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MDM2 EXPRESSION IN CANINE LIPOSARCOMA

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Canine liposarcoma (LP) is an uncommon soft tissue sarcoma (STS) that arises more frequently in the subcutis. Three variants of LP have been described: well differentiated (WDLP), myxoid (MLP) and pleomorphic (PLP). In man, LP is the most common STS and is classified in WDLP/atypical lipomatous tumor (ATL), dedifferentiated LP (DDLp), MLP and PLP. WDLP/ALT and DDLp are considered different morphological presentations of the same biological entity bearing the amplification of the genes encoding for mdm2 and CDK4, and overexpressing these proteins. The aim of this study is to assess by immunohistochemistry (IHC) the expression of mdm2 in the different subtypes of canine liposarcoma.

Cases were selected from the pathology archives of the Universities of Bologna, Milano and Perugia. Hematoxylin and Eosin stained sections were evaluated and cases were classified according with the WHO classification of tumors of domestic animals when possible. IHC was performed with anti-human mdm2 mouse monoclonal antibody (clone 2A10). Cross reactivity of the antibody was assessed by western blot analysis. According with the guidelines applied in human medicine, cases were scored as positive if at least 10% of neoplastic cells had nuclear staining.

A total of 47 canine liposarcoma were collected: 19 were WDLP, 18 were PLP, and 7 were MLP. Three cases did not fit in any of the categories and had histological features consistent with DDLp. A total of 15/47 cases were mdm2 positive. WDLPs expressed mdm2 in 12/19 cases (63.2%), MLPs in 1/7 cases (12%), PLPs in 0/18 cases (0%) and DDLPs in 2/3 cases (75%). Taken together WDLPs and DDLPs expressed mdm2 in 14/22 cases (63.6%).

These preliminary results suggest that WDLP/DDLp can represent a biological entity characterized by mdm2 overexpression and distinct from MLP and PLP, paralleling what is reported in human medicine. Nevertheless, several WDLP were mdm2 negative. This result may be related to a low sensitivity of IHC. Since a greater sensitivity and specificity of fluorescent in situ hybridization (FISH) has been reported compared to IHC in evaluating mdm2 expression in human LP, further studies may clarify the level of mdm2 gene amplification in canine LP.

Gross et al., Skin diseases of the dog and cat: clinical and histopathologic diagnosis. 2nd ed. 2005

Goldblum et al. Enzinger & Weiss's Soft tissue tumors. 6th ed. 2013

Fisher et al. Diagnostic pathology: soft tissue tumors. 1st ed. 2011

Hendrick et al. Histological classification of mesenchymal tumors of skin and soft tissues of domestic animals. 2th Series. 1998

oncology

canine, liposarcoma, mdm2

CYTOLOGIC GRADING OF CANINE AND FELINE SPINDLE-CELL SARCOMAS OF SOFT TISSUES AND ITS CORRELATION WITH HISTOLOGIC GRADING

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The grading of soft tissue spindle cell sarcomas (STSCS) is considered more useful than histologic histotype to assign a specific therapy and determine the prognosis in humans (Enzinger et al., 2001). Histologic grading is considered an important prognostic factor in canine STSCS as it may predict the onset of local recurrence and metastatic potential. In human pathology and in the veterinary practice, fine needle aspiration cytology (FNAC) of soft tissue mass lesions can be an accurate and minimally invasive method for the initial pathologic diagnosis of soft tissue masses. The aim of our study was to assess the utility and the accuracy of the cytologic grading on FNAC smears, in comparison to the histologic grading in STSCS

Over a period of 2 years (2009-2011) 33 cases of cytologically diagnosed STSCS (20 canine and 13 feline) with a following histological diagnosis were retrospectively separately reviewed. The FNAC smears were graded without prior knowledge of the histologic grade, using the scheme proposed by Weir et al., (1999), which is a three-tier system based on nuclear atypia, nuclear overlap, mitosis, and necrosis. The corresponding histological sections were graded using the Coindre et al.,(1988) criteria in a blind fashion. The degree of concordance was established using the Cohen's K coefficient. (Palmer et al., 2001).

In the canine SCSTS there was an overall cytologic and histologic concordance for grading in 12/20 cases (60%). The concordance was observed in 4/8 (50%) cases of histologic grade 1, in 8/12 (67%) cases of grade 2, and in 0 cases of grade 3. In the feline species the concordance was 11/13 cases (85%). The concordance was observed in 5/6 (83%) tumors of histologic grade 1, in 4/4 (100%) tumors of grade 2, and in 2/3 (66.6%) cases of grade 3. The overall concordance in the entire study population of canine and feline STSCS was 70%. The gradewise concordance was 65% in grade 1 cases, 75% in grade 2 cases, and 66% in grade 3 cases.

