICAL EXPRESSION OF COX-2 IN FELINE INJECTION SITE SARCOMA

25.

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Introduction

COX-2 is an inducible isoform of COX which is expressed in inflamed and neoplastic tissues. This study sought to determine the expression of COX-2 in feline injection site sarcoma (FISS).

Material and Methods

Thirty-one cases of FISS were graded according to the histological grading system proposed by Couto et al. (2002). Seven cases were classified as grade I, thirteen as grade II and eleven as grade III. The expression of COX-2 was analysed by immunohistochemistry. Staining was evaluated semi quantitatively for percentage and intensity, and graded on a scale as follows: percentage <5% = 0; 5-25% = 1; 26-50% = 2; 51-75% = 3; >75% = 4; intensity: low = 1; moderate = 2; strong = 3. Each COX-2 score represented the product of percentage of positive tumour cells and intensity. Scores from 1 to 4 were considered as low COX-2 expression, from 5 to 7 as intermediate COX-2 expression, and greater than 8 as high COX-2 expression.

Results

Thirty FISS (97%) were immunopositive for COX-2. The score level varied from low (12/30; 40%) to intermediate (9/30; 30%) and high (9/30; 30%). No correlation was observed between COX-2 score and histological grade.

Conclusion

The results of this study demonstrate that COX-2 is widely expressed in FISS. Thus, COX-2 inhibitors may have high potential as chemotherapeutics agents.

26. PATHOLOGICAL IMPLICATIONS OF CALLUS FORMATION AND THEIR ROLE IN THE ETIOLOGY OF OSTEOSARCOMA: A CASE REPORT

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Introduction

Osteosarcoma is a highly malignant bone tumour, which can sometimes develop after deficient callus formation.

Materials and Methods

The patient studied was a 12-year-old female German Shepherd dog affected by a chronic condition of the tibia of the left hind leg. Clinical, radiographic, cytologic and histologic examination were performed.

Results

The radiographic examination showed an oblique non-union fracture of the left tibial diaphysis. A periosteal reaction and a non homogeneous radiopacity of the medullary cavity were visible. The cytologic examination of the bone marrow smear aroused a suspicion of osteosarcoma, which was subsequently confirmed by histology, leading to the diagnosis of osteoblastic osteosarcoma.

Conclusions

Chronic osteomyelitis following osteosynthesis may be due either to the metal implant being rejected by the bone tissue as incompatible or to a non-union. Failure to comply with aseptic and antiseptic techniques during the surgical procedure may be involved as well. The chronic inflammatory process which was present at the fracture site, along with the non-union, resulted in the fracture subsequently developing into a neoplastic process.

COMPARISON OF HIGHLY MALIGNANT CANINE AND HUMAN MAMMARY CANCER: IMMUNOHISTOCHEMISTRY OF AROMATASE AND STEROID HORMONE RECEPTORS.

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Introduction

Steroid hormones, especially estradiol, have an important role in the pathogenesis of breast cancer. The cytochrome P450-Aromatase (Aro, CYP19A1) is responsible of the conversion of androgens to estrogens. Canine mammary tumours (CMT) have been proposed as a model to study human breast cancer (HBC). The aim of this study was to compare the immunohistochemistry expression of Aro and ER α , ER β , PR and AR in both species in order to know the validity of the canine model from a hormonal point of view.

Materials and Methods

Three groups of patients were established: 21 HBC with clinical malignancy (lymph node affectation, group A), 13 HBC with high malignant histological grade (grade III, group B) and 19 CMT with both clinical and histological malignancy (lymph node affectation and histological grade III, group C). Immunohistochemistry of Arom, ER α , ER β , PR and AR was performed using antibodies with known reactivity in both species and following the same methodology.

Results

Aromatase and steroid receptors immunoexpressions were similar when comparing groups B and C; nevertheless, group A showed higher immunoexpression of ER α (p=0.005), ER β (p=0.058) and AR (p=0.03) than group C. Arom expression was associated with menopausal status (HBCA: p=0.01), ER α (A: p=0.075) and inversely with the intensity of AR (A: p<0.01, B: p=0.04).

Conclusion

Canine malignant mammary tumours are comparable to HBC, in terms of hormonal status and arom expression, only if tumours of high histological malignancy are considered.

DIFFERENT MORPHOLOGICAL TYPES OF SKIN BENIGN TUMOURS CHEMICALLY INDUCED IN MICE AND THEIR ASSOCIATION WITH KERATIN MODIFICATIONS

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Introduction

Tumours developed in mouse using induced multistage skin carcinogenesis show similarities with those spontaneously arose in humans. Our purpose was to establish the relationship between the morphology and the keratin expression profile of chemically induced tumours.

Methods

Nineteen adult NMRI mice were treated with a two-stage carcinogenesis protocol. Skin samples were routinely processed. Sections were immunolabelled by ABC-P method with antisera to keratins K5, K6, K1, K10, K13.

Results

Morphologically different types of tumours were diagnosed: flat (papule, villous and finger-like growths) or pedunculated squamous cell papillomas and keratoacanthomas. Incidence and multiplicity were significantly higher in papillomas than in keratoacanthomas. The number of papule papillomas and keratoacanthomas overexpressing K5 (indicating cellular proliferative capacity) and expressing K6 (indicating hyperproliferation) was significantly higher than in the other subtypes. In addition, a significantly reduction in K1 and K10 expression was seen. K13 expression (early marker of malignant expression) was significantly higher in keratoacanthomas, followed by villous and pedunculated papillomas.

Conclusion

There is a clear association between morphology and keratin characterization of carcinogen-induced skin tumours; this can be used to evaluate the tumour development and its tendency to regression or to malignant progression, vital data for assessment of studies on skin cancer prevention.

EXPRESSION OF CYCLIN E IN TRANSITIONAL CELL CARCINOMA

29.

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Introduction

During the cell cycle expression of cyclins is changing. In this study we analyzed the expression of cyclin E in transitional cell carcinoma. The aim of this research are analysis of the expression of cyclin E in transitional cell carcinoma and comparison of the expression of cyclin E with stage and histological grade of cancer.

Material and methods

In this study we used biopsy material which is diagnosed at the Institute of Pathology, Medical Faculty in Belgrade. We analyzed 24 cases of transitional cell carcinoma of the urinary tract, which were different histologic grade and stage. For the analysis was used immunohistochemical method. All cases were statistically processed and analyzed semiquantitatively scored.

Results

In this sample, statistical test, showed no statistically significant correlation between stage and expression of cyclin E ($p > 0,05$). Using this method it was determined that there is no statistically significant correlation between grade of transitional cell carcinoma and expression of cyclin E ($p > 0,05$).

Conclusion

Based on research results in the expression of cyclin E in transitional cell carcinoma, loss of expression was found in tumor tissue. Expression of cyclin E decreases with increasing histologic grade and increased tumor stage.

POSTER SEMINAR TOUR
SESSION 3A - INFECTIOUS DISEASES

A SUSPECTED CASE OF HALICEPHALOBUS SPP. ENCEPHALITIS IN A HORSE

31.

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Introduction

An 18- year- old male Holsteiner horse with acute onset of neurological signs was referred to the University Horse Clinic. Clinical examination confirm that horse is susceptible to encephalitis. The horse died despite therapy and necropsy was performed.

Materials and Methods

During the necropsy the tissue samples of the lung, myocardium, liver, spleen, stomach, intestine and brain were fixed, routinely processed and stained by HE, PAS and Luxol fast blue. The brain was sampled for microbiology.

Results

Necropsy examination revealed large atelectatic areas in the left lung, dilation of the right heart ventricle, spleen and liver congestion with villous projections on the organs surface. In cranial cavity the cerebellum subarachnoideal edema was evident. Microscopically, in the leptomeninges, in the brain stem, medulla oblongata and mesencephalon mostly perivascularly, rhabditiform nematodes with small body diameter and intensively stained, were observed.

Conclusion

Nematode morphology and location was most compatible with opportunistic saprophagous parasite *Halicephalobus* (syn. *Micronema*) sp.

32. IMMUNOHISTOCHEMICAL DETECTION OF BETA CATENINE IN TRANSITIONAL CELL CARCINOMA

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Introduction

Different immunohistochemical methods have been used aiming at more precise diagnosis and prognosis of transitional cell cancer. Our study aims to evaluate the expression of beta catenine in different histological grades and stages of transitional cell carcinoma.

Material and methods

24 diagnosed biopted cases were analyzed at the Institute of Pathology of the Faculty of Medicine in Belgrade. 6 pyelon, 2 ureter and 15 urinary bladder carcinomas were analyzed for the purpose of this research. For the analyses we have used the immunohistochemical method. Immunohistochemical marking of the analysed samples was graded by semiquantitative method.

Results

Moderate and diffuse beta catenine expression was noted in 70,83% (17/24), while 16,66% (4/24) was beta catenine antibody negative. The biggest expression of beta catenine was noticed in stage 1 it decreased due to the increase of the tumor stage ($p < 0,01$). With the increase in tumor grade, expression of beta catenine decreased from the normal value ($p > 0,05$).

Conclusion

Increasing grade and stage of the tumor, decreases the expression of beta catenine. In beta.catenins, there was a significant correllation between the expression of these molecules and tumor stage, and therefore, furthes studies involving a larger number of cases would be necessary.

HISTOPATHOLOGICAL FINDINGS IN DOMESTIC PIGS 33. WITH NATURAL HEV INFECTION

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Introduction

In 1997, a novel virus was discovered in swine and designated as swine Hepatitis E virus (HEV). Microscopically infected pigs had evidence of hepatitis characterized by mild to moderate multifocal and periportal lymphoplasmacytic hepatitis with mild focal hepatocellular necrosis. The aim of our study was to clarify the presence of HEV and described histopathological lesions in Croatia.

Materials and methods

A 37 fatteners were completely post-mortem examined. During the necropsy samples of bile were collected and tested by nested PCR for presence of HEV. The liver tissue was collected in 10% buffered formalin, routinely processed, embedded in paraffin blocks and stained with H&E.

Results

HEV RNA was detected in 3 (8.1%) of total 37 pigs. Lymphoplasmacytic hepatitis was detected in these 3 animals. A moderate inflammatory reaction was present in periportal spaces around bile ducts and in interstitial tissue, as well as centrolobar necrosis and degeneration of hepatocytes with mild lymphoplasmacytic reaction. Histopathological lesions were not observed in the samples of the 34 animals detected HEV-negative by RT-PCR.

Conclusion

This study confirmed presence of HEV infection in Croatian large pig production units. Histopathological lesions of liver in HEV positive animals were similar to findings described in previous publications.

34. IMMUNOHISTOCHEMICAL STUDY OF THE INFLAMMATORY INFILTRATE ASSOCIATED WITH SWINE HEPATITIS E VIRUS INFECTION

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Introduction

Hepatitis E virus, causative agent of swine infectious hepatitis, is present in swine farms of many geographical areas. A number of evidences have demonstrated the zoonotic nature of hepatitis E virus (HEV). Although many studies have described histological alterations of the liver, little is known about the mechanism of cell damage or the type of local immune response by the host cells to the HEV.

Materials and Methods

Formalin fixed and paraffin embedded liver samples from 12 slaughtered pigs which were RT-PCR positive for HEV in the rectal swab and in liver, were examined by light microscopy, using haematoxylin and eosin stain and immunohistochemistry for CD3 and CD79.

Results

Mild and moderate multifocal lymphohistiocytic hepatitis in the portal tracts and/or irregularly distributed in the liver was observed. The infiltrate comprised predominantly of mononuclear cells, occasionally observed close to area of necrosis of the hepatocytes. Councilman bodies which represent areas of focal eosinophilic condensation in the cytoplasm were also observed. The portal and lobular lymphocytes were predominantly CD3+ T cells, whereas CD 79+ B lymphocytes formed distinct aggregates within the regions of T-cell infiltration.

Conclusions

These findings provide further evidence for an immune mediated pathogenesis in hepatitis E virus infection in swine.

PREVALENCE AND PATHOGENICITY STUDY OF INTESTINAL TRICHOMONADS IN PIGS

35.

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Introduction

Infections with protozoal parasites from the order Trichomonadida are common in veterinary medicine. In pigs, two different trichomonad species (*Tritrichomonas foetus* and *Tetratrichomonas buttreyi*) have been described as commensals in the large intestine. In this study, the prevalence and the pathogenicity of trichomonads in pigs should be determined.

Materials and Methods

Chromogenic in situ hybridization (ISH) was performed on paraffin embedded colon sections of 192 pigs. First, all tissue sections were analysed using a probe specific for all Trichomonadida (OT). Afterwards, all positive samples were further examined using the OT probe and another probe detecting all known Tritrichomonads (Tritri) on two consecutive sections.

Results

100 of the 192 pigs were tested positive with the OT probe. Of these positive pigs, 14% showed moderate to high-grade parasitic load with trichomonads invading the lamina propria. In the second ISH, the positive result with the OT probe was confirmed in 91 pigs, whereas only 48 pigs showed positive signals using the Tritri probe.

Conclusion

The results suggest the presence of two different trichomonad species, presumably *T. foetus* and *T. buttreyi*, in the colon of pigs. Since trichomonads were found to emigrate into intestinal mucosa, their pathogenicity needs to be further re-evaluated.

DETECTION OF CASPASE ACTIVITY AND APOPTOSIS IN NATURALLY INFECTED SHEEP WITH PESTE DES PETITS RUMINANTS

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Introduction

Peste des petits ruminants (PPR) is an economically significant and infectious disease of small ruminants. PPR virus (PPRV) is classified in the Morbillivirus genus of the Paramyxoviridae family. In recent years, virus induced apoptosis or eliminating of infected cells by apoptotic mechanism have been detected some of the virus infections.

Materials and Methods

Tissues from 14 sheep from 3 PPR outbreaks were examined. All of the 14 animals were found positive for PPRV RNA by RT-PCR. Tissue samples were fixed in 10% neutral-buffered formalin and embedded in paraffin wax. Viral antigens and caspase 3, 8, 9 were detected by avidine biotine peroxidase technique and apoptosis by TUNEL methods.

Results

Immunohistochemically viral antigens were detected in the alveolar macrophages, pneumocytes and syncytial cells of lungs. In the spleen, tongue, buccal cavity, lips and soft palate intense diffuse or granular immunolabelling was also observed. TUNEL positive signals and caspase 3, 8, 9 immunopositive cells were seen in the same tissues.

Conclusion

Apoptosis of the infected cells and caspase activity may appear to play an important role in the pathogenesis of PPR in naturally infected sheep.

PATHOLOGICAL STUDIES ON GASTROINTESTINAL LESIONS IN PORCINE CIRCOVIRUS 2 ASSOCIATED DISEASE

37.

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Introduction

Porcine circovirus 2 (PCV-2) associated gastrointestinal lesions were investigated to clarify histological characteristics, localization of PCV-2 and target cells of the virus.

Materials and Methods

Systemic organs of 25 PCV-2 infected pigs were examined by light microscopy and immunohistochemistry.

Results

PCV-2 associated lesions were found in 6 of 19 stomachs and 8 of 25 intestines. In the gastric mucosa, erosion, dilation of gastric glands, and single cell necrosis of the gastric gland epithelium were observed in association with lymphocytic or lympho-granulomatous infiltration. PCV-2 antigens were frequently localized in the epithelium of isthmus cervix gastric gland and found more frequently in the nuclei than in cytoplasm. Many macrophages laded PCV-2 antigen. Intestinal lesions were less severe than gastric ones. PCV-2 laden macrophage infiltrated in the lamina propria and Peyer's patches showing lymphoid depletion with localization of PCV-2 antigens. Villous atrophy, epithelial degeneration and nuclear fragmentation in the mucosa were observed in association with PCV-2 antigen in the crypt epithelium. Cleaved caspase-3 and TUNEL positive cells were present in these lesions.

Conclusion

Epithelial cells of isthmus, cervix gastric glands and intestinal crypts might be targets of PCV-2 and their injury might be partially attributed to apoptosis by the virus.

38. SPONTANEOUS BLEEDING SYNDROME IN NEONATE CALVES: DESCRIPTION OF 12 CASES IN FRANCE

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Introduction

Bovine Neonatal Pancytopenia (BNP) is an emerging bleeding syndrome affecting neonate calves, firstly described in Germany, and widely distributed in Europe yet. The etiology is unknown but excludes Bovine Viral Diarrhea Virus (BVDV). We describe 12 fatal cases of spontaneous bleeding syndrome in calves of 5 breeds and both genders.

Materials and methods

Hematology, Serology, Necropsy, Histopathology, Virology (Bluetongue Virus (BTV), BVDV in blood, spleen, and bone marrow) and genotyping (BVDV).

Results

The 12 cases were characterized clinically by an acute onset, before 3 weeks of age; spontaneous bleeding through skin, mucous membranes; severe nonregenerative anemia and thrombocytopenia; and rapidly fatal outcome. Gross lesions in all cases included mucous and serosal petechial hemorrhages. Microscopic lesions were acute hemorrhages without vasculitis, bone marrow aplasia, and facultative lymphoid hypoplasia in lymph nodes and spleen. While all cases were negative for BTV infection, 2 bone marrow samples were positive for BVDV (BVDV1e type). The 10 remaining cases correspond to BNP, and included 9 calves born from dams vaccinated against BVDV (as classically reported in BNP).

Conclusion

BVDV infection should still be investigated in spontaneous bleeding syndromes in calves. The etiology of BNP remains unknown, although viral or immunological factors are likely involved.

TRYPANOSOMA CONGOLENSE (FOREST-TYPE) INFECTION IN A DOG AFTER A STAY IN SENEGAL

39.

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Introduction

In a two-year-old crossbred Belgian shepherd bitch presented for exhaustion and weight loss, severe hypoglycemia (0.12 g/l), anemia (red blood cells: 2.6 x 10¹²/L) and thrombocytopenia (45 x 10⁹/L) were diagnosed. The dog came back from a six-month stay in Senegal and sub-Saharan Africa. We present here up-to-date knowledge on pathogenesis of canine African trypanosomiases.

Materials and methods

Examination of blood smear, lymph node aspiration and PCR on whole blood.

Results

Blood smears showed red blood cell anisocytosis and polychromasia, macrothrombocytes and numerous free protozoa. The parasites were 10 to 20 µm in length, had a central nucleus, a medium-sized kinetoplast located at the margin of the body, just in front of the posterior extremity (marginal and subterminal kinetoplast), a poorly-developed undulating membrane and no free flagellum. This morphology is consistent with *Trypanosoma congolense*. Numerous trypanosomes were also present in lymph node aspiration. PCR analysis revealed an infection with *Trypanosoma congolense* forest-type.

Conclusion

Cytological examination and PCR confirm that the dog was chronically infected by *Trypanosoma congolense* forest-type. In cattle, strains of *T. congolense* differ greatly in virulence: *T. congolense* savannah-type produces rapidly fatal infection, *T. congolense* forest-type causes chronic infection and *T. congolense* kilifi-type produces unapparent infection.

42. DISTRIBUTION PATTERNS OF AVIAN BORNAVIRUS IN PSITTACINE BIRDS SUFFERING FROM PROVENTRICULAR DILATATION DISEASE

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Introduction

Recently, different avian bornavirus genotypes (ABV) have been identified in psittacines that died from Proventricular dilatation disease (PDD).

Materials and Methods

Brain, spinal cord, retina, N. ischiadicus, crop, gizzard, proventriculus, intestine, myocard, liver, kidney, pancreas, skin and skeletal muscle were investigated in 65 birds (7 different species) that were suspicious for PDD. H&E-staining was used for histological evaluation, ABV-antigen was demonstrated immunohistologically applying an anti-phosphoprotein antibody. Data were compared to the detection of viral RNA by real time RT-PCR in (38/53 birds), ABV-specific antibodies (6/9 birds) and isolation of infectious virus (16/19 birds).

Results

Virus antigen was restricted to the central nervous system (CNS) in 4 birds but was found also in the periphery in 20 animals. In 22 of the birds virus antigen was detectable only in the periphery, in 19 no viral antigen was found. Viral antigen detection correlated with the presence of viral RNA in 28 cases, with detection of ABV-specific serum antibodies in 6 animals and with isolation of infectious virus in 16 birds.

Conclusion

Whether different viral distribution patterns were due to infection with different viral genotypes, represent different stages of infection or are mainly determined by host factors have to be further investigated.

44. PULMONARY LESIONS FOLLOWING INTRAVENOUS INOCULATION WITH A HIGH CONCENTRATION OF STAPHYLOCOCCUS AUREUS IN PIGS

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Introduction

Following intravenous inoculation of *Staphylococcus aureus* pigs are prone to develop low grade sepsis. The porcine lung plays an important role in clearing systemic bacterial infections. We investigated pulmonary changes after increasing the concentration of *S. aureus* in the inoculum.

Material and methods

Five pigs were intravenously inoculated with *S. aureus* (1×10^8 CFU/kg body weight), two control animals were sham-inoculated. Blood samples for bacterial cultivation were taken at regular intervals before and after inoculation. By 48 hours all animals were euthanized, necropsied and tissue was sampled for histological examination.

Results

All five infected animals had disseminated pulmonary abscesses with thickening of surrounding alveolar septa. Furthermore, areas of necrosis, haemorrhage, fibrinous exudation and oedema were seen in two pigs.

After 6 hours most infected animals had cleared the bacteria from the blood, however high concentrations were found in the lungs post mortem.

The pulmonary lesions were more severe compared to those of earlier studies using the same bacterial dose suspended in a higher volume.

Conclusion

With increased inoculum concentration of *S. aureus* more severe pulmonary changes are observed, possibly creating a focus of cytokines from the lungs.

POSTER SEMINAR TOUR
SESSION 3B - INFECTIOUS DISEASES

45. ORIGIN OF THE WORLD FROG PANDEMIC:
EVIDENCE FROM EAST ASIA SUPPORTS THE
NOVEL PATHOGEN THEORY.

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Introduction

The pandemic caused by the chytrid fungus (*Batrachochytrium dendrobatidis*;Bd) is considered to be partly responsible for the precipitous decline of amphibian populations in the world. Nevertheless, its effects have not yet been seen in Asia. We searched for the reason why no effects of Bd have been seen in Japan.

Materials and Methods

We conducted a pathological and molecular biological examination on 28 native Japanese giant salamander (*Andrias japonicus*;AJ), 10 collected from 2005 to 2008 and 18 collected from 1902 to 1937, to clarify the prevalence of Bd. Results: Bd infection was confirmed in 13. Of these, the earliest specimens were 7 collected in 1902. The base sequence of the ITS -1 region of the amplified PCR product (DDBJ No.AB435220) was slightly different from that of Bd registered with DDBJ. However, the genetic base sequence of the 18S and 28S rRNA specific gene amplified from AJ was the same as that of the registered Bd.

Discussion

Till now, the earliest recorded occurrence of Bd anywhere in the world was assumed to be that found in *Xenopus laevis* in South Africa in 1938. The present paper reports a much earlier occurrence of Bd, i.e., in 1902. We also identified more than 30 haplotypes in wild frogs in Japan (Goka2009, 2010), but only a few haplotypes have been found in Panama and Australia.

Conclusions

Our findings concerning the earliest specimens and diversity of Bd suggest an Asian origin for this amphibian-killing fungus.

SYSTEMIC MYCOSIS DUE TO CANDIDA ALBICANS IN A DOG

46.

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Introduction

Systemic mycotic infection was diagnosed in a 2-year-old female hovawart presented with generalized lymphadenopathy. Pyogranulomatous inflammatory reaction was found in lymph nodes, spleen, liver, kidneys, adrenal glands, peritoneum, lungs, myocardium and mammary gland.

Materials and Methods

Tissue samples were collected for histopathological examination, fixed in buffered 10% neutral formalin, dehydrated, embedded in paraffin wax, sectioned on a microtome at a thickness of 4 µm, and stained with hematoxylin and eosin (H&E), Ziehl-Neelsen (ZN) and periodic acid Schiff (PAS) stain for the detection of infectious agents in tissues. Samples of all affected organs were taken for fungal culture.

Results

At necropsy, whitish nodules of various sizes were found in lymph nodes, spleen, liver, kidneys, adrenal glands, peritoneum, lungs, myocardium and mammary gland. Histopathologically, the pyogranulomas consisted of neutrophilic granulocytes, macrophages and multinucleated giant cells, often with necrotic centres. In all pyogranulomas were present fungi-like structures, which were subsequently demonstrated in affected organs using PAS stain.

Conclusion

Diagnosis of generalized candidiasis was settled after *Candida albicans* infection was confirmed by culture.

47. AVIAN TUBERCULOSIS IN A CAPTURED RUPPELL'S GRIFFON VULTURE (GYPS RUPPELLII)

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Introduction

Avian tuberculosis was diagnosed in a captured female Ruppell's griffon vulture (*Gyps ruppellii*) with granulomatous splenitis and hepatitis.

Materials and Methods

Tissue samples were collected for histopathological examination, fixed in buffered 10% neutral formalin, dehydrated, embedded in paraffin wax, sectioned on a microtome at a thickness of 4 µm, and stained with hematoxylin and eosin (H&E) and Ziehl-Neelsen (ZN) for the detection of acid-fast bacilli in tissues. The liver and spleen were taken for culture and quantitative Real-Time PCR examinations.

Results

At necropsy, whitish to yellow nodules of various sizes were found in the spleen and liver. Histopathologically, the granulomas appeared to be typical of avian tuberculosis. In some granulomas, necrotic centres surrounded by a variable layer of palisading epithelioid macrophages and multinucleated giant cells with variable admixture of lymphocytes and plasma cells were present. Signs of mineralization in granulomas were not observed. Using Ziehl-Neelsen staining acid-fast bacilli were demonstrated in organs affected by granulomatous inflammation.

Conclusion

Diagnosis of *Mycobacterium avium* subsp. *avium* infection was confirmed by culture and quantitative Real-Time PCR examination for the presence of specific insertion sequences for avian tuberculosis (IS901 and IS1245) in the liver and spleen.

UNUSUAL MANIFESTATION OF BHV-1 INFECTION IN NEONATAL CALVES

48.

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Introduction

Bovine herpes virus 1 (BHV-1) is a worldwide pathogen of cattle, causing clinical syndromes of infectious bovine rhinotracheitis (IBR), infectious bovine pustular vulvovaginitis, abortion, and meningoencephalitis.

Materials and methods: Necropsy was performed on the carcass of an 11-day-old calf from a group of animals exhibiting clinical signs of dyspnoea, epiphora, and keratitis with multiple mortalities.

Results

Main gross findings included fibrinonecrotising laryngotracheitis, with raised white plaque like lesions with hyperaemic borders in the oesophagus, which histologically appeared as raised areas of necrotic epithelium, with occasional eosinophilic intranuclear inclusion bodies present in oesophageal epithelial cells, consistent with Herpesvirus infection. BHV-1 DNA was detected via PCR performed on splenic samples.

Conclusions

Gross differentials for raised oesophageal lesions in cattle include infections with bovine papular stomatitis virus, bovine papillomavirus, *Candida albicans*, and *Fusobacterium necrophorum*; these were excluded on the basis of pathology and microbiological findings. Although erosions/ulcerations of the upper alimentary tract have been described with BHV1 infection, this case represents an unusual presentation (appearing proliferative grossly). BHV1 infection should therefore be considered as a differential diagnosis for oesophageal lesions of this kind in cattle.

49. INFILTRATES OF T CELLS APPEAR IN OBEX OF SHEEP DURING CLINICAL PHASE OF SCRAPIE

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Introduction

The aim of the study was to determine if in addition to characteristic histopathological lesions, infiltrates of T and B cells appear in the obex of sheep naturally infected with scrapie.

Materials and methods

42 sheep were divided into three groups: 1) healthy sheep, 2) sheep in the clinical phase of scrapie, and 3) sheep in the preclinical phase of scrapie; and subgroups with differently susceptible PrP genotypes: VRQ/VRQ, ARQ/ARQ and ARR/ARQ. The paraffin tissue sections of the obex were immunohistochemically stained for prions (2G11, Institut Pourquier), B cells (CD79 α , DAKO) and T cells (CD 3, DAKO). Histopathological lesions, intensity of immunohistochemical reaction for prions and the number of B and T cells, counted under magnification of 200 x, were recorded.

Results

The average number of T cells in healthy sheep and sheep in the preclinical group was low (from 0.48 to 0.68 cells per visible field). The number was significantly higher in the clinical group ($P < 0.05$), where 5.60 T cells per visible field were counted in VRQ/VRQ genotype and 4.53 cells were counted in ARQ/ARQ genotype. The number of T cells was in positive correlation with the level of spongiform lesions (ρ 0.7493, $P < 0.001$) and the intensity of immunohistochemical reaction for prions in the obex region (ρ 0.6571, $P < 0.001$).

The number of B cells in the obex of all sheep was very low; in average it was lower than 0.05 cells per visible field.

Conclusion

During clinical phase of scrapie, infiltrates of T cells appear in the obex of naturally infected sheep. The number of T cells is correlated to the level of spongiform lesions and the intensity of immunohistochemical reaction for prions, but is not linked to the PrP genotype.

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Introduction

Histoplasmosis is a systemic fungal infection caused by *Histoplasma capsulatum* and is distributed worldwide. Cutaneous and subcutaneous infections can occur as a part of the disseminated diseases or by direct implantation of fungi by trauma.

In this paper skin lesions in three dogs without apparent clinical signs of systemic infection are described.

Material and methods

Skin biopsies of three nodules, 0.5-2 cm in diameter, from thorax, foot pad and perineal region from the Puli, Shar Pei and German Shepherd dog respectively, were examined. Formalin fixed and paraffin wax embedded tissues were stained with HE and periodic acid –Schiff (PAS).

Results

Microscopically, the dermis, panniculus, and occasionally the deeper subcutis contained nodular to diffuse infiltration of large macrophages, admixed with variable number of neutrophils, lymphocytes and plasma cells. Many of the macrophages were filled with small (1-3 μm) punctiform, intracytoplasmatic, dark, oval bodies (yeast), which were well demonstrated with PAS reaction.

Conclusion

Cutaneous histoplasmosis in dogs causes granulomatous or pyogranulomatous inflammation in the dermis and subcutis. Differential diagnoses include cutaneous infections of other opportunistic fungi and algae, micobacterial infection, sterile granuloma and pyogranuloma syndrome and neoplasia.

Intracellular findings of the PAS positive, punctiform yeast could confirm the diagnosis.

51. DISTRIBUTION OF MYOFIBROBLASTS, TRANSFORMING GROWTH FACTOR- β 1 AND , TRANSFORMING GROWTH FACTOR- β 1 RECEPTOR I IN GRANULOMA CAUSED BY MYCOBACTERIUM AVIUM COMPLEX IN PIGS

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Introduction

Recently, it has been suggested that Transforming Growth Factor- β 1 (TGF- β 1) plays an inhibitory role in macrophage activation in Mycobacterium avium complex (MAC) infection. Our study was designed to evaluate the distribution of myofibroblasts, TGF- β 1, TGF- β 1 receptor I (TGF- β 1 RI) in mesenterial lymph nodes in pigs with MAC infection. An additional reason for our research was the fact that macroscopic lesions are not correlated with microscopic findings.

Materials and methods

Formalin fixed and paraffin embedded samples of 90 mesenterial lymph nodes, which were positive for Mycobacterium avium subsp. hominissuis by RT-PCR, were examined by light microscopy using haematoxylin and eosin, Masson-trichrom staining and immunohistology for α SMA, desmin, TGF- β 1, TGF- β 1 RI and CD3 T Ly.

Results

In one third of the lymph nodes with histological changes characteristic for granulomatous lymphadenitis no macroscopically visible changes were established.

Myofibroblasts which expressed α SMA were observed in the outer wall of granuloma. TGF- β 1 was expressed mostly on epithelioid cells in granuloma, while TGF- β 1 RI were demonstrated predominantly on myofibroblasts.

Conclusion

These findings could explain pathogenesis, morphogenesis and the immune response in granulomatous lymphadenitis in pigs caused by Mycobacterium avium subsp. hominissuis.

EVALUATION OF HEPATIC DAMAGE IN CATTLE VACCINATED WITH RECOMBINANT CATHEPSIN L1 AND PEROXIREDOXIN AND CHALLENGED WITH FASCIOLA HEPATICA

52.

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Introduction

There is an international interest in developing vaccines against fasciolosis in ruminants due to the increase of antihelmintic resistance to most efficient drugs. The aim of the present work was to evaluate the hepatic lesions and fluke burdens in cattle vaccinated with recombinant cathepsin L1 (CL1) and recombinant peroxiredoxin (Prx) and challenged with *F. hepatica*.

Material and Methods

Twenty-one male, 4-month age, Holstein Friesian calves were allocated into three groups of 7 animals each. Group 1 was immunized twice with CL1 in adjuvant Quil A, group 2 was vaccinated twice with Prx in Quil A. Group 3 was immunized only with Quil A. All animals were infected 8 weeks post-immunization with 500 metacercariae from *F. hepatica*, and killed 15 weeks after infection. At necropsy the liver was removed for fluke burdens and samples were collected, fixed in formalin and embedded in paraffin for histopathological study.

Results and conclusions

No significant differences for fluke burdens were found between the three groups, but the number of flukes was slightly lower in the Prx vaccinated group versus the adjuvant control group. Histopathological changes such as portal fibrosis, hyperplasia of bile ducts, infiltrate of lymphocytes, plasma cells and eosinophils were slightly less severe in the vaccinated groups.

53. OCURRENCE OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) IN CASES OF ANTIBIOTIC NON-RESPONSIVE DIARRHOEA IN PIGS

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Introduction

The aim of the study was to analyze the presence of PCV2 in cases of antibiotic non-responsive diarrhoea in pigs.

Materials and Methods

Internal organs and feces from 101 pigs, from 52 farms were collected. Sections of lymph nodes and intestines were analyzed for presence of PCV2 DNA by in situ hybridization test (ISH) and hematoxylin-eosin (HE) stained. Feces were tested for presence of *Brachyspira hyodysenteriae* and *Lawsonia intracellularis* by PCR.

Results

In samples from 23 farms large amounts of PCV2 DNA, indicating PMWS, were detected in lymph nodes by ISH. In this group in 19 farms PCV2 was also found in samples of ileum. The animals from remaining 4 farms, PCV2-positive in lymph nodes, were negative in ileum. In samples from 3 farms lymph nodes were negative for PCV2 in ISH and ileums were positive. In HE stained sections of lymph nodes lesions characteristic for PMWS were identified. Similar lesions were observed in PCV2-positive samples of ileum. DNA of *Lawsonia intracellularis* was found in feces from 13 farms, negative for PCV2. Infection by both *Brachyspira hyodysenteriae* and *Lawsonia intracellularis* was detected in 1 farm.

Conclusion

In PMWS-affected pigs similar lesions can be observed both in lymph nodes and in ileum. Presence of PCV2 in ileum may correlate with diarrhoea in PMWS- free animal.

MULTIFOCAL GRANULOMATOUS MENINGOENCEPHALITIS IN A DOG ASSOCIATED WITH SPOROBOLOMYCES ROSEUS

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Introduction

German shepherd dogs appear to be predisposed to mycotic encephalitis. A 9-year old German shepherd presented with sudden onset of central nervous signs. CSF analysis and brain MRI scan revealed inflammatory meningoencephalitis.

Materials and methods

A complete necropsy was performed. Samples of brain tissue were routinely fixed and embedded in paraffin wax, cut and stained with standard HE and PAS. DNA was extracted from paraffin embedded brain tissue and the ITS2 region was amplified and sequenced (Turenne et al., 1999)*.

Results

At necropsy, a focal white-grey malacic lesion was noted in the mesencephalon. Histologically, multiple granulomatous lesions were discovered in the diencephalon and mesencephalon. Intravascular growth of organisms was prominent with fibrinoid necrosis and thrombosis. PAS staining demonstrated intralesional presence of multiple irregular septate, 5-7 μm wide, nondichotomously branched hyphae with thin parallel walls.

The amplified ITS2 sequence was compared to all sequences submitted in Genbank using the BLAST software at NCBI and showed 96% homology with *Sporobolomyces roseus*.

Conclusion

This report describes the first case of intracranial sporobolomycosis in an animal.