The overall concordance is quite similar to that reported in human literature. Although a wider population is required to strengthen our findings, these results suggest that cytologic grading of STSCS obtained by FNA may be a useful tool for therapeutic approaches and as a prognostic indicator.

Coindre KM, Nguyen BB, Bonichon F, et al. *Cancer*. 1988; 61:2305-2309

Enzinger FM, Weiss SW, Goldblum JR. *Soft tissue tumors*, 4th ed. St. Louis, Mosby; 2001: 10-13.

Palmer HE, Mukunyadzi P, Culbreth W, Thomas JR. *Diagn Citopathol*. 2001; 24(5):307-316

Weir MM, Rosemberg AE, Bell DA. *Am J Clin Pathol*, 1999; 112:784-790

diagnostic cytopathology, small animal oncology
cytologic grading, soft tissue sarcoma, dog and cat

EFFECTS OF 17-AAG TREATMENT IN CANINE OSTEOSARCOMA CELL LINES: APOPTOSIS, AUTOPHAGY AND MITOPHAGY EVALUATION.

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Canine osteosarcoma is highly resistant to current chemotherapy so we wanted to research how tumour cells resist therapy. We tested a geldanamycin derivate prototype of an Hsp90 inhibitor, 17-AAG, on canine osteosarcoma cell lines D22 and D17, derived from primary tumour and secondary tumours respectively, with the aim of understanding the interplay between apoptosis, autophagy and mitophagy, given the dual effect of this process in regulating cancer cell viability.

Canine osteosarcoma cell lines D22 and D17 were treated with different 17-AAG concentration, 0.5µM and 1.0 µM, for 24 and 48 hours.

For ultrastructural analysis of cell lines, cell pellets were fixed and embedded in epoxy resin; ultrathin sections were obtained and double stained with uranyl acetate followed with lead citrate to be examined by means of an EM 900-ZEISS electron microscope. For apoptosis quantification cells were stained with AnnexinV-FITC Kit and analyzed by flow cytometry. For autophagy quantification cell culture were fixed and probed with rabbit polyclonal anti-LC3 for autophagosome count. Mitophagy were evaluated with colocalization of LC3 dots and mitochondria. Images were acquired with confocal microscope.

Results were normalized to DMSO-treated cells and data analyzed using the odds ratio. In the D22 cell line, growing dose and time of 17-AAG treatment caused apoptosis increase; in D17 cell line tendency was the same except for apoptosis decrease after 48h of treatment at maximum dosage 1.0µM. In between cell lines, in terms of autophagy and mitophagy, number of cytoplasmic dots per cell increased in presence of drug, especially after 48h of treatment and difference between cell lines were significant.

Further investigation is necessary to understand if induction of autophagy and mitophagy at high dosage treatment have a protective role in D17 cell survival. Our results highlight important differences between cell tumour type in resistance to Hsp90 inhibitors, underlining the potential of inducing apoptosis and simultaneously enhancing or inhibiting autophagy thus improving the efficacy of Hsp90 inhibitors in treatment.

Gazitt Y, Kolaparthi V, Moncada K, Thomas C, Freeman J. Targeted therapy of human osteosarcoma with 17AAG or rapamycin: characterization of induced apoptosis and inhibition of mTOR and Akt/MAPK/Wnt pathways. *Int J Oncol.* 2009 Feb;34(2):551-61

Samarasinghe B, Wales CT, Taylor FR, Jacobs AT. Heat shock factor 1 confers resistance to Hsp90 inhibitors through p62/SQSTM1 expression and promotion of autophagic flux. *Biochem Pharmacol.* 2014 Feb 1;87(3):445-55. Epub 2013 Nov 28

Bassam Janji, Elodie Viry, Joanna Baginska, Kris Van Moer and Guy Berchem. Role of Autophagy in Cancer and Tumour Progression. *Intech*, Chapter 9. 2013

disciplinare

osteosarcoma, 17-AAG, HSP90

THE EXPRESSION OF MATRIX METALLOPROTEINASE 2 (MMP-2) AND 9 (MMP-9) AND THEIR POSSIBLE PROGNOSTIC ROLE IN FELINE INJECTION-SITE SARCOMAS (FISS)

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Aggressive cancer is often characterized by high expression of gelatinases, namely matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9). These enzymes play a pivotal role in tumor invasion and metastasis by degradation of extracellular matrix (ECM) leading to cancer spread both to contiguous tissues and distant organs. Feline injection-site sarcoma (FISS) is an aggressive subcutaneous tumor that develops a variable period after vaccination. It is believed to arise as a result of fibroblasts and myofibroblasts proliferation at sites of chronic inflammation induced by the vaccine's adjuvants, its antigens, or both. Histologically, ISS are characterized by inflammatory peritumoral infiltration, proliferation of atypical spindle cells and multinucleated giant cells. Aim of this study was to investigate the expression of MMP-2 and MMP-9 in FISS and assess their possible role as prognostic markers to predict tumor recurrence or metastasis after excisional surgery.