55. INTERACTIONS OF VIRULENT AND AVIRULENT
YERSINIA RUCKERI STRAINS WITH ISOLATED GILL
ARCHES AND INTESTINAL EXPLANTS OF RAINBOW
TROUT (*ONCORHYNCHUS MYKISS*, WALBAUM)

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Introduction

The port of entry in *Yersinia ruckeri* infections in salmonids has not yet been fully elucidated. We have studied the interactions of *Y. ruckeri* with gills and gut explants using standardized perfusion models.

Materials and Methods

Avirulent and virulent *Y. ruckeri* strains (approximately 108 CFU/ml) were added to isolated perfused gill and gut explants. The adhesion and invasion capacity were determined by bacteriological examination of tissues and efferent perfusion fluid, respectively. Histology and immunohistochemistry were performed to visualize adherent and invading bacteria.

Results

Bacteriological examination indicated that both virulent and avirulent *Y. ruckeri* isolates adhere to and invade both tissues without significant differences. Histopathological changes were not observed in the tissues. *Y. ruckeri* was found in the mucus of the primary and secondary lamellae, closely associated with the gill epithelium and some invading in the lamina propria. In gut, *Y. ruckeri* bacteria were present in the crypts, attached to the villi, within the epithelial layer of gut segments and few in the lamina propria mucosae.

Conclusions

Y. ruckeri was shown to colonize and invade gills and gut tissue of rainbow trout, indicating that both organs can be the port of entry.

CHARACTERISATION OF SLOVENIAN OVINE ATYPICAL SCRAPIE CASES

56.

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Introduction

Atypical scrapie is a form of scrapie described in sheep at first in 1998 in Norway (NOR 98), and later in a number of other countries, including very recently in New Zealand and Australia. The first cases of atypical scrapie were found in Slovenia in 2010 and their characteristics are presented.

Materials and Methods

Brain samples were collected during routine monitoring for TSEs from sheep that died or were killed on farms. Atypical cases were found with rapid test, after that, several methods for characterisation were applied - three additional rapid tests, histopathology, immunohistochemistry, genotyping, and BSE/TSE discriminatory test.

Results

All tests which necessitate proteinase K digestion were negative, BSE and classical scrapie were excluded, but immunohistochemistry revealed pattern characteristic for NOR 98 atypical scrapie. Beside that, several ovine cases have been found which show somewhat different atypical immunostaining of cerebellum, and which were concluded as unconfirmed for TSE (not negative) after additional examination in the CRL for TSE.

Conclusions

Atypical scrapie can be expected in small ruminants in all countries but detection requires appropriate testing. Slovenian ovine atypical scrapie cases are similar to NOR 98 however we should be aware that other forms of atypical scrapie may appear.

57. SISTEMIC VENOUS THROMBOSIS IN BABESIA CANIS CANIS INFECTION OF TWO DOGS

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Introduction

A male poodle dog (case 1) and female mixed breed dog (case 2) were treated with imidocarb dipropionat due to babesiosis. Clinical signs related to case 1 included anemia, jaundice, renal failure, bilirubinemia and increased serum amylase. Dog case 2 presented multiple organ dysfunction syndromes with leucocytosis, thrombocytopenia and bilirubinemia. After initial improvement both dogs died 12 and 6 days after the beginning of treatment.

Material and methods

Necropsy was performed and the tissue samples were collected for routine histopathology. DNA was extracted from both dog spleens for confirmation with PCR and subsequent sequencing.

Results

Apart from gross findings distinctive for hemolytic anemia in canine babesiosis as hemoglobinuric nephrosis, thrombosis of splenic vein followed by multiple infarctions in both cases was also observed. In case 2, multifocal necrotizing dermatitis was seen. Histopathology revealed fibrous thrombi in spleen, pancreas and tongue in case 1 and also fibrinous in spleen, skin and esophagus in case 2. Sequence analysis of the PCR fragment revealed a sequence 100% identical to *Babesia canis canis* in both cases.

Conclusion

Hemolysis, disseminated intravascular coagulation and vascular damage are consistent occurrence in *B. canis canis* infection. We presume that ATIII loss through damaged glomeruli due to hemoglobinuric nephrosis led to venous thrombosis in presented cases.

LACK OF ASSOCIATION BETWEEN SCRAPIE GENETIC SELECTION AND VISNA/MAEDI VIRUS ANTIBODY TITERS 58.

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Introduction

Scrapie and Visna/maedi (VM) are chronic and fatal small ruminant infections. Antibody detection against VM virus (VMV) by ELISA is used for detection of VM infection within a flock and for implementation of control and eradication programs. Resistance/susceptibility to scrapie is related with 3 main codons of the PRNP gene, namely 136, 154 and 171. Selecting breeding favoring scrapie resistance based in the individual genotype has been performed since 2002. This genetic selection could have unexpected effects in other conformational, reproductive or disease-related traits.

Material and methods

We used individual data from 10,602 Rasa Aragonesa sheep distributed in 17 flocks (flock seroprevalence ranging between 23,71% and 87,37%). Using multifactorial statistical analysis (SAS) we studied the possible relation between VMV antibody titer and the different groups of resistance/susceptibility to scrapie.

Results

The VMV serum antibody titer from scrapie genetically resistant animals is not statistically different from scrapie genetically sensitive sheep.

Conclusion

Scrapie genetic selection for resistance does not seem to influence individual VMV serum antibody titers and therefore it has no impact in ELISA-based, VMV control and eradication programs.

59. IMMUNE RESPONSE IN THE UPPER RESPIRATORY TRACT DURING COINFECTIONS BVDV/BHV-1

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Introduction

Many investigations indicate that both bovine viral diarrhoea virus (BVDV) and bovine herpesvirus type 1 (BHV-1.1) impairs the immune response of cattle inducing immunosuppression.

Materials and Methods

The study of the nasal mucosa and tonsils, as organs of primary viral entry and replication, is crucial to deepen in the pathogenesis of these viral processes. Calves were inoculated intranasally with non-cytopathic BVDV and 12 days later with BHV-1.1. Immunophenotypic characterization and quantification of subpopulations of immune effector cells and their biosynthetic activity was performed using flow cytometry and immunohistochemistry techniques.

Results

We observed a significant decrease in the number of CD4+ and CD8+ circulating and an intense activation of lymphoid structures in the studied organs, leading to a recovery of these subpopulations. During the BVDV infection, it observed an initially establishing of a type II immune response characterized by the expression of IL-4, but after BHV-1.1 infection does not establishing a correct cellular response (type I), and they kept the type II immune response.

Conclusion

This fact, join to the decrease in CD8+ lymphocytes facilitates maintenance of BHV-1.1 in the upper respiratory tract and tonsils.

Supported by grants (AGL 2006-01536 and PO9-AGR-467) from Spain.

BLUETONGUE IN GOATS NATURALLY INFECTED WITH SEROTYPE-1: ROLE OF PROINFLAMMATORY CYTOKINES IN THE DEVELOPE OF EDEMA

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Introduction

A generalized subcutaneous edema appeared in goats during the outbreak of BT (serotype-1) that took place in the south of Spain in 2007.

Materials and Methods

In order to clarify the pathogenetic mechanisms involved in the appearance of edema, the role of proinflammatory cytokines (TNF α and IL-1 α) and their relationship with viral infection, tissue samples from infected goats were routinely processed for histopathologic and immunohistochemical studies.

Results

Microscopical examination showed the presence of a perivascular edema around small vessels with vacuolated endothelium, next to the presence of perivascular and interstitial mononuclear infiltrates constituted mainly by macrophages and lymphocytes. BTV detection on tissue samples by immunohistochemistry (MoAb 2E9) displayed that arteriolar and capillary endothelial cells, together with macrophages and lymphocytes, were the main target cells of the virus. Vacuolated endothelium as well as perivascular and interstitial mononuclear infiltrates were immunolabeled against TNF α and IL-1 α .

Conclusion

The viral infection of endothelial cells and neighbouring mononuclear cells (macrophages and lymphocytes) might induce an increase in the biosynthetic activity of these cells as well as the release of proinflammatory chemical mediators involved in an increased permeability of vessels and edema appearance.

(Work supported by MICINN, Spain. AGL2009-13174-C02-01)

**POSTER SEMINAR TOUR
SESSION 5A - WILDLIFE DISEASES**

61. MASSIVE TOXOPLASMA ABORTIONS IN A DAIRY SHEEP FLOCK

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Introduction

Toxoplasmosis has not been diagnosed in Greek sheep or goat flocks to date, although many seropositive animals have been found in recent seroprevalence studies.

Materials and Methods

Massive abortions (300/500 pregnant sheep) at 110-130 days of gestation and subsequent reduced milk production were observed in a dairy sheep flock in Greece. Mother and fetal serology, and fetal brain cytology were employed to establish diagnosis. Two treatment regimes were subsequently evaluated in the remaining 200 pregnant sheep. Sulfadimidine was administered at a dose of 20 (Group 1) or 33 (Group 2) mg/kg every 2 days, 4 times, i.m.

Results

Toxoplasmosis was confirmed by mother and fetal serology, and by fetal brain cytology. Treatment was effective in both groups, as the abortion rate was reduced and the gestation period prolonged, resulting in more animals having normal milk production, but was significantly more effective in Group 2.

Conclusions

To our knowledge, ovine toxoplasmosis abortions with such massive abortion rate have not been reported previously. This is also the first report of reduced milk production in a dairy flock attributed to toxoplasmosis abortions.

PATHOMORPHOLOGICAL CHANGES IN BROILERS INFECTED WITH ORNITHOBACTERIUM RHINOTRACHEALE

62.

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Introduction

In this paper, results of examination of *O. rhinotracheale* presence and pathomorphological changes in broilers in epizootiologic region of South Banat are presented.

Materials and Methods

Three flocks of broilers were chosen for examination, one target flock with manifestation of respiratory disorders and two control flocks without respiratory disorders. For these flocks, whose state of health was observed, gross changes and microscopic changes of macroscopically changed respiratory organs were examined as well as the presence of specific antibodies to *O. rhinotracheale*.

Samples of blood serum were examined by ELISA (IDEXX). For pathohistological examination sections of lungs and trachea after standard processing were stained using standard hematoxylin-eosin method (HE).

Results

In the target flock with manifested respiratory disorders high seroprevalence of specific antibodies for *O. rhinotracheale* was found. Autopsy found frequent changes in trachea, air sacs and lungs, which were manifested as: tracheitis catarrhalis, aerosacculitis fibrinosa and pneumonia fibrinosa. In two control flocks, with low seroprevalence of specific antibodies for *O. rhinotracheale*, there were no clinical signs and autopsy found lower number of chickens with the lesions in the respiratory organs.

Conclusion

Our investigations determined that *O. rhinotracheale* infection was spread in intensive broiler production.

COMPARATIVE EVALUATION OF IMMUNOHISTOCHEMISTRY AND IN-SITU HYBRIDIZATION FOR THE DETECTION OF ENCEPHALITOOZON CUNICULI IN RABBIT TISSUES

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Introduction

Infections with the obligate intracellular and sporogenic eukaryote *Encephalitozoon cuniculi* are widespread in pet rabbits. The aim of this study was a comparative evaluation of two specific detection methods on tissue sections for the use in postmortal routine diagnostics.

Materials and Methods

Immunohistochemistry (IHC) with a commercially available polyclonal antibody was performed on paraffin embedded sections of brain and kidney of 81 rabbits (71 with suspicious clinical signs and/or pathohistology and 10 negative controls). Additionally, the eyes of three further patients were examined. All samples which tested positive by IHC were subjected to in-situ hybridization (ISH) using an oligonucleotide probe targeting a portion of the 18S rRNA gene.

Results

Of the 71 rabbits 17 brains, four kidneys and two eyes were tested positive by IHC. Only six of the 17 IHC-positive brains and all four kidneys showed unequivocally positive signals in the in-situ hybridization. However, several of the examined samples showed partial staining of the pathogen or unclear results.

Conclusion

Based on these results, IHC can be recommended for the detection of *E. cuniculi* in routine diagnostics. In contrast, in-situ hybridization failed to detect all developmental stages of the organism and thus is an inappropriate diagnostic method.

BUDGERIGAR FLEDGLING DISEASE IN AN 8-YEAR-OLD 64. BLUE-FRONTED AMAZON PARROT (AMAZONA AESTIVA AESTIVA) - CASE REPORT

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Introduction

The incidence of budgerigar fledgling disease (BFD) in the blue-fronted amazon parrot is relatively low and it concerns birds aged up to 14 weeks.

Material and methods

8-year-old blue-fronted amazon parrot (BFAP), died after showing symptoms of depression, anorexia and diarrhoea, followed by dyspnoea, ataxia and paralysis. Samples of liver, kidneys, spleen and bursa Fabrici were stained with HE.

Results

Macroscopic examination revealed dystrophic feathers, deformed beak and hypohydraemia. Numerous extravasations in the skeletal muscles and skin were observed, together with ascites, hepatomegaly with irregular yellow-white mottling, splenomegaly, oedema renum, hydropericardium, oedema pulmonum, extravasation under epicardium and in the central nervous system. Microscopic lesions in the liver were typical for BFD virus: karyomegaly with amphophilic intranuclear inclusion bodies, multifocal necrosis with heterophilic and plasmatic cell infiltration and extravasations. Necrosis of renal tubules epithelium, interstitial cell infiltration, hyperaemia and extravasation was observed. Atrophy of lymphocytic cells was observed in the spleen and bursa Fabrici. The pattern of disease, and the presence of amphophilic inclusion bodies in the nuclei of hepatocytes, indicates the incidence of BFD.

Discussion

BFD virus infection in adult BFAPs is asymptomatic. Immunosuppression caused by changing of environment could be a reason of symptoms appearance.

65. ABNORMAL DEVELOPMENT OF THE UPPER INCISORS IN THE NOSE OF A ROCK HYRAX (*PROCAVIA CAPENSIS*) WITH A *BACILLUS DASSIE* INFECTION.

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Introduction

Hyraxes are rabbit-sized, hooved African mammals that share a common ancestor with elephants and seacows. Tooth eruption in the nose may cause recurrent or chronic pathologies but sometimes stays asymptomatic.

Material and Methods

A 3 year-old male Rock Hyrax was euthanised after long wasting, with a suspicion of tuberculosis. At autopsy, prominent cahexia could be noticed along with a swelling of the upper palate at the level of the upper incisors. The head was consequently treated in order to obtain the skull. Samples from lung, liver, spleen and mesenteric lymph nodes were taken for bacteriological examination.

Results

There was an abnormal positioning of the upper right incisor that erupted in the right nose cavity. Additionally, mycobacteria were detected by microscopy and a 16S-based PCR for the *Mycobacterium tuberculosis* complex (MTC) from the 4 organs sampled, and isolated from lung samples. They were identified by spoligotyping as belonging to the *bacillus dassie* type.

Conclusion

This is the first time this pathology is described in a rock hyrax. Additionally, the hyrax-specific MTC species *bacillus dassie* was isolated from lung samples. No causal link could be established either between ectopic upper incisor, or colonisation with this bacterium, and the wasting process.

THE EVALUATION OF YERSINIA ENTEROCOLITICA PRESENCE IN SLAUGHTERED SWINE CARCASSES AND TONSILS 66.

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Introduction

The detection of *Yersinia enterocolitica* in swine carcasses has become a practice in slaughterhouse inspection. The research was oriented to establish some correlations between transformation of histological structures and *Yersinia enterocolitica* detection.

Materials and methods

Were investigated 147 samples of tonsils from commercial slaughtered swine. Were performed histological exams, inoculation of culture media and homogenates for RT-PCR analysis. Was studied the correlations between macroscopic modified areas, histopathological aspects (paraffin inclusions; trichromic Masson coloration), the isolation of *Yersinia enterocolitica* through classic bacteriological methods and identification of *ail* gene using amplification with a Light Cyclor 2.0.

Results

The identification of some transformation of tissues or organs correlated with *Y. enterocolitica* isolation may constitute a way of suspecting the yersiniosis. From analyzed samples *Y. enterocolitica* was isolated in 24 cases through bacteriological classic methods and in 68 cases by using RT-PCR. In contaminated tonsils was observed an erythrocyte infiltrate associated with crypt epithelium degeneration and mononuclear infiltrate.

Conclusions

RT-PCR methods allowed *Y. enterocolitica* identification from a larger number of samples than classic method; there haven't been observed any histopathological lesions but the hemorrhagic infiltrate and the mononuclear one may induce the contamination suspicion.

67. GASTRIC HELICOBACTERS AND MUCOSAL HISTOLOGY OF THE PYLORIC GLAND REGION IN DOMESTIC DOGS

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Introduction

Helicobacters are commonly found in the stomach of dogs, but their pathogenic significance remains enigmatic. The aim of this study was to evaluate the prevalence of helicobacters and morphological reaction of mucosa in the pyloric gland region of domestic dogs.

Materials and Methods

Gastric mucosal samples were taken from dogs during necropsy and obtained from five sites of the pyloric gland region of each stomach. They were stained with hematoxylin & eosin and Diff – Quick staining method to evaluate the amount of mucous cells, plasma cells and lymphocytes in the mucosal samples with different amount of helicobacters.

Results

Our previous research results did not show marked differences in the amount of the cells in the gastric mucosa of the cardiac and fundic gland region in association of the amount of helicobacters. Whereas in the pyloric gland region, there is significantly more superficial mucous cells, plasma cells, intraepithelial and lamina propria lymphocytes, but less pyloric gland mucous cells in the mucosal samples with mild, moderate and severe colonization density of helicobacters.

Conclusion

There is remarkable proliferation of superficial mucous cells and infiltration of plasma cells and lymphocytes in the mucosa of the pyloric gland region with higher amount of helicobacters.

ERYSIPELOTHRIX RHUSIOPATHIAE INFECTION ASSOCIATED WITH AN OUTBREAK OF MORTALITY IN PSITTACINE BIRDS IN A MIXED SPECIES AVIARY

68.

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Introduction

Erysipelothrix rhusiopathiae (ER) septicemia was associated with an outbreak of mortality in captive psittacines. A rainbow lorikeet (*Trichoglossus haematodus*) and one eclectus parrot (*Eclectus roratus*) died peracutely with no premonitory signs in a mixed species aviary within two weeks.

Material and methods

The main gross findings after necropsy were severe dehydration (2/2), severe hepatomegaly (1/2), a large area of cardiac dark red discoloration (1/2), multifocal hemorrhage in several tissues (1/2) and severe inner pectoral musculature pallor (1/2). Relevant microscopic findings included thrombosis, bacterial embolism and/or necrosis with fibrinohemorrhagic or heterophilic inflammation in myocardium (1/2), musculature (1/2) and several tissues (2/2), and dermatitis with vasculitis and bacterial and fungal embolism (1/2).

Results

Gram stain revealed Gram-positive bacilli in both cases. ER was cultured in frozen hepatic tissue of the rainbow lorikeet. Immunohistochemistry (IHC) with an antiserum against serotypes 1a, 1b and 2 of ER was performed and was positive in the eclectus parrot. Bacterial genome was detected by multiplex real-time PCR in paraffin-embedded tissues of both parrots.

Conclusion

Both, the IHC assay and the PCR assay could be used as future retrospective diagnostic tool of historical cases. This report is one of the rare descriptions of septicemic infection by ER in psittacine birds.

69. ABOUT HISTOPATHOLOGICAL CHANGES IN SWINE AFFECTED WITH PORCINE CIRCOVIRUS-2

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Introduction

Among pigs, there are two types of circovirus described: PCV1 that is non-pathogenic and PCV2 that has been associated with a number of disease syndromes and today it has been described as an infectious agent causing post-weaning multi-systemic wasting syndrome (PMWS). The clinical signs of PMWS include growth retardation and wasting, dyspnoe, lymphadenopathy, diarrhoea and pallor or icterus, but the severity of the disease varies considerably and the diagnosis can only be determined by certainly at necropsy demonstrating typical gross lesions (lymphocyte depletion and granulomatous inflammation) in close association with an abundance of PCV2 antigen.

Materials and methods

The materials were collected from pigs at age of 6 to 14 weeks. The organ specimens (lungs, lymph nodes and spleen) were tested by RT-PCR. The tissue samples were fixed in 10% neutral buffered formalin. Histological specimens were stained with haematoxylin-eosin and immunohistochemical stainings.

Results

The present study describes the detection of PCV2 infection in 10 Estonian swineherds; nine of them were infected with PCV2 and had clinical manifestation. Most common pathological changes in lymph nodes were granular multifocal inflammation, lymphoid tissue depletion and presence of giant cells. Lung tissue samples had changes similar to the sub-acute interstitial pneumonia and some samples had changes similar to the hemorrhagic inflammation.

Conclusions

PCV2 infection was spread in Estonian swineherds and the cause was PMWS with pathological changes in different organs.

THE CASE OF DIROFILARIOSIS IN POLAND – DESCRIPTION OF MICROSCOPIC LESIONS IN THE DOG INFECTED WITH DIROFILARIA REPENS

70.

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Introduction

Dirofilaria repens is a parasite occurring in subcutaneous or intramuscular connective tissue of dogs and some other carnivores.

Material and methods

The internal organs from 8 years old male mongrel dog, were put to histopathological examination. Slides were stained with H&E, AB-PAS, van Gieson and von Kossa methods. Blood was examined by the hematocrit capillar method by Kingston-Morton 3 days before euthanasia. Skin and subcutaneous tissue were examined for the presence of adult nematodes.

Results

Parasitological examination showed numerous microfilariae of *Dirofilaria repens* in circulating blood, and some adult nematodes of this species in subcutaneous connective tissue. Examination of blood parameters showed increased level of ALT and bilirubin and decreased level of whole protein, albumins and creatinine. Microscopic examination of liver, kidneys, lungs, hearth muscle and intestines showed the presence of microfilariae in parenchyma and/or blood vessels of mentioned organs and significant lesions of liver (cirrhosis), kidneys (nephritis) cardiac muscle (carditis). The necrotic foci and infiltrations of inflammatory cells in different level of intense with eosinophils in internal organs were observed.

Conclusion

Significant lesions in different internal organs with accompanied them presence of numerous microfilariae *D. repens* can suggest the bigger participation of microfilariae than adult specimens in diseases process.

71. SALMONELLOSIS AND MACRORHABDIOSIS IN PASSERINE BIRDS IN AUSTRIA DURING WINTER 2009-10

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Introduction

In Austria suddenly widespread deaths of wild-living birds of the order Passeriformes, family Fringillidae, appeared during the winter months in 2009-10. The Siskin (*Carduelis spinus*) was the species most often affected, followed by Bullfinch (*Pyrrhula pyrrhula*) and Greenfinch (*Carduelis chloris*).

Materials and Methods

The carcasses were mainly found at private feeding places and were sent to the Institute of Pathology and Forensic Veterinary Medicine in Vienna for macroscopic, histopathologic and bacteriologic examination.

Results

Almost all birds were emaciated or in poor body condition. The most noticeable findings were multifocal to confluent yellow-white nodules in the crop. Histologically severe diptheroid-necrotizing inflammation of the entire crop mucosa, submucosa and lamina muscularis together with a huge amount of bacteria was found. *Salmonella typhimurium* (U277) was isolated in large numbers from these lesions. In a large proportion of these birds *Macrorhabdus ornithogaster* could be demonstrated histologically on the surface epithelium of the proventriculus.

Conclusion

Infection with *salmonella typhimurium* sometimes combined with macrorhabdiosis was responsible for the mass mortality of passerine birds in many parts of Austria in winter 2009/10.

IMPLICATION OF THE PULMONARY MACROPHAGES 72. IN THE INNATE IMMUNE RESPONSE IN A MIXED INFECTION (BVDV/BHV-1.1)

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Introduction

Bovine viral diarrhoea virus (BVDV) is considered a predisposing factor to secondary respiratory infections in cattle. Our objective was to study and compare the quantitative and biosynthetic changes in the pulmonary macrophages of coinfecting calves with BVDV and bovine herpesvirus type 1.1 (BHV-1.1) versus single inoculations.

Materials and Methods

An animal group was experimentally infected with NCP BVDV (BVDV group), other group with BHV-1.1 (BHV-1.1 group) and a third group with a combination of both (BVDV+BHV-1.1 group). The animals were killed sequentially in batches of two. A non-inoculated control group was killed at the end of the experiment. Lung samples were processed for further histopathological and immunohistochemical studies (MAC387, TNF α and IL-1 α).

Results and conclusion

The most significant result is the scarce presence of alveolar macrophages in the BVDV+BHV-1.1 group, whereas the BHV-1.1 group showed an increase in this cell population. Despite these differences, we did not detect significant changes in the secretion of proinflammatory cytokines between the different groups, except for a greater expression of IL-1 α in the BHV-1.1 group. The proinflammatory capacity of TNF α would be limited by the low number of IL-1 α -producing cells in the BVDV+BHV-1.1 group, resulting in enhanced susceptibility to secondary agents.

Supported by grants from Spain (AGL 2006-01536 and PO9-AGR-467)

73. MYOCARDITIS BY NEMATODES INFECTION,
PRESUMABLY SARCONEMA EURYCERCA, IN A WILD
WHOOPEER SWAN (CYGNUS CYGNUS) IN KOREA

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A dead whooper swan was found in an area of cropland near a stream and was submitted to the National Veterinary Research and Quarantine Service (NVRQS) in Korea. The affected animal was in relatively good condition. Grossly, the heart was enlarged and had pale and dark red stripes. A white, elongate parasite was seen on the cut surface of the heart. Histopathologically, severe lymphohistiocytic inflammation, myocardial necrosis, many adult heart worms and microfilariae were observed in the myocardium. Hemorrhage, lymphocytic inflammation, mineralization, and myocardial degeneration were also seen around the adult worms. No bacteria or viruses were isolated from the affected bird. These pathological findings indicate that the whooper swan was infected with nematodes, presumably *Sarconema eurycerca*, resulting in non-suppurative myocarditis. To the authors' knowledge, this is the first reported case of *Sarconema eurycerca* infection in a whooper swan in Korea.

CANINE DISTEMPER VIRUS INFECTION WITH INTESTINAL COCCIDIOSIS IN FENNEC FOXES (VULPES ZERDA)

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Fifteen 8-month-old fennec foxes imported from Sudan showed fever, mucopurulent ocular discharge, diarrhea, severe emaciation, seizures, and generalized ataxia, and died. Three of the 15 animals were presented for diagnostic investigation. Severe dehydration, brain congestion, and gastric ulcers were observed in all animals. In one animal, the lungs had failed to collapse and were multifocally dark red in appearance. Histopathologically, there were lymphohistiocytic meningoencephalitis with malacia, mild interstitial pneumonia, lymphoid depletion of lymphoid tissues and organs, and intestinal villous atrophy with intralesional coccidia. There were many intracytoplasmic and/or intranuclear inclusion bodies in the epithelial cells of the medullary velum, lungs, liver, kidneys, trachea, pancreas, stomach, gall bladder, urinary bladder, and ureters, and in macrophages of malacia foci and lymphocytes and macrophages of lymphoid organs. Additionally, intestinal coccidia were confirmed to be *Isospora* species by a fecal test. To our knowledge, this is the first report of canine distemper with intestinal coccidiosis in fennec fox.

PATHOMORPHOLOGICAL LESIONS AND DISTRIBUTION 76. OF VIRAL ANTIGEN IN BIRDS INFECTED WITH THE PATHOGENIC STRAIN OF H5N1 AVIAN INFLUENZA VIRUS

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Introduction

Avian influenza is considered to be one of the greatest threats to modern poultry production and human health. The aim of this study was to determine pathomorphological lesions in organs of birds infected with H5N1 avian influenza virus, presence and distribution of viral antigen in tissue samples and a degree of their correlation.

Materials and Methods

Examination was conducted on the carcasses of seven mute swans and one domestic rooster. After the necropsy, tissues of the brain, trachea, lungs, liver, spleen, pancreas, kidney and intestine were sampled for histopathology and immunohistochemical examination. LSAB method and H5N1 avian influenza virus nucleoprotein polyclonal antibodies were used for detection of virus antigen in examined tissues.

Results

Most constant gross lesions were haemorrhages and necroses of the pancreas. Major histological lesions were multifocal necroses in pancreas, liver and spleen, encephalitis non purulenta, with neuronal dystrophy and neuronophagia. Viral nucleoprotein was immunohistochemically proven in pancreas, brain, liver, lungs, and in one swan in kidney.

Conclusion

High correlation between pathomorphological lesions and distribution of viral antigen was determined. Lesions in mute swans and domestic rooster were similar in character and distribution, which is about equal sensitivity of these two species on H5N1 avian influenza virus.

77. PULMONARY NEMATODIASIS COMPATIBLE WITH
AELUROSTRONGYLUS ABSTRUSUS INFECTION IN A
WILD CAT (*FELIS SILVESTRIS*)

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²*Pygarvusvet, Lda, Portugal*

The authors report a case of an adult wild cat (*Felis silvestris*) hunted by dogs and submitted to necropsy examination at the Peneda-Gerês Natural Park. Tissue samples were fixed in formalin and sent to the Histopathology Laboratory of the University of Trás-os-Montes e Alto Douro (UTAD) where it was processed for light microscopy according to standard procedures.

The lung showed multiple incubation nodules with alveoli packed with eggs and larvae and inflammatory infiltration. Lung arteries showed medial hypertrophy. This parasitary pneumonia is compatible with pulmonary infection by *Aelurostrongylus abstrusus*, a common lungworm of cats. To the best of our knowledge this is the first report of a pulmonary nematodiasis, compatible with *Aelurostrongylus abstrusus*, in a wild cat in Portugal.

**POSTER SEMINAR TOUR
SESSION 5B - ORGAN PATHOLOGY**

78. COMPARATIVE PATHOMORPHOLOGICAL PATTERN OF THE LIVER IN BROILER CHICKENS OF TWO BREEDING LINES

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Introduction

The aim of the study was to show the intensity and type of morphological lesions in the liver of broiler chickens of two breeding lines at various times of rearing.

Material and Methods

The experiment was conducted on 72 broiler chickens (Ross, Cobb lines, n=6). On day 3, 10, 17, 24, 31 and 38 of the rearing, healthy animals were euthanised and liver samples were HE, Red Oil O and PAS stained (McManus).

Results

Parenchymatous, adipose, vacuolar degeneration and focal necrosis were the most frequent lesions. Progressive lesions included hyperplasia and hypertrophy of biliary ductules cells, hyperplasia of muscular layer cells of arterioles and endothelium cells of blood vessels as well as connective tissue around blood vessels and biliary ductules. The following lesions were observed with a slightly lower frequency: infiltration of lymphoid cells around blood vessels and biliary ductules, enlargement of lymphatic follicles, infiltration of heterophiles around proliferating biliary ductules. Additionally observed circulatory disturbances.

Discussion

Morphological changes were observed in healthy chickens, with regressive lesions dominating, during all the periods of rearing, with particular intensity on days 10 and 38 of the rearing. The Ross-line birds were more susceptible to the lesions.

This study is financed as a research project (NN308230236).

COLONIC ADENOMATOUS POLYPOSIS IN A VERVET MONKEY (*CHLOROCEBUS AETIOPE*) 79.

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Introduction

A polyp is a sessile or pedunculate growth or mass protruding from a mucous membrane. It may be resulted of hyperplasia or neoplasia. In histopathologic examination adenomas are differentiated from nonneoplastic lesions because they show degrees of dysplasia. Familial adenomatous polyposis (FAP) is a term used in human medicine for a dominantly inherited precancerous condition associated with deletion of the adenomatous polyposis coli gene that results in the development of more than 100 polyps in the colon and rectum. The disease has not described in animals.

Material and Methods

During a routine necropsy examination, performed on a male vervet monkey carcass, multiple small round polyps, about the size of a match head, protruding from of whole length of the colon's wall and, with minor involvement, on the wall of the caecum were noticed. The polyps were mostly sessile or slightly pedunculated (Fig. 1). Samples of colon and caecum were collected and referred to the department of pathology of Razi vaccine and serum research institute, Karaj-Iran.

Results

In histopathologic examination of the polyps, in colon and caecum, they showed short pedicle of delicate connective tissue covered with normal mucin-secreting colonic mucosa, in larger polyps, or had a broad base in smaller polyps. However, the top of the polyp were covered with a thick layer of basophilic cells which form very irregular glands and have mostly lost their mucin-secreting capabilities (Fig. 2,3). High-power examination showed that those are dysplastic cells with nuclear pleomorphism, changes from elongated to rounded. Nuclei lied in the center of the cells and occupied a large area. Rarely mitotic activity with a poorly-formed and ill-defined basement membrane was seen. Based on macroscopic and microscopic examinations the mentioned lesions seemed closely resemble to FAP in humans and classified as: adenomaous polyposis.

Conclusion

In author knowledge this is the first report of the adenomatous polyposis in vervet monkey. Further investigations are needed for suggesting this species as a model in researches on FAP and colonic cancer in human.

FELINE MYOCARDIAL HYPERTROPHY, A RETROSPERSPECTIVE STUDY

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Introduction

Hypertrophic cardiomyopathy (HCM) is the most common cardiomyopathy in cats. Nevertheless, the clinical correlates of this pathologic entity are not clear. The purpose of this study was to establish a database that ultimately may allow a better understanding of the clinical significance of HCM.

Materials and methods

295 cats presented for autopsy, aged at least 3 months old and over (mean age 2.2 years) were included in the database.

Results

28.5% of the cats that died spontaneously were macroscopically suspected of HCM (narrow lumen and hypertrophic wall of the left ventricle and septum). 46.4% of all suspected cats were neutered males (vs 27.1% in the whole population). The mean weight of HCM suspected cats was 4.1 kg (vs 3.1 kg in the whole population). Mean heart weight was 20.6 grams (vs 15.9 grams in the whole population). The mean thickness of the left ventricular wall of the suspected cats was 10.6 mm (7.7 mm in the whole population). 58% of the cats that died during or immediately after anaesthesia were macroscopically suspected for HCM.

Conclusion

HCM is a frequently occurring condition in cats and could be an important factor in post anaesthetic mortality.

CONGENITAL NUTRITIONAL MYODEGENERATION IN A CYPRUS MOUFLON (*OVIS ORIENTALIS OPHION*) LAMB

81.

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Introduction

Nutritional myodegeneration (white muscle disease, WMD) results from selenium (Se) and/or vitamin E deficiency and affects a wide range of species. The Cyprus mouflon or agrino (*Ovis orientalis ophion*) is a wild sheep species endemic and unique to Cyprus.

Materials and Methods

A 5-days-old mouflon lamb was found dead in the Platania mouflon enclosure in Cyprus. No signs of disease were observed prior to death. Gross, histopathological and biochemical analyses were performed.

Results

Necropsy revealed generalised pale discolouration of skeletal muscles, as well as the myocardium. Histopathology showed severe myodegeneration. Liver Se levels and liver vitamin E were 0.23 mg/Kg (f.w.) and 2.1 mg/100 g (f.w.) respectively.

Conclusions

Unlike most WMD cases, the present WMD case was attributed solely to vitamin E deficiency. Although reference values are not available in mouflon species, liver Se values were within the normal levels of other ruminants, while vitamin E levels were lower than the reported reference values for lambs, albeit not as low as in lambs with clinically manifested vitamin E deficiency, a finding that may indicate increased requirements for vitamin E in the Cyprus mouflon. WMD had not been diagnosed in agrino or other mouflon species to date.

82. MORPHOMETRIC EVALUATION OF THE CEREBRAL ISCHEMIA-REPERFUSION INJURY AFTER EXPERIMENTALLY CARDIAC ARREST IN PIGS

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Ciprian Ober, Cosmin Muresan

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Introduction

Acute hypoxia in time of cardiac arrest induces coagulative necrosis of the brain neurons. The reperfusion is associated with oxidative stress and neuronal apoptosis.

Materials and Methods

Fifteen male pigs were divided in two groups. Control group (5) was submitted to general anesthesia and vascular catheterization. The experimental group (10) was submitted to the same procedure and to cardiac arrest by electrical shock (ventricular fibrillation). After six minutes of assisted cardiac arrest the pigs were resuscitated. Five experimental pigs for 72 hours when were killed under anesthesia. The brain samples were processed for histopathology - haematoxylyne-eosine, cresyl violet staining, IHC (caspase 3) and confocal microscopy (Fluoro Jade B and Propidium Iodide).

Results

The most damaged cerebral regions were CA3 of hippocampus, temporal cortex (layers 2,3,6) and cerebellum (Purkinje). The distribution of neuronal loose in CA3 was similar in all pigs while in the other regions was inconsistent.

Conclusions

The most suitable coloration for counting of dead neurons is HE. IHC for caspase 3 completes this with the early apoptotic neurons. Image analysis is suitable after cresyl violet staining, IHC and fluorescent labeling of dead neurons

INFLUENCE OF SACCHARIN ON CHANGES OF RAT PANCREAS AND LIVER HISTOLOGY, GLYCEMIA, FOOD INTAKE AND WEIGHT

83.

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Introduction

Artificial sweeteners are widely used. In animal subjects, as in humans, sweet taste increases insulin secretion. Artificial sweeteners have been reported to increase cephalic-phase insulin secretion, hunger, food intake and body weight.

Material and methods

24 Wistar rats had free access to normal diet and were kept under constant conditions. They were divided randomly into 2 groups and treated for 6 weeks. Group 1 was given drinking water and Group 2 received 0.005% saccharin in drinking water. Food intake was measured daily while animals were weighted weekly. Glycemia was determined from capillary blood samples. After sacrificing tissues were routinely processed and 5 µm thick sections were stained with hematoxylin and eosin.

Results

There were no statistically significant differences in food intake between groups. Glycemia was statistically higher in Group 1 and female rats. Weight increased significantly in Group 2. Pathohistological analysis of liver and pancreatic islets showed no changes. Dense, homogenous exocrine secretion in pancreatic ductal system was observed in Group 2 samples.

Conclusions

Saccharin use lead to increased glycemia. Although food intake wasn't statistically different, weight gain was statistically higher in Group 2 related to insulin effect on adipose tissue. Endocrine pancreas showed no changes, but impaired outflow of exocrine secretions was observed.

84. EPITHELIOGENESIS IMPERFECTA IN CHURRA LAMBS

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Introduction

Epitheliogenesis imperfecta or aplasia cutis is a rare condition in sheep, characterized by an inherited congenital discontinuity of squamous epithelium. We report the main pathological findings observed in several cases of this disease.

Materials and methods

A total of 19 newborn lambs, belonging to three different flocks of Churra sheep, sharing males, were grossly examined, and samples from skin, tongue, oral mucosa and oesophagus were microscopically studied.

Results

In all the cases, at birth, a complete absence of the epidermis and a very congestive dermis was seen in different areas of the body. All the lambs showed lesions in the distal parts of the limbs and, in 11 lambs, the detachment of one or several hooves was seen. Secondly, the pinnae were affected, uni or bilaterally, in 16 animals, together with shortening and fusion of the auricular cartilage. The tongue and hard palate were affected in 12 cases. In a lower number of lambs, the tail, thigh, periocular region or wide areas of the back, were also affected. Histologically, the absence of the epidermis and hair follicles and glands was observed.

Conclusions

Differences in the severity and location of the lesions are observed among lambs affected of epitheliogenesis imperfecta.

GASTRIC POLYPS AND SPONTANEOUS HELICOBACTER SPP. INFECTION IN DOGS

85.

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Introduction

The objective of this study was to evaluate the morphological aspects of gastric polyps and the prevalence of *Helicobacter* spp infection in dogs with these lesions.

Material and methods:

Ten dogs (5 males and 5 females) with a mean age of 11 (range 9 - 16) years were diagnosed with gastric polyps on routine necropsy examination. The polyps were removed and examined histologically. *Helicobacter* spp. presence was investigated by Giemsa method and immunohistochemistry technique, using polyclonal antibody anti-*Helicobacter pylori* (ab7788, ABCAM).

Results

The polyps were classified as hyperplastic (6/10) and benign lymphoid (2/10). The leiomyoma was detected in two cases. Giemsa method revealed the presence of *Helicobacter* spp. in eight cases. Positive reaction for *H. pylori* by IHC was detected in 3 cases.

Conclusion

Gastric polyps are found in dogs but more frequently diagnosed during necropsy examination of the stomach. The histological study is important for differentiated the type of gastric polyps. We found a correlation between the development of the gastric polyps and spontaneous gastric infection with *Helicobacter* spp. in dogs.

A CASE REPORT OF VALVULAR AND PARIETAL ENDOCARDITIS IN A BENGAL TIGER

87.

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Introduction

Considering captive tiger pathology, previous studies reported gastrointestinal disease due to general adaptation, bacterial (anthrax, tuberculosis, salmonellosis) and viral diseases (canine distemper, rhinotracheitis, Calicivirus, panleukopenia, feline infectious peritonitis) as the most frequent encountered in this species.

Materials and Methods

A 16-year-old female of *Panthera tigris* clinically diagnosed with chronic renal failure was submitted for necropsy. Cytological investigations were focused on transudate sampled from peritoneal cavity and thorax (May Grünwald Giemsa stained). Fragments of organs were used for routine histopathology (Masson trichromic stain). Gaffney's one-hour Giemsa stain was used for bacterial colony identification.

Results

Blunted teeth with pulp cavity exposure associated with periodontal disease and gingivitis, endocarditis confined to tricuspid, aortic valve and parietal endocardium of right ventricle, myocardial fibrosis, ascites, hydrothorax, cardiac cirrhosis, interstitial nephritis and proliferative glomerulonephritis, uremic enteropathy and gastropathy, hemorrhages and infarction of spleen, embolic pneumonia and bronchointerstitial pneumonia were the most important gross and histological lesion. Bacterial colonies were identified in endocardium and lung.

Conclusion

Prolonged antigenemia that produces high concentration of soluble immune complexes is associated with dental and endocardial injuries. Thus, glomerulonephritis may be the consequence of periodontal disease and endocarditis. Bacterial endocarditis and subsequent cardiac failure are also responsible for pulmonary inflammation and cardiac cirrhosis.

88. PATHOMORPHOLOGICAL PATTERN OF THE LIVER IN CARP (CYPRINUS CARPIO L.) DEPENDING ON THE FISH PRODUCTION TECHNOLOGY

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Introduction

The morphological pattern of the carp liver in fish reared by a semi-extensive (natural feed) and low-intensity (natural feed supplemented with granulated cereal fodder) fish production technology has been compared.

Material and Methods

The study was conducted on 120 2- and 3-year-old carp divided into 4 groups (n=30): control – fish fed with natural feed (benthos), coming from two fishing farms; fish fed according to low-intensity technology, additionally fed with granulate - Aller-Aqua, (originating from two other farms). HE, PAS staining and ultrastructural examinations were performed.

Results

In all groups parenchymatous degeneration and steatosis simplex were the most frequently lesions (especially in 2 and 4 groups). Other lesions were present sporadically in 3 and 4 carp group. The glycogen content in hepatocytes of fish was similar. Among the ultrastructural lesions in carp liver in all the groups, steatosis simplex dominated, especially in fish of groups 2 and 4. In addition, small changes in mitochondria and in rough endoplasmic reticulum were observed, especially in older carp.

Conclusion

Slightly fewer morphological lesions were observed in the carp fed with natural feed than in those fed with granulate. Less morphological lesions were observed in fish aged 2 than in those older by 1 year.

90. DESCRIPTION OF INTESTINAL EMPHYSEMA (IE) LESIONS AMONG PIGS FROM A SLAUGHTERHOUSE IN OKLAHOMA, UNITED STATES

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Introduction

IE is a low incidence but well recognized lesion of slaughter pigs, first described in 1737. Little is known about its histology, and the cause is undetermined.

Materials and Methods

Over 3 days, 54,000, 110-125kg pigs were examined. Cases of IE were identified, and the viscera were examined. Samples of intestines, liver, heart, mesenteric lymph node, spleen and pancreas were collected for light microscopy. Samples were stained with H&E, Gram, GMS and Acid fast techniques, and tested by PCR for *Mycobacterium* spp.

Results

Seven cases clustered during times of collection, but pigs came from a wide geographic distribution. Mild chronic serositis was common, but two cases also were associated with multiple abdominal abscesses. All pigs had a granulomatous mural enteritis and serositis with air trapped presumably in lymphatics, and lymph nodes. The enteric mucosa and all other organs examined were normal, except by hepatic and splenic pyogenic abscesses in two cases. These abscesses seemed unrelated to the IE. Mycobacterial testing and special stains were negative.

Conclusions

IE is a segmental and granulomatous enteric and peritoneal condition of swine. It is not an artefact of slaughter because it is associated with a granulomatous process that exists prior to time passed during transport and killing.

NEUROGENESIS IN THE DENTATE GYRUS OF A YOUNG DOG WITH EPILEPTIC SEIZURES

91.

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Introduction

A young dog with a history of epileptic seizures exhibits extensive cell proliferation in the granule cell layer of the dentate gyrus.

Materials and Methods

The dividing cells in the dentate gyrus were characterized by immunohistochemical markers for developing and mature neurons. Furthermore, immunohistochemistry was carried out to rule out infectious diseases such as rabies, canine distemper and parvovirus infection.

Results

Whereas gross alterations in the brain were not present, abundant mitotic figures were detected in the dentate gyrus histologically. The mitotic cells were negative for the astrocytic marker glial fibrillary acidic protein (GFAP) and doublecortin, a protein that is expressed by immature neurons. The weak positive staining for neuronal nuclear antigen (NeuN), a marker for mature neurons, categorizes the mitotic cells as members of the neuronal lineage.

Conclusion

To date, it is accepted that neurogenesis is a physiological feature that lasts until adulthood in the mammalian brain. In rodent models, an increase in neurogenesis has been observed after acute seizures. This is the first report of an increased mitotic rate in the dentate gyrus of a dog suffering from spontaneous seizures. Whether this represents a general reaction pattern in the brain of epilepsy dogs has to be further investigated.

92. SEASONAL EQUINE MYOPATHY IN GRAZING HORSES IN THE NORTH OF SPAIN

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Introduction

Degenerative myopathy in horses has been related to different etiologies. For decades, high mortality outbreaks have been appearing in grazing animals and the “atypical myopathy” of unknown etiology has been described. In this study, an acute myopathy outbreak in young animals kept exclusively on pasture after an autumn of high temperatures is described. Most of the herds were affected and the horses showed acute clinical signs and death within 48 hours.

Material and methods

Necropsies were performed on three animals, and tissue samples were processed using special histochemical techniques.

Results

A pale appearance was observed in several skeletal muscles and myocardium. Histopathologic examination revealed a multifocal and monophasic rhabdomyolysis, characterized by a cytoplasmic swelling and vacuolation, hyaline degeneration, segmental necrosis and mineralisation as well as lipid droplets and positive PAS material storage in the most affected muscle fibers. Postural and respiratory muscles showed the most severe lesions while the damage of other muscles, including the myocardium was less severe. However, Purkinje myocardial cells showed multifocal necrosis. An hepatic centrolobulillar steatosis and nephrosis were the other two main lesions.

Conclusions

The histopathological findings and the seasonal nature of the outbreak might be associated with “Seasonal pasture myopathy”.The etiology is discussed.

POSTER SEMINAR TOUR
SESSION 7A - CLINICAL PATHOLOGY

VISCERAL GOUT IN JUVENILE CHINESE SOFT SHELLED TURTLE (PELODISCUS SINENSIS)

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Introduction

Diathesis urica (Gout) is a common disease among reptiles. When the concentration of the free uric acid and the urate salts becomes elevated in the blood (hyperuricemia) or in other body fluids, the uric acid crystallizes, forming insoluble precipitates that are deposited in the joint (articular gout) and in other internal tissues (visceral gout). Gout can be caused by medications, kidney diseases, starvation, improper diet or dehydration.

Materials and Methods

The postmortem examination of a 10 month-old male Chinese Soft Shelled Turtle with no clinical history was performed. Tissue samples of the liver, kidneys and serous membranes were collected, fixed in 10% neutral buffered formalin and in absolute alcohol and dyed with HE and De Galantha's method for urates.

Results

The microscope research revealed the presence of monosodium urate crystals (small white nodules, called tophi, clearly visible to the unaided eye) and concretions deposited in the liver, kidneys and serous membranes. The liver and kidneys were swollen and degenerated. The high number of inflammatory cells in liver and kidneys were described

Conclusion

Although gout frequently occurs in the reptiles, is a rare phenomenon in juvenile carnivorous turtles living in the aquatic environment.

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Introduction

In August 2009 numerous eroded paraplant-embedded samples were accidentally detected in our archive. Macroscopically, some of the tissues were infested with mould fungus and webs of moths.

Material and Methods

After checking the whole archive, profoundly infested material was disposed. Mycological and parasitological investigations were carried out. Treatment of less affected material was tested by formalin-bath (1,5% formalin) and gamma rays.

Results

The paraplant-embedded material showed a high-grade infestation with webs of corn moths as well as six different species of mould fungi. While fungal growth could not be confined by formalin-bath, gamma ray treatment was highly effective. Therefore, the entire paraplant-embedded material of our Institute was sent to radiation. To optimise the conditions of storage an air dehumidifier was installed after archives' decontamination.

Conclusions

Altogether 8484 samples had to be disposed, which caused an extensive loss of material for research purpose as well as for retrospective forensic questions. Therefore we advise a routine check of archives regarding air humidity and paraplant embedded material.

96. DETECTION OF APOPTOTIC CELLS AND IMMUNOHISTOCHEMICAL STUDY OF BCL-2, BAX AND CASPASE-3 IN CANINE MAMMARY TUMORS

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Introduction

Cell proliferation and apoptosis are the key determinants of the growth of canine mammary tumors. The aim of this study was to investigate the role of apoptosis relationship with histologic type, tumor grade, tumor size and p53 status, proliferation activity assessed by Ki-67 and mitotic index.

Materials and Methods

Samples of 112 mammary tumors of epithelial origin were fixed in 10% buffered formalin and routinely processed (H-E method). Expression of Bax, Bcl-2, caspase-3 and p53, Ki-67 was determined by immunohistochemistry and apoptosis was evaluated by TUNEL technique.

Results

The positive immunostaining for Bcl-2, Bax, caspase-3 and p53 was noted in 72%, 54%, 62%, 38% of all canine mammary tumours. High rate of apoptotic index and caspase-3, Bax protein expression were positively correlated with tumor size, histologic type and tumor grading, proliferative activity and p53 protein expression. However, level of proliferative activity was higher than apoptotic rate. No significant difference was observed between Bcl-2 and Bax expression.

Conclusion

The increased apoptotic index and expression of apoptosis-associated proteins (caspase-3 and Bax) might play an important role in the development of canine mammary tumors and be useful in tumor grading.

THE IMPORTANCE OF CYTOLOGIC EXAMINATION IN DOG ORAL NEOPLASIA 97.

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Introduction

The incidence of oral neoplasia in dog is relatively high, early diagnosis being a key factor for assessment of prognosis and therapy.

Methods and materials

A number of 594 dogs were diagnosed with tumors in 2009. 36 cases of them presented oral neoplasms, diagnosed by cytological examination. Samples were taken by fine needle aspiration, biopsy and scraping and routinely stained (May Grünwald Giemsa).

Results and discussions

The diagnosis of fibromatous epulis was based on numerous typical and dysplastic fibroblasts and a small number of typical keratinocytes. Acanthomatous ameloblastoma was diagnosed following the observation of typical or dysplastic odontogenic epithelial cells. Squamous cell carcinoma was featured by anaplastic keratinocytes. Fibrosarcoma was diagnosed due to the pleomorphous spindle cells. The presence of cytoplasmic melanin granules and the identification of epithelioid and spindle cells represent the features of diagnosis in malignant melanoma.

Conclusions

The occurrence of dog oral neoplasia was 6%, 48% of cases being benign tumors and 52% malignant. The lesions diagnosed by cytological examination were: 12 cases of fibromatous epulis (33%), 5 cases of acanthomatous ameloblastoma (15%), 4 cases of squamous cell carcinoma (11%), 6 cases of fibrosarcoma (16%), 9 cases of melanoma (25%). In 92% of cases histopathological examination confirmed the cytological diagnosis.

98. MORPHOLOGICAL EXAMINATION OF T-2 TOXICOSIS IN BROILERS FED DIETS WITH DIFFERENT FEED ADDITIVES

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Introduction

This research was done with the aim to investigate efficacy of inorganic (Minazel plus-Mz), organic (Mycosorb-Ms) and mixed (Micofox-Mf) adsorbents in prevention and/or alleviating adverse effects of T-2 toxicosis in broilers.

Materials and methods

Experiment was performed on 160 one day old broiler chicks "Ross" proveniention, divided into 8 groups. Animal sacrifice was done from all experimental groups on the 21. day of the trial. Formalin fixed and paraffin embedded samples of liver, small intestine and bursa of Fabricius were examined by light microscopy using hematoxylin and eosin staining and immunohistology for PCNA.

Results

Pathohistological examination showed negative consequences of T-2 toxin in all inspected organs as degenerative changes in oral and small intestine mucosa, enterocytes and hepatocytes necroses, as well as lymphocytes depletion in bursa Fabricii. Using immunohistology investigations adverse effects of T-2 toxin could be seen on cell proliferative activity in intestine and bile duct mucosa and also on lymphocytes in bursa Fabricii.

Conclusion

Disparately from inorganic (Mz) and organic (Ms) adsorbents which didn't provoke protective effects, in liver, intestine and bursa Fabricii of broilers who were given feed with T-2 toxin and mixed adsorbent (Mf) mostly preserved structure of these organs could be noted.

METASTASISING DIFFUSE SPLENIC SARCOMA ORIGINATING FROM RESIDENT MACROPHAGES (ERYTHROPHAGOCYTTIC HISTIOCYTTIC SARCOMA): HYPOPROTEINAEMIA AS THE MAIN CLINICAL FEATURE

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Introduction

Dogs develop a range of primary splenic histiocytic neoplasms; histiocytic sarcoma, malignant histiocytosis and, rarely, haemophagocytic histiocytic sarcoma. While the former are of dendritic cell origin, the latter seems to arise from sessile macrophages in the red pulp.

Materials and Methods

A 6-year-old Flat Coated Retriever was hospitalised with lethargy and anorexia. After extensive clinical examination and unsuccessful therapy, it was euthanased and submitted for post mortem examination with suspected protein losing enteropathy. Thorough morphological examination (including immunohistology and TEM) and some functional studies were performed.

Results

The clinical examination identified hypoalbuminemia, hypoglobulinaemia, hypocholesterolaemia and regenerative anaemia. Gastrointestinal endoscopy and histology were unremarkable. The post mortem examination revealed moderate anaemia, jaundice, hepatosplenomegaly and acute renal infarcts. The histological examination identified diffuse infiltration of the splenic red pulp by CD11d, CD18 and lysozyme positive polymorphic macrophages that exhibited marked erythrophagocytosis. These cells were also evident diffusely in the liver and, in smaller numbers, the bone marrow.

Conclusions

A diagnosis of metastasising diffuse splenic erythrophagocytic histiocytic sarcoma was made. The suspected cells of origin and their specific characteristics as well as the potential cause of the hypoalbuminaemia will be discussed.

CYTOLOGICAL PITFALLS: DIFFERENTIAL DIAGNOSES FOR CYTOPLASMIC GRANULATION

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Introduction

The cytology of two rare benign tumors in dogs, Merkel cell tumor and myxoma, mimics the cytology of two common malignant tumors; poorly differentiated mast cell tumor (MCT) and melanoma. This comparison was done in order to retrospectively seek differentiating cytological features for these tumors.

Materials and methods

Fine needles aspirates from a Merkel cell tumor and a myxoma, both histologically confirmed, were compared to the typical cytology of MCT and melanotic melanoma.

Results

The cytology of Merkel cell tumor is that of a round cell tumor with purple cytoplasmic granulation. The cell borders of the neuroendocrine cells are indistinct, the cytoplasmic granules often peripheral and occasionally rod-shaped. The cytology of this myxoma is consistent with a mesenchymal spindle cell tumor with cytoplasmic dark black granulation. Scant eosinophilic extracellular mucoid matrix is seen, nuclear morphology is consistent and melanophages are lacking in granulated myxoma.

Conclusion

The specific location and shape of the cytoplasmic granules along with the morphology of cell borders aid in the cytological differentiation between Merkel cell tumor and poorly differentiated MCT. The presence of extracellular matrix and mainly uniform nuclei along with lack of melanin phagocytosis, distinguish myxoma with cytoplasmic granules from melanotic melanoma cytologically.

THE QUANTIFICATION OF HISTOLOGICAL AND BIOCHEMICAL CHANGES AFTER BILE DUCT LIGATION IN WISTAR RATS

101.

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Introduction

The cholangiocyte proliferation is triggered during extrahepatic bile duct obstruction induced by bile duct ligation, which is a common *in vivo* model used for the study of cholangiocyte proliferation and liver fibrosis. The aim of this study was the quantification of liver lesions in rats with bile duct ligation.

Materials and Methods

A total of 10 male Wistar rats, with an average weight of 200 g, were used. The samples were gathered and evaluated biochemically and histologically after 1, 2, 3, and 4 weeks. The histological examination was performed using the Knodell scoring system.

Results

The histological changes including portal inflammation, necrosis, and fibrosis were seen in the bile duct ligated group. The Histological Activity Index scores of these groups were significantly higher than those of the Sham operated group.

A strong increase of the bile duct diameter and a thickness of the bile duct wall were noticeable, many proliferating connective tissue cells being observed. Also, a strong inflammatory infiltrate dominated by neutrophils was noticed. The serum bilirubin and liver enzyme levels were significantly increased after BDL.

Conclusion

The most obvious lesion was the ductular reaction, the highest number of biliary ducts being found at 2 weeks after BDL.

102. THE BIODISTRIBUTION AND ACUTE SYSTEMIC TOTOXCITY OF SINGLE WALLED CARBON-NANOTUBE FUNCTIONALIZED WITH SINGLE-STRAND DNA IN WISTAR RATS

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Introduction

The aims of this study were to evaluate the pharmacokinetics of single walled carbon nanotubes (SWCNT) and the oxidative stress induced in Wistar rats.

Materials and Methods

SWCNT were functionalized with Cy3-labeled ss-DNA through ultrasonication and the aqueous suspension was injected intraperitoneally in 65 male Wistar rats, in different doses. The body distribution of SWCNT was analyzed using Confocal Laser Microscopy and Raman spectroscopy into the tissues after 3, 6, 24 and 48 hours. The oxidative stress (seric and hepatic MDA, carbonilated proteins, total antioxidant capacity and thiol groups) was measured in parallel.

Results

High quantities of SWCNT were found especially in reticulo-endothelial system (RES) from mesentery, omentum and abdominal parietal lymph nodes. A discrete presence was observed in spleen, lung, kidney and liver. No evidence of accumulation in pancreas and encephalon. Excepting the presence of SWCNT, no morphological changes were recognized. All markers of oxidative stress were found increased, regarding control rats, in a dose dependent increasing manner, with maximum intensity at 24 hours and slow recover after.

Conclusions

Intracellular accumulation of SWCNT induces a dose depend elevation of oxidative stress markers in serum and liver tissue, oxidative stress being a dominant mechanism in the SWCNT toxicity.

STUDY OF TOXICOPATHOLOGIC EFFECTS OF DIAZINON ON HISTOPATHOLOGICAL, BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN RABBITS BY DERMAL EXPOSURE OF TOXIN

103.

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Introduction

Diazinon is one of the organophosphate pesticides widely used. The present study was carried out to investigate the toxic pathologic effects of diazinon in male rabbits through dermal exposure.

Material and Methods

12 adult male rabbits were divided into two equal Groups. The treatment group received diazinon through dermal exposure for 28 days. The control group was given ethyl alcohol. Blood samples were collected from marginal ear vein on day 0 and later on days 7th, 14th, 21th, 28th of treatment. On day 28 the rabbits were euthanized.

Results

The analysis obtained from the data revealed that diazinon affects the hematological and biochemical parameters. In fact, there was a significant decrease in red blood cell count and hemoglobin in the treated rabbits. Diazinon increased alkaline ALP, ALT, and AST level in the serum and cholesterol level. Diazinon also significantly decreased triglyceride and total protein level of the serum in the treatment group. Histopathology revealed fatty degeneration and necrosis of hepatocytes, interstitial pneumonia, nephrosis, myocardial necrosis, degeneration in testis, and finally suppurative dermatitis in skin.

Conclusion

This study showed Diazinon had numerous toxic effects. Therefore, there is risk of human contact to this toxin and produce serious toxicity.

104. ADENOMA OF THE UTERUS IN A GUINEA PIG

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Introduction

Benign epithelial tumours of the uterus are very rare in domestic animals. Endometrial adenocarcinoma is rare excepting in the cattle and rabbits.

Material and methods

A surgical excision of a Guinea pig, female, 3 years of age, consisting in a uterine segment with 4 cm long with representation of an ovary. A routine processing was made and staining with HE. Immunohistochemistry was performed by avidin-biotin peroxidase complex method, using vimentin and large spectrum cytokeratin as primary antibodies.

Results

The ovary presented multiples cysts mainly in the hilar area that compressed ovarian tissue around. In the uterus we observed a papillary neoforation, with 1 cm in diameter, consisting in white fasciculate tissues. Microscopically, this consisted in a papillary area of epithelial tissue with a septum of conjunctive tissue. The epithelial cells were organized in acinar pattern, with abundant cytoplasm and basal nucleus. No mitosis were observed. The conjunctive tissue presented some atipia and cells with granular, eosinophilic content. The tumour cells were positive to cytokeratin and the stroma was positive to the vimentin.

Conclusion

Based on the morphological and immunohistochemical results, we concluded that this lesion was an adenoma of the endometrium.

DIFFERENTIAL DIAGNOSIS OF GRANULOMATOUS LESIONS IN WILD GREY MULLET'S POPULATION FROM LIGURIAN SEA IN ITALY 105.

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Introduction

Mycobacterium spp. and *Photobacterium damsela*e are recognized as the more frequent agents of granulomatous lesion in fish. Although episodes of mycobacterial infections have been frequently reported in wild fish worldwide, to date few sporadic cases were described in Italy.

Materials and Methods

115 wild grey mullet (*Mugil cephalus* and *Liza aurata*) were fished from the Eastern coast of Ligurian Sea, then sacrificed and necropsied in order to investigate the presence of lesions referable to mycobacteriosis and to identify the mycobacterial species involved. Samples from liver and spleen were collected from all fish for histopathological, histochemical (Ziehl-Neelsen stain) and microbiological examinations. Furthermore molecular analysis for *P. damsela*e ssp. *piscicida* identification were performed.

Results

At gross examination granulomatous lesions were observed in liver and spleen of only one animal; microscopically, 35% of fish displayed granulomas with different histological features, partly positive at Ziehl-Neelsen staining and confirmed by microbiological examination. The identified colonies were characterized as *M. fortuitum*, *M. abscessus*, *M. flavescens*, *M. chelonae*. Forty-seven percent of animals resulted positive for *P. damsela*e ssp. *piscicida*.

Conclusions

These data suggest that mycobacterial infections in wild fishes are widely diffuse as well as *P. damsela*e infections. Moreover the pathogenicity of some mycobacterial species, previously considered as saprophyte, was proved.

CHOLESTEROL DIET EFFECTS ON ATHEROSCLEROTIC PLAQUE COMPOSITION AND BINDING CAPACITY OF THE MITOCHONDRIAL 18 KDA TRANSLOCATOR PROTEIN IN AORTA OF APO E KO MICE

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Introduction

Apo E has long been known to play a key role in cholesterol transport and metabolism. In addition, it has indicated that 18 kDa translocator protein (TSPO) is involved in the regulation of cholesterol transport into mitochondria in relation to bile production and steroidogenesis. Dysregulation of apoE in humans and animals is associated with several conditions that in separate studies also have been linked to TSPO function.

Materials and Methods

With the present study we sought to address in the 5 weeks old animal model (C57BL/6 Apolipoprotein E knockout mice), whether Apo E deficiency and 3% cholesterol supplementation in the diet for a period of 10 weeks, can be correlated with altered TSPO binding characteristics in aorta tissue. Biochemical and histopathological examination were carry out.

Results

Feeding ApoE KO mice with high -cholesterol diet results with significant thickening of aorta's wall, accelerated atherosclerosis and deposition of cholesterol crystals with calcifications. The in vivo findings in apoE knockout mice revealed that TSPO levels appear to be reduced in aorta of Apo E KO mice treated with cholesterol diet comparing to the control counterparts.

Conclusion

Although these data suggest reduced density of TSPO in aorta of Apo E KO mice, the potentially modulating role of 18kDa TSPO in the arterial wall deserves further attention.

INTERVERTEBRAL DISKS DEGENERATION: PRELIMINARY STUDIES ON PATHOLOGICAL FEATURES OF 42 SURGICAL SPECIMENS

107.

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Introduction

Intervertebral disks (ID) can exhibit normal aging changes and degenerative alterations leading to disk extrusion into the spinal canal.

Materials and Methods

The authors report the histological features of n. 42 canine spinal surgery specimens comparing the observations between spinal level and breed - chondrodystrophoid (CD) and non chondrodystrophoid (NCD) dogs - by means of the Fisher test.

Results

N.22 ID of CD and n. 20 ID of NCD, aging from 3 to 12 years old, were evaluated. N. 34 samples were collected in thoracic/lumbar spine, and n. 8 in cervical spine. N. 40 samples were composed mainly by nucleus pulposus; two specimens showed lamellar structures of anulus fibrosus or cartilaginous laminae. Chondroid clusters in degenerate matrix occurred frequently (72.7% of CD e 45 % dei NCD). Chondroid metaplasia was detected in 80.5% of cases; it was significantly more frequent in CD (94.7%), but it was also present in 64.7% of NCD. Calcium deposits were observed in 100% of CD (80% I NCD). Granulation tissue and neovascularisation were detected in 71.43% of cases. In contrast with the literature (Hansen, 1952) the most interesting features were chondroid metaplasia and calcium deposits in NCD.

Conclusion

A breed predisposition could induce the chondroid metaplasia, but probably a multifactorial aetiology induces disk degeneration. Further investigations are in progress in order to verify this hypothesis.

108. ELEMENTS OF EPIDEMIOLOGY OF CANINE
MAST CELL TUMOR DIGNOSED BY CYTOLOGIC
EXAMINATION

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Introduction

Preoperative diagnosis of mast cell tumor represents an important step in the assessment of the therapy.

Methods and materials

150 dogs were diagnosed with mast cell tumors between 2002-2009. Samples were taken by fine needle aspiration or by scraping of the excised masses and were May Grúnwald Giemsa stained.

Results and discussions

The Boxer is the most affected (25.33%), followed by other 32 breeds. The dogs submitted to the study had ages between 15 years and less than 1 year. The females have the highest susceptibility (58.67%). Solitary lesions (84%) are more frequent than multicentric ones, located mostly on the limbs (41.33%), followed by thorax and abdomen (each with 8.66%), head (8%), cervical region (6%), lymphnodes (4%), perineal and preputial regions (each with 2%), base of the tail (1.37%), rarely in the spleen, mammary gland and bone marrow (each with 0.66%). Grade I mast cell tumor has the highest incidence (44%).

The lesion debuted predominantly in December (12%) and infrequently in February and October (each with 5.33%).

Conclusions

The cytologic examination of the mast cell tumors represents a reliable diagnosis method and a valuable tool in epidemiological studies.

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Introduction

Gonadoblastoma is very rare neoplasm that includes germ cell and Sertoli cell elements. In animal, this neoplasm has only been reported in two dogs. We report a rare case of gonadoblastoma in a rabbit.

Materials and Methods

A male 6-year-old unknown-breed pet rabbit was presented with enlargement of the left testicle. Bilateral orchietomy was performed. The enlarged left testicle was approximately 1.7 cm in length, globular and firm.

Results

The tumor showed proliferating pattern forming discrete tubular structures composed of large round cells similar to germ cell and spindle cells with elongated nucleus similar to Sertoli cell. Large round cells had abundant clear cytoplasm and large nuclei. Spindle cells arranged in palisading pattern around large round cells, and sometimes surrounded eosinophilic amorphous material (Call-Exner bodies). Non-neoplastic Leydig cells were also scattered within the intertubular stroma. Immunohistochemistry, large round giant cells were positive for germ cell markers such as c-kit and PLAP, whereas spindle cells had positive reaction for Sertoli cell markers such as vimentin and WT-1. Ultrastructurally, eosinophilic materials consisted of duplicated basal laminae corresponding to those of Call-Exner body.

Conclusion

The morphological, immunohistochemical and ultrastructural characters of this tumor were suggestive of gonadoblastoma.

110. ONION EFFECTS ON SPERM QUALITY PARAMETERS AND TESTIS TISSUES IN DIABETIC WISTAR RATS

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Introduction

It is well known Free radicals have most important role in Diabetes pathogenesis, experimental diabetes induced by Stereptozytocin causes spermatogenesis defects. Some studies have been reported Onion helps spermatogenesis via its antioxidants on normal tissue but not on diabetic ones.

Material and Methods

An experimental study was designed on thirty 8week-old vistar rats, divided into 3 groups. Diabetes induced by 55mg/kg stereptozytocin intraperitonially in treatment groups, then one ml of Onion extract was given orally to one of treatment group and the other received water instead, for 28 days. Blood samples were collected and rats were euthanized .Testis tissues were fixed for Histopathological studies.

Results

Sperm quality parameters were measured like motility, viability, TAD, MDA and number of sperms. Microscopic micrographs of tissues also were observed. Significant improvement was detected in sperm quality and regenerative aspects in seminiferous tubules in treatment group with onion.

Conclusion

Onion extract can be useful in decreasing the disorders caused by sterptozytocin and diabetes in testis via its antioxidants like flavonoides and can be considered as a plant drug in treatment of infertility in diabetes.

**POSTER SEMINAR TOUR
SESSION 7B - FREE TOPICS**

113. MALIGNANT LYMPHOMA IN A DOMESTIC GUINEA PIG (A CASE REPORT)

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Introduction

Spontaneous tumors in guinea pigs (*Cavia porcellus*) are rather rare according to literature data. Spontaneously arising neoplasia occur in guinea pigs with a reported prevalence of 0,4 % to 1,4 % in animals under 3 years of age and 0,5-30% in older animals. Leukemic and non-leukemic form of malignant lymphoma is described in the guinea pig population. The leukemic form occurs more frequently.

In this case report we present clinical features, diagnostic procedure, and necropsy examination of a 3 years old male domestic guinea pig with leukemic form of lymphoma.

Material and Methods

A 3- year- old male guinea pig was presented at the University of Veterinary Medicine in Olsztyn with a history of long term dyspnoe, apathy, weight loss, bilateral swelling of conjunctiva. Clinical examination revealed lymphadenomegaly.

Radiological and ultrasound examination, blood examination, fine-needle aspiration (FNA) of the mandibular lymph node and pathological examination was performed. Samples were fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with HE.

Results

Blood examination revealed leukocytosis with lymphocytosis. Radiological and ultrasound examination showed free fluid in the thoracic cavity, hepatomegaly, splenomegaly and enlargement of the mesenteric lymph nodes. Cytological examination by fine needle aspirate from mandibular lymph node revealed pleomorphic population of medium to large lymphoblastic cell. Pathological examination revealed generalized enlargement of peripheral and mesenteric lymph nodes, free fluid in the thoracic cavity, hepatomegaly and splenomegaly. Histological evaluation of enlarged lymph nodes showed a pleomorphic population of medium to large mononuclear cells in the scant stroma.

Conclusion

Pleomorphic population of medium to large lymphocytes in the biopsy and pleomorphic population of medium to large mononuclear cells in the scant stroma are characteristic for malignant lymphoma, which confirmed the clinical diagnostic.

PHENOTYPIC CHARACTERIZATION OF SPONTANEOUS MAMMARY TUMORS IN PET RABBITS

114.

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Introduction

Molecular-based subtypes consisting of two receptor-positive (ER and/or PR) types (luminal-like A and luminal-like B) and three hormone receptor-negative types (HER-2-expressing, basal-like, and normal-like) have been recently investigated in mammary gland tumors of the dog by immunohistochemistry (IHC). The present study is aimed to apply an IHC panel to a series of 12 rabbit mammary tumours to verify the molecular-based classification in this species and to evaluate the rabbit as an animal model of human breast cancer

Material and methods

Three hyperplastic mammary glands, 2 adenomas and 10 adenocarcinomas, underwent IHC stains with the following panel of antibodies: estrogen receptor- α (ER- α), HER-2, cytokeratin 5/6 (CK 5/6). According to Sassi et al.(2009), luminal-like (A and B), basal-like, HER-2 and normal-like patterns were determined.

Results

Seven cases were ER- α - positive (2 hyperplasias, 2 adenomas and 2 adenocarcinomas). HER-2 positivity was observed in 2 cases (1 adenoma, 1 adenocarcinoma). Cytokeratin 5/6 positivity was identified in 3 cases (1 adenoma, 2 adenocarcinomas). Among the 10 carcinomas, 5 normal-like, 3 luminal-like A, 1 basal-like and 1 HER-2 patterns were identified.

Conclusion

In the rabbit all the 5 molecular subtypes were identified with normal-like and luminal-like as the most represented. The rabbit seems to be the best animal model for human breast cancer if basal and luminal-like patterns are considered.

115. THE ROLE OF MYOFIBROBLASTS IN THE
PATHOGENESIS OF PARASITIC LIVER FIBROSIS IN
FALLOW DEER

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Jovanovic Milijan, Knežević Milijana

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Introduction

Increase of extracellular matrix in humans, as well as in animals, occurs as a consequence of myofibroblasts activation. The role of these cells in the development of liver fibrosis, caused by migration of giant liver fluke (*Fascioloides magna*), is the subject of this paper.

Materials and methods

Formalin-fixed and paraffin-embedded liver samples from 60 hunted fallow deers, infected with flukes, were examined by light microscopy, using H&E and Masson-trichrom staining and immunohistology for α -smooth muscle actin (α -SMA) and Desmin.

Results

Pathohistologic examination of the livers revealed intensive proliferation of interstitial fibrous tissue and regenerated lobules, proliferated bile ducts and pigmentation. Distribution of myofibroblasts depend on the degree of liver fibrosis. In cases of liver cirrhosis in hyperplastic pseudolobules hepatic stellate cells (HSCs) were located at its periphery. Cells of different shapes and size were positive to α -SMA imunoreactivity and desmin.

Numerous septal and portal myofibroblasts also stained positive with these antibodies.

Conclusion

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

Supported by project OI156010.

EARLY HEPATIC CHANGES IN GOATS IMMUNIZED WITH RECOMBINANT GLUTATHIONE S TRANSFERASE (SIGMA CLASS) AND CHALLENGED WITH FASCIOLA HEPATICA 116.

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Martínez-Moreno Álvaro¹, Brophy Peter², Pérez José¹

¹*University of Cordoba, Spain*

²*Aberystwyth University, United Kingdom*

Introduction

Understanding mechanisms of host response against *F. hepatica* is crucial for improving vaccine efficiency against this parasite. While in rats protective response occurs during early stages of infection, in ruminants no studies have investigated early changes in vaccinated animals. The aim of the present work was to study the hepatic changes and host response in goats immunized with recombinant GST-sigma class (sGST).

Material and Methods

Twenty-five, 4 month-old, parasite free goats were divided into 3 groups; group 1 (n=10) was immunized twice with sGST in Quil A, group 2 (n=10) was immunized with adjuvant Quil A, and group 3 (n=5) was used as uninfected control. Groups 1 and 2 were orally infected with 100 metacercariae of *F. hepatica*. Three goats from each group were killed at 7-9 days post-infection (dpi) and remaining animals were killed at 15 weeks post-infection (wpi).

Results

At 7-9 dpi, sGST vaccinated animals showed lower hepatic necrosis, higher iNOS expression by peritoneal macrophages, and higher number of eosinophils surrounding migrating larvae than Quil A immunized animals.

Conclusion

The results of the present work suggest an early host response in vaccinated animals and encourage new studies using higher number of animals.

117. APPLICATION OF IN-SITU HYBRIDIZATION FOR THE DETECTION AND IDENTIFICATION OF AVIAN MALARIA PARASITES IN PARAFFIN-EMBEDDED TISSUES FROM CAPTIVE PENGUINS

Dinhopl Nora, Mostegl Meike, Richter Barbara, Weissenboeck Herbert

University of Veterinary Medicine, Austria

Introduction

Avian hemosporidian blood parasites including species of *Haemoproteus*, *Plasmodium* and *Leucocytozoon* are transmitted by blood-sucking dipteran insect vectors and all three but most often *Plasmodium* sp. are referred to as avian malaria. In tissue samples of dead birds diagnosis of infection may prove complex. To overcome this problem a chromogenic in situ hybridization (ISH) was established, which facilitates detection of parasites directly within the tissue.

Materials and Methods

Various formalin fixed, paraffin wax-embedded tissues from 29 penguins with a suspected diagnosis of avian malaria were examined in this retrospective study. For ISH a digoxigenin-labelled oligonucleotide probe was designed, targeting a segment of 18S ribosomal RNA.

Results

Avian malaria parasites were readily identifiable by a distinct purple to black signal in various tissues including liver, spleen, lung, kidneys and brain.

Conclusion

Using this diagnostic approach, for the first time specific, simple and cost-efficient detection of avian malaria parasites in paraffin-embedded tissues is possible by chromogenic ISH.

CRYPTOSPORIDIUM PARVUM NATURAL INFECTION IN GOAT KIDS FROM CLUJ COUNTY, ROMANIA: HISTOLOGICAL AND MORPHOLOGICAL CHARACTERIZATION

118.

Pompei Bolfa, Bejan Alexandru, Cornel Catoi, Taulescu Marian, Vasile Cosma, Cuc Cosmina

USAMV, Romania

Introduction

Few studies were conducted to investigate the pathogenesis and histopathological alterations in the mucosa of goatlings with criptosporidiosis.

Material and methods

58 kids were presented to the necropsy, and the feces of another 83 goatlings were studied, with ages between 1 day and 3 weeks of age. Infection was confirmed by Ziehl-Neelsen stain modified by Henriksen. Grossly exam was followed by histopathological exam (Hematoxylin-Eosin stain), scanning electron microscopy exam and immunofluorescence exam.

Results

Pathological changes occurred mainly in the ileum, but also caecum and colon at the apical surface of intestinal epithelial cells. Different parasitic stages appeared as round or oval formations, sometimes covering the free border, which appeared atrophic and with denudation of intestinal villi.

Conclusion

Most histological and morphological characteristics of goatling infection with *C. parvum* are common on the whole with other *Cryptosporidium* spp.

119. VERMINOUS ARTERITIS OF CRANIAL MESENTERIC ARTERY OF HORSES - THE ROLE OF ARTERIAL SMOOTH MUSCLE CELLS

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²*Faculty of Veterinary Medicine, Department of Pathology, Serbia*

Introduction

Morphological characteristics of verminous arteritis of the cranial mesenteric arteries of 16 sacrificed necropsied horses and the role, origin and distribution of smooth muscle cells (SMC) in its development are described in this paper.

Materials and methods

After macroscopic examination tissue samples for pathohistological examinations were routinely processed and stained with hematoxylin eosine (H&E), Weigert van Gieson, Masson-trichrome and Periodic-Acid-Schiff (PAS) staining. Immunohistochemical staining was performed on selected sections using α -smooth muscle actin (α -SMA), desmin and vimentin.

Results

Enlarged, thickened cranial mesenteric arteries with a hard-elastic consistency and narrowed lumen, were macroscopically evident in all examined sick horses. Live larvae of *Strongylus vulgaris* were noted macroscopically. Inflammatory and fibrous changes were seen microscopically and were present in the intima, media and adventitia of the blood vessel. The inflammatory infiltrate in the wall of the cranial mesenteric artery consisted of eosinophils, macrophages, plasma cells, lymphocytes and rare multinucleated giant cells and was situated mostly near the *Strongylus vulgaris* larvae. Beside inflammatory changes, intimal fibrosis and extension, characterized by increased proliferation of α -SMA and vimentin positive cells was notable.

Conclusion

All described changes are characteristic for cranial mesenteric artery verminous arteritis.

HEPATIC AMYLOIDOSIS IN DAIRY CATTLE ASSOCIATED WITH CHRONIC BRONCHITIS (A CASE REPORT)

120.

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Amyloidosis can be a consequence of chronic respiratory diseases.

A 5- year-old Holstein cattle with a history of chronic respiratory problems was examined in farm. Coughing, tachypnea, mucopurulent discharges in nostrils, respiratory rale and pasty stool was observed.

The cow was slaughtered because of poor body condition and irresponsiveness to treatment.

Necropsy revealed consolidation of lung lobules with a nodular pattern except in caudodorsal region of diaphragmatic lobes. Airways were filled with a green mucopurulent exudates. Pleural adhesion was observed. Mediastinal lymph nodes were very large without any pus on cut surface. Other organs were congested and edematous.

Histopathology revealed severe chronic bronchitis and bronchiolitis. Air ways had denuded epithelium. Wall of them was infiltrated with mononuclears. Air ways cartilages were completely destroyed. Lymphoplasmacytic interstitial pneumonia and pleuritis with extensive fibrosis was observed. Small intestine had severe edema. Congestion, extensive hyaline change, hepatocellular atrophy were our findings in liver .Congo red staining confirmed severe hepatic amyloidosis. Masson trichrom staining revealed fibrosis of portal areas and areas around central veins.

We think the cause of pasty stool was fluid secretion to intestinal lumen because of severe congestion of liver and portal system that was a consequence of cor pulmonale.

121. STUDY OF LEUKEMOID REACTIONS IN SOME HEMOPARASITOSIS CONCERNING DOGS AND CATS

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Introduction

The authors present the importance of cytomorphological examination in some hemoparasitosis (*Babesia* sp., *Bartonella* sp., *Hemobartonella* sp.) in dogs and cats, both of these examinations being used in diagnostic purposes and to identify leukemoid reactions.

The aim of this study is to demonstrate the achievement of a differential cytomorphological diagnosis between leukemoid reactions and Vera leukemia in dogs and cats infected with pathogens transmitted by fleas and ticks.

Material and methods

This study was conducted in the Clinic Faculty of Veterinary Medicine Bucharest on a number of 328 dogs and cats positively diagnosed with one of three pathogens. Blood collected was displayed on the slide, which was colored May Grundwald-Giemsa and then examined under a microscope.

Results

Cytomorphological examination found that 28.6% of cases of all animals developed leukemoid reaction (55% dogs, 45% cat).

Leukemoid reactions had the following basic cell proliferation: for dogs was neutrophil granulocyte, eosinophilic, lymphocytic, monocitaric leukemoid reactions, and in cats appears in addition an leukemoid reaction with NK cell response.

Conclusions

It has been demonstrated that leukemoid reactions are ephemeral (against leukemia-Vera Status) by the disappearance of proliferating cell base following specific treatment of these morbid conditions.

HETEROTOPIC SALIVARY GLAND TISSUE IN THE RETROBULBAR SPACE IN A DOG

122.

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University of Veterinary and Pharmaceutical Sciences, Czech Republic

Introduction

A 10-year-old female German shepherd was presented with retrobulbar mass compressing the eyeball. Histopathological examination showed differentiated seromucinous gland tissue morphologically corresponding with salivary gland.

Materials and Methods

Biopsy samples were collected for histopathological examination, fixed in buffered 10% neutral formalin, dehydrated, embedded in paraffin wax, sectioned on a microtome at a thickness of 4 μm , and stained with hematoxylin and eosin (H&E). For comparison the tissue samples of normal unaltered salivary glands (gl. parotis, gl. submandibularis), lacrimal gland and third eyelid gland were taken for histopathology.

Results

Histopathological examination of the retrobulbar mass revealed seromucinous tubuloalveolar gland with predominance of mucinous component and presence of intercalated and striated ducts. In some parts of the parenchyma cystic dilatation of alveoli and ducts was observed. There were no signs of neoplastic changes in examined tissue.

Conclusion

Diagnosis of salivary gland heterotopia in the retrobulbar space was settled after the comparison of biopsy with normal salivary, lacrimal and third eyelid glandular tissue.

123. PRO - AND AGAINST ELEMENTS FOR CYTOLOGICAL INVESTIGATION OF MAMMARY GLAND TUMORS IN QUEEN AND BITCH

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Ionascu Iuliana, Cranganu Dan

Faculty of Veterinary Medicine, Romania

Introduction

The role of cytological exam in mammary gland tumors is controversial for the specialists and pets' owners. In this study, our aim was to establish the grade of positive correlation between cytological and histopathological exams.

Materials and methods

Mammary gland tumors from 39 queens and 88 bitches were considered in this study. The diagnosis was established by cytological investigation (fine needle aspiration, imprints MGG stained) and histopathological sections (Masson trichromic) for all tumors. The histopathological diagnosis was made according to WHO classification.

Results

The cytological criteria (morphology, presence of the secretory and ductal epithelial cells, mesenchymal cells with and without metaplasia, the presence of inflammatory cells and the background), which were the basis for the mammary gland tumors diagnosis were presented. The diagnosis disparity was 7.7 % of the cases in queens and it was correlated with the nude nuclei, inflammatory process and with the lack of clinical data. The diagnosis disparity was at 10.2 % of the cases in bitches and it was correlated with inflammatory process, stromal metaplasia and the structural complexity of the tumors.

Conclusions

Cytological diagnosis of mammary gland tumors has a bigger accuracy in queen than in bitch. For the second species, diagnostic cytology via fine needle aspiration has a limited value of diagnosis, but it is a useful method for lymph node metastasis.

THE POULTRY HEPATOPATHIES IN CORRELATION WITH FOOD SAFETY 124.

Militaru Manuella, Laurentiu Tudor, Ghimpeteanu Margarita Oana, Ciobotaru Emilia,
Dinescu Georgeta

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Introduction

The typology of the liver lesions, correlated with the isolation of pathogenic and conditional pathogenic microorganisms can provide information about general health status of the flock and the potential damage of consumer's health. The aim of this study was to link the different types of hepatopathy diagnosed in rejected organs with the presence of some microorganisms.

Materials and methods

411 livers, weighting 18.4 kg, from young abattoir slaughtered poultry were grossly investigated. Cytological (May Grünwald Giemsa stain), histological (Masson trichromic and Congo red stains) and microbiological exams were performed for 22 livers considered representative for gross lesion expression.

Results

Two major types of lesions were grossly observed: non-specific hepatopathy with diffuse degenerative features associated with vascular reaction and multifocal miliary necrotic hepatitis. Cytologically, the degree of hepatocyte degeneration and the typology of inflammatory cells were investigated. Histologically, different types of lesions were described: hepatosis, miliary necrotic hepatitis, interstitial diffuse infiltrate and amiloidosis. The most common microorganisms were *E. coli*, *Pseudomonas* spp. and yeasts.

Conclusions

94.4% of samples were represented by non/specific hepatopathy and 5.6% by multifocal miliary necrotic hepatitis. Miliary necrotic hepatitis is associated with *Pseudomonas* spp. (5/7) while non-specific hepatopathies is correlated with *E. coli* (8/15), yeasts (8/15) and *Enterococcus* spp. (7/15).

125. OBSERVATIONS CONCERNING CADMIUM AS POLLUTANT ELEMENT IN SOIL AND FORAGES AND ITS INFLUENCE UPON ANIMALS IN THE SOUTHERN AREA OF BUCHAREST

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Faculty of Veterinary Medicine Bucharest Romania, Romania

Introduction

Cadmium is one of the pollutant elements emitted in the eastern area of Bucharest. It is described an accumulation of the metal into internal organs: liver, kidney, heart, brain.

Materials and methods

There were collected soil samples from different depth and distances from the pollution source, forages samples and organs collected from horses, pigs and ruminants. The cadmium concentration analyzing method was atomic absorption spectrophotometry. For animals, there was used clinical examination procedures and for internal organs study, the macroscopic and histopathological examination.

Results

Cadmium concentrations exceed the maximum admitted limits by 2.3 times in soil samples collected from depths under 10 cm and at 200 m distance of the pollution source; by 3,57 times in forages samples and in organs the limits were exceeded by 9 times in horse liver and almost 40 times in horse kidney.

Cadmium high values were related with clinical simptomatology, expressed mainly by digestive and respiratory troubles, and with major lesions of the kidney and liver (nephritis, hepatosteatosi).

Conclusion

The correlation between cadmium high concentration in soil, plants and organs and the clinical and anatomopathological findings proves a chronic cadmiun toxicosis of the animals in the eastern area of Bucharest.

NEURONAL CEROID LIPOFUSCINOSIS IN A TIBETAN TERRIER: PATHOLOGICAL FINDINGS 126.

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Introduction

The neuronal ceroid lipofuscinoses (NCLs) are a group of inherited (autosomal recessive) progressive lysosomal storage diseases characterized by the accumulation of autofluorescent inclusions (lipopigment) mainly in neurological tissue, retina and muscle, resulting in progressive neurodegeneration and premature death.

Clinical case

We made the diagnosis of canine ceroid lipofuscinosis based on clinical (including gene test) and histopathological findings in a 10 years old Tibetan Terrier. The animal was first time presented at the age of 6 years with strong anxiety, nervousness, irritability and biting. Despite therapy with tranquilizers, the disease progressed. The dog was last time presented with strong distinct symptoms (extreme anxiety, amnesia, aggression, loss of learned behavior, nyctalopia) at the age between 9 and 10.

Results

Macroscopic findings revealed a moderate dilation of the lateral brain ventricles. The cerebrum and cerebellum showed a slightly yellowish discoloration of the parenchyma. Microscopically, within the cytoplasm of neurons and macrophages of the brain and spinal cord was marked accumulation of finely pale brown-greyish (H&E), in some neurons eosinophilic, autofluorescent granular material. Special stainings (PAS, LFB, ZN) confirmed lipopigment storage.

Conclusions

The macroscopic and microscopic findings together with clinical data and gene test are indicative for canine form of late-onset NCL in a Tibetan Terrier.

127. BONY FUSION AFTER REIMPLANTATION OF
REMOVED AUTOGENOUS CANCELLOUS BONE IN A
DOG TREATED SURGICALLY FOR DISK ASSOCIATED
WOBLER SYNDROME BY A DISTRACTABLE
VERTEBRAL TITANIUM CAGE.

Wegge Beatrice, De Decker Steven, Caemaert Jacques, Gielen Ingrid,
Van Ham Luc, Chiers Koen

Ghent University, Belgium

Introduction

Disk associated wobbler syndrome (DAWS) is the most common cause of cervical spondylomyelopathy in dogs. DAWS is generally treated by surgery. In this case we evaluated the use of a distractable titanium cage for surgical treatment in dogs.

Materials and methods

A 10-year-old Dalmatian with DAWS was submitted for surgical treatment. After total discectomy of C5-C6 and C6-C7, an median corpectomy of C6 was performed by a pneumatic drill. The removed cancellous bone was carefully collected to act as an autogenous cancellous bone graft. Subsequently, the titanium implant was placed and affixed in the defect. Finally it was filled and covered with the collected cancellous bone. The dog improved and survived for 22 months and was euthanised because of lumbosacral stenosis. The intervertebral tissue surrounding the implant was removed and submitted for histopathological examination to confirm a bony fusion of the bone graft with the lateral edges of the vertebral body.

Results

Samples of the lateral and ventral sides showed mature cancellous bone. The spinal cord section at the level of the implant showed no signs of compression.

Conclusion

This is the first veterinary report demonstrating bony fusion after reimplantation of mechanically removed cancellous bone after a vertebral corpectomy.

MORPHOLOGICAL STUDY OF THE OPTIC TECTUM OF BEARDED DRAGON 128.

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²Autonomous University, Spain

Aim

The Optic Tectum (OT) is one of the most complex structures in the central nervous system (CNS) of the lizards and receives both visual and auditory inputs. This work analyzes the OT of bearded dragon (*Pogona vitticeps*), using both histochemical and immunohistochemical techniques.

Material and methods

formalin-fixed and paraffin-embedding CNS samples of three healthy adults were used. Serial samples of the mesencephalon were consecutively stained for HE, Nilss, Kluver-Barrera staining techniques, silver impregnation and nervous tissue markers as neuronal (anti neuron-specific enolase (NSE), PGP 9.5, neurofilaments), and glial (GFAP, olig2, myelin proteolipid protein (PLP) and myelin basic protein (MBP)) antibodies. The immunohistochemical study was carried out using ABC method.

Results and discussion

Correlation of the staining patterns demonstrated the high complexity in neurons and nerve fibers distribution; all antibodies showed cross-reactivity with the lizard CNS counterparts. NSE and PGP 9.5 reacted with numerous neurons and axons through the tectal layers; neurofilaments 200, strong and diffusely reacted with fibers; PLP and MBP antibodies reacted with myelinated fibers of different length and thickness. Joint analysis point out new data of the OT cytoarchitecture in *Pogona vitticeps*, which appears similar to that found in some lizard as *Anolis sagrei* and *Eublepharis macularis* as well as in birds.

129. **INCLUSION BODY DISEASE IN SNAKES: TISSUE
DISTRIBUTION AND MORPHOMETRIC ANALYSIS OF
THE EOSINOPHILIC INCLUSIONS**

Mozos Mora Elena, Ruiz M Josefa, Jiménez Carlos R, Pérez José, Zafra Rafael,
Pérez-Écija Alejandro

University of Cordoba, Spain

Introduction

Inclusion body disease (IBD) is a disorder of uncertain origin described in captive snakes (boids and vipers). IBD diagnosis is based in the observation of intracytoplasmic eosinophilic bodies (IBs) which could be present in virtually any cell type; nonetheless, in vivo IBD diagnosis is a challenge.

Aim

to analyze the tissue distribution and size of IBs in order to obtain data that facilitate criteria for new diagnostic procedures.

Material and methods

Formalin-fixed and paraffin-embedded samples from all organ systems of two Boas constrictor presenting chronic and subacute clinical onset of IBD have been used. Data were obtained counting the number of IBs-containing cells and measuring IBs diameters in 10 fields (x400) selected in 5 serial sections using the Image Pro Plus software.

Results and Conclusion

Variable number and size of IBs were found in the majority of tissue and organs in both animals; however, the Harderian gland (75% and 80% of affected cells per field, respectively), the vomeronasal organ (49% and 50%), kidney (26% and 39%) and liver (28% y 20%) were the most affected organs. In relation to IBs size (diameter means), renal tubules showed the largest IBs (1.8 μm), followed by the liver (1,68 μm), the Harderian gland (1,52 μm) and the vomeronasal organ (1,2 μm). In conclusion, the Harderian gland and the vomeronasal organ seem to be suitable to carry out in vivo and postmortem IBD diagnosis.

Vercauteren Griet, Saey Veronique, Van Loon Gunther, De Bruijn Marco,
Delasalle Catherine, Chiers Koen

Ghent University, Belgium

Introduction

Aortic rupture is very rare in animals. However, in Friesian horses this condition is more frequently encountered, suggesting this is a hereditary disorder. A congenital aneurysm, copper deficiency, long-term degenerative disease with weakening of the aorta, migration of *Strongylus vulgaris* and extreme hypertension in breeding stallions have been suggested. Here we describe 3 unusual manifestations of aortic rupture.

Materials and methods

Three Friesian horses were submitted for necropsy with a suspicion of sudden aortopulmonary fistulation based on history, clinical signs, echocardiography and cardiac catheterisation. Histological examination of the aortic wall was performed.

Results

Grossly, the aortic wall was ruptured close to the ligamentum arteriosum with fistulation into the pulmonary artery. Perivascular hematomas were extensive. Histological examination revealed a thickening of the tunica media by increase of mucinous stroma. Focal areas were necrotic and occasionally thrombosis of vasa vasorum was present.

Conclusion

This defect could be associated with dysregulation of matrix metalloproteinases in Friesian horses. The cause of this condition is not known, although the presented histological lesions resemble cystic medial necrosis of the aorta, as seen in humans.

131. EFFECTS OF LOW-FREQUENCY PULSED DIRECT CURRENT ON CAPTIVE-HOUSED SEA FISH

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²*Institute for Agricultural and Fisheries Research, Belgium*

Introduction

To reduce the unwanted by-catch in shrimp trawling, alternative stimulation techniques such as electricity, which selectively invokes a reaction in shrimp, might be used. The purpose of this study was to evaluate the effects of such electric pulses in sea fish.

Materials and Methods

Plaices (n=21), soles (n=22), dragonets (n=21), pogges (n=21), codfish (n=20), armed bullheads (n=14) and fivebeard rocklings (n=8) were exposed to electric pulses (60V, 0.5 milliseconds, 5Hz) during 10 seconds. Behaviour and mortality were recorded. All fish were euthanized and necropsied 24h after exposure. Control animals consisted of equal numbers of the same species which were not exposed to electric pulses.

Results

All fish survived the experiment. In almost all exposed fish, minor and brief fright reactions were observed. In general, gross and histological abnormalities were rarely present in exposed and control fish. In one control and two electrical exposed plaices, small multifocal cutaneous haemorrhages were observed. In one electrical exposed sole, a small focal interstitial haemorrhage in the muscle was present.

Conclusion

Alternative techniques such as electric pulses for catching shrimp could be promising since this appears to have low impact on fish, at least under experimental conditions.

EFFECTS OF CASTRATION ON RENAL INJURY IN OSBORN-MENDEL RATS

132.

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Kamiie Junichi, Shirota Kinji

Azabu University, Japan

Introduction

Osborne-Mendel (OM) rats develop progressive glomerulonephropathy in early of life. In males, segmental glomerulosclerosis develops from 15 weeks of age, and progresses to global sclerosis until 20 weeks of age. The glomerular injury of males is more intense than in females suggesting a pivotal role of sex steroids in such gender difference. We investigated the effects of castration on renal injury.

Materials and Methods

Male OM rats were castrated at 3 weeks of age and their renal injuries were histopathologically compared with age-matched intact males until 20 weeks of age.

Results

Hyaline droplets in the podocytes and adhesion of capillary loop to the Bowman's capsule developed in intact males at 7 weeks of age. Such glomerular lesions had been exacerbated until 20 weeks of age. Castration attenuated such renal injuries. By immunohistochemistry, androgen receptor (AR) was expressed in mesangial cells and podocytes in the glomeruli of intact male rats. At 20 weeks of age, expression of glomerular AR in castrated rats was lower than that in intact age-matched males.

Conclusion

Besides the mechanisms involved in development of glomerular injury, down-regulation of AR in the glomerular cells might play a role in progression of glomerular injury.

133. SEASONAL OUTBREAKS OF TYZZER'S DISEASE IN MALAGASY GIANT JUMPING RATS (HYPOGEOMYS ANTIMENA) IN THE RIGA ZOO

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Introduction

Two outbreaks of sudden mortality in Malagasy giant jumping rats have occurred in Riga's Zoo. During the first outbreak in fall of 2004 7/8 rats died. In the second outbreak (fall of 2009) 2/2 rats died. All ages of animals were affected.

Materials and methods:

Necropsies, histopathology, special staining, and PCR were used to determine the cause of outbreaks.

Results

Acute hepatic necrosis was detected in 3/4 rats examined from 2004 outbreak and in both rats from 2009 outbreak. Large, slender bacilli, suggestive of *Clostridium piliforme* were seen in Warthin-Starry silver stained sections. In 2009 cases *C. piliforme* DNA was found in formalin-fixed paraffin-embedded liver tissues by PCR. Sequencing of conserved region of *C. piliforme* showed that the strain was 99% homologous to mouse isolate. These findings support Tyzzer's disease as the cause for both outbreaks.

Conclusions

Occurrence of both Tyzzer's disease outbreaks in the fall indicates that wild rodents which start to move indoors during cooler nights may be carriers of *C. piliforme*. While the susceptibility of Malagasy giant jumping rats to *C. piliforme* is not known, these two outbreaks suggest that this rodent species may have increased sensitivity to Tyzzer's disease.

ATAXIA DUE TO A VERTEBRAL BODY HEMANGIOSARCOMA IN AN 18-YEAR OLD GELDING

134.

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Introduction

Neurological symptoms are rarely caused by neoplasms involving the spinal cord in horses. Hemangiosarcomas occur most frequently in dogs, but have been infrequently reported in horses. Only a few studies report of vertebral hemangiosarcomas in horses.

Materials and methods

An 18-year-old Dutch warmblood gelding with spinal ataxia was euthanized. Necropsy revealed an epidural hemorrhagic mass at C3 with compression of the spinal cord and osteolysis of C3 and C4. Thorough examination of other organs revealed no other tumors. The mass was fixed and analysed by histology and immunohistochemistry (von Willebrand Factor).

Results

Histological examination of the mass at C3 showed cavities filled with erythrocytes and lined by poorly differentiated endothelial cells, which were confirmed immunohistochemically. These cells were pleomorph with indistinct cell borders. The nuclei showed anisokaryosis with a prominent nucleolus. Neoplastic endothelial cells were invading the vertebral bodies of C3 and C4 and multifocal osteonecrosis was present.

Conclusion

The histological and immunohistochemical results are consistent with a diagnosis of hemangiosarcoma. It was unclear whether the origin of the mass was in the vertebral body with infiltration of the epidural space or vice versa.

135. IMMUNOHISTOCHEMICAL DETECTION OF HEPATITIS E VIRUS IN NATURALLY AFFECTED PIGS

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Introduction

A swine hepatitis E virus (HEV) was recognized as causative agent of subclinical hepatitis. HEV-infected pigs had evidence of multifocal and periportal lymphoplasmacytic hepatitis with focal hepatocellular necrosis. Viral infection was detected by in situ hybridization in liver, mesenteric lymph nodes, kidneys and in enterocytes. The aim of this study was to detect HEV by immunohistochemistry (IHC) and to determine infected organs and cell types.

Material and methods

Paraffin blocks of lung, myocardium, liver, spleen, intestines, kidney and submandibular, mediastinal and mesenteric lymph nodes were investigated. The samples were collected from 3 pigs previously found HEV-positive by nested RT-PCR. For IHC detection a mouse anti-HEV Mab (USBiological®) is used.

Results

HEV antigen was detected in liver samples of all 3 pigs in periportal inflammatory cells and in hepatocytes in centrolobar degeneration process. Positive signal was more intensive near liver hilum and gall bladder but also found in mesenteric lymph nodes.

Conclusion

The presence of HEV was detected in liver and mesenteric lymph nodes. The other investigated organs were found negative. IHC is a useful tool and highly specific method for HEV detection. By our results, parts of liver near hilum and gall bladder can be specific and suitable place for sampling.

MACROSCOPIC AND MICROSCOPIC LESIONS IN PIGS 136. WITH EXPERIMENTALLY INDUCED NON-BACTERIAL THROMBOTIC ENDOCARDITIS

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Introduction

Infective endocarditis models have been established in several laboratory animals. However, this disease model has only sparsely been described in pigs despite advantages such as cardiovascular, anatomical, and physiological similarities to humans, the possibility of collecting large blood volumes and to study prosthetic valve endocarditis. The purpose of this study was to further develop a method for induction of non-infectious thrombotic endocarditis in pigs, which in future studies will be used as a basis for experimental bacterial endocarditis.

Materials and Methods

Eight pigs underwent surgical placement of a catheter from the carotid artery to the left ventricle, by ultrasonographic guidance. The catheter remained in situ until euthanasia 2, 4, 5, and 6 days later, followed by a post-mortem macroscopic and microscopic examination.

Results

In six pigs the catheter was placed in the left ventricle, whereas in two pigs it was located extracardially. The six pigs all developed mural endocarditis lesions and four of these also had valvular endocarditis. Histologically, lesions consisted primarily of an endothelial disruption with overlying fibrin and thrombotic material.

Conclusion

The results are promising for the development of an infective endocarditis model, though there is a need for refinement regarding the catheter placement.

137. SYSTEMIC CRYPTOCOCCOSIS IN A FREE-RANGING CYPRUS MOUFLON

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Introduction

The Cyprus mouflon or agrino (*Ovis orientalis ophion*) is a wild sheep species endemic and unique to Cyprus.

Materials and Methods

A 3-year-old male agrino was found dead in the Paphos forest. Gross and histopathological, and molecular analyses were performed.

Results

The animal was cachectic and had a 3-cm³ swelling on the right maxillary area. Large quantities of whitish tumour-like tissue occupied most of the nasal cavity. Similar nodules were observed throughout the lungs, together with parasitic pneumonia lesions. Microscopically the lesions contained large clusters of thin-walled fungal organisms surrounded by a wide halo. India Ink and Nigrossin stained smears showed spherical yeasts surrounded by a thick unstained capsule. In Mucicarmine stained sections, the fungal capsule, not visible in HE sections, stained pink or red, a finding diagnostic of *C. neoformans* infection, a basidiomycetous encapsulated yeast that may cause life-threatening infections. The diagnosis of severe, multifocal cryptococcal rhinitis and pneumonia was established. Sequence and phylogenetic analysis of partial genomic regions from two different capsule associated genes (CAP59 and CAP64) classified the strain as *C. neoformans* var. *neoformans* (serotype D).

Conclusions

This report describes the first case of *C. neoformans* infection in any species in Cyprus, the first concerning a mouflon worldwide, and one of few systemic cryptococcoses reported concerning ruminants.

MULTIFOCAL SARCOMA IN PET RABBITS IN CENTRAL EUROPE

138.

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Introduction

The number of surgical specimens from rabbits is rapidly increasing, but the knowledge of specific disease processes in this species is sparse.

Materials and Methods

Since autumn 2008 six cases of disseminated multifocal dermal to subcutaneous sarcomas in pet rabbits were submitted to our laboratory. Histopathology, immunohistochemistry and electronmicroscopy were performed with formalin-fixed and paraffin-embedded samples.

Results

All rabbits exhibited disseminated cutaneous neoplasms. The trunk was most significantly affected. The neoplasms were situated intradermally with variable infiltration of the subcutis. In the cases, in which follow-up was possible, spontaneous death or deterioration occurred in the following weeks.

The lesions presented histologically as intradermal to subcutaneous accumulations of round to plump spindle-shaped cells. The cells exhibited moderate cellular pleomorphism and a variable proliferative rate reaching 2/ hpf. Proliferative activity of epidermal keratinocytes was mild and confined to the margins of the ulcers.

The immunohistochemical pattern indicates an origin from mesenchymal cells (positivity for vimentin). A number of cells in all cases also expressed smooth-muscle actin. Scattered cells exhibited lysozyme, but the majority was negative.

Viral particles could not be detected electronmicroscopically.

Conclusion

The lesions resemble virally induced tumors.

139. MACROPHAGES FROM DOGS WITH HISTIOCYTIC COLITIS SHOWED AN IN VIVO AND IN VITRO ALTERATE ACTIVITY

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Introduction

Histiocytic ulcerative colitis (HUC) is a progressive inflammatory bowel disease (IBD) that occurs predominantly in boxer. The lesions are mainly characterized by mixed inflammatory cell infiltrate with the main presence of PAS-positive macrophages into the intestinal wall suggesting a defective immune regulation.

Material and Methods

Colonic biopsies were obtained from 10 boxer, 5 HUC + and 5 unaffected dogs. Serial sections were analyzed for macrophages stain into inflammatory, for NFκB expression and for macrophages apoptotic index evaluation. For in vitro study, canine blood monocytes isolated from peripheral blood by the Bøyum method were cultured O-N in media containing PMA, LPS or MD. The abilities of non- and PMA-stimulated macrophages to reduce NBT was estimated. LPS or MD stimulated macrophages were utilized for RT-PCR analysis in attempt to amplify the TNF-α, IFN-γ, TGF-β, IL-1β, IL-4 and IL-12 interleukins transcripts.

Results

Elevated NFκB activity in intestinal epithelial cells and macrophages was observed in HUC+ dogs, and an high percentage of these macrophages are apoptotic. The capacity of non- and PMA-stimulated monocytes to reduce NBT was found to be significantly decrease in HUC+ dogs derived macrophages to respect controls; additionally HUC+ dogs derived macrophages exhibited a different cytokines pattern as a response to LPS and MDP stimulation, with low levels of IFN-γ and elevated TGF-β /IL-4 secretion.

Conclusions

High apoptosis rate of macrophages strictly related to cellular level of phosphorilate NFκB form, observed in HUC+ macrophages, is related to an increased susceptibility to bacterial-products. This different sensitivity is confirmed by the spontaneous and induced NBT tests and cytokines profile of stimulate HUC+ cells and adequately reflect the status of the HUC+ dog.