24 cases of FISS were selected from the archive of the Department of Veterinary Medicine of Perugia. Tumor dimension, completeness of excision, mitotic index, inflammation, presence of lymphoid follicles, necrosis and the presence of multinucleated giant cells were evaluated. Immunohistochemistry was performed for MMP-2 and MMP-9. Moreover, veterinarians were contacted to get additional information about the patients' follow-up after tumor excision.

MMP-2 showed a variable expression in FISS, predominantly in spindle mononucleated cells and its expression was higher in recurrent tumours, even if histological margins were not infiltrated. On the other hand, positivity for MMP-9 was seen more frequently in giant cells of the tumors and in a lower percentage of mononucleated spindle cells. As for MMP-2, the expression of this gelatinase was higher in the group of tumors that recurred. Recurrent FISS were also associated with a larger size at the moment of first excision, with a mean higher degree of necrosis and a higher mitotic index, whereas inflammation and lymphocytic follicles were not significantly related to the lesions' follow-up.

Taken together, the expression of MMP-2 and MMP-9 seems to be associated with a higher risk of tumor recurrence. The size of the tumor at the moment of excisional surgery seems to be another important factor, all along with mitotic index and tumor necrosis. The results from our study would suggest that gelatinases can be used as useful prognostic immunohistochemical markers in FISS.

1) Martano M et al. *Vet J.* 2011 May; 188:136-41.

2) Giudice C et al. *Vet J.* 2010 Oct; 186:84-8.

3) Benassi MS et al. *Ann Oncol.* 2001 Jan; 12:75-80.

Oncologia

feline, sarcoma, metalloproteinases

IMMUNOHISTOCHEMICAL DETECTION OF CD25+ LYMPHOCYTES IN FELINE INJECTION-SITE SARCOMA**Magi G.E.*^[1], Petini M.^[1], Berardi S.^[1], Rossi G.^[1]**^[1] *Università di Camerino ~ Matelica*

CD25 (IL-2Ra) is the α -subunit of the membrane-bound interleukin-2 receptor (IL-2R), which is mainly expressed by regulatory T cells (Tregs). Tregs play a pivotal role in the homeostasis of the immune system and in the modulation of the immune response (1,2). Two type of Tregs are known: natural Treg and adaptive Treg. Normally these cells regulate immune system activity and prevent autoimmunity. Imbalanced function or number of these cells, either enhanced or decreased, might lead to tumor development and autoimmunity, respectively (1,2,3,4). In the present study we hypothesized the presence of CD25+ cells among tumor infiltrating lymphocytes (TILs) that are normally associated with feline injection-site sarcoma (FISS), a type of tumor considered a good model to study a link between inflammation and neoplasia. To this end the CD25 protein expression levels were analyzed by immunohistochemistry in eighteen cases of FISS.

4 μ m sections were immunostained by avidin-biotin technique for CD25 (Thermo Scientific, mouse monoclonal antibody). Staining was evaluated semi-quantitatively for percentage of positivity (10 fields at 40X) and the expression was considered as follows: percentage <20% = weak positivity; 21-50% = moderate positivity; >50% = strong positivity.

All the cases studied (18/18) had TILs positive for CD25. The immunolabeling appeared as distinct membrane staining or as diffuse cytoplasmic expression. 4 cases weakly expressed CD25 protein, 5 cases had moderate expression and 9 cases strongly expressed CD25. The medium expression percentage for all the 18 cases was of 55%.

The present study identified large proportions of CD25+ cells among tumor-infiltrating lymphocytes in FISS. The exact function and molecular mechanism by which CD25 is associated with carcinogenesis is still unclear. As observed in experimental animal model, CD25+ lymphocytes inhibit Th1 and CD8+ immune response and consequently they could represent a permissive and stimulatory factor for tumor development. The knowledge on their induction, activation and function opens the possibility for their selective in vivo manipulation as an attractive immunotherapeutic approach in a tumor such as FISS.

1) Sakaguchi S. Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu Rev Immunol* 2004; 22:531–562.

2) McHugh R.S., Whitters M.J., Piccirillo C.A., et al. CD4(+)CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity* 2002; 16:311–323.

3) Dieckmann D., Bruett C.H., Ploettner H., Lutz M.B., Schuler G. Human CD4(+)CD25(+) regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent type 1-like regulatory T cells. *J Exp Med* 2002; 196:247–253.

4) Nishikawa H., Sakaguchi S. Regulatory T cells in cancer immunotherapy. *Curr Opin Immunol* 2014; 27:1–7

VETERINARY ONCOLOGY

CD25, sarcoma, immunohistochemistry

CHARACTERIZATION OF A NOVEL AUTOPHAGIC VACUOLAR MYOPATHY IN MICE

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The aim of this study was to describe histopathological and molecular findings in a group of 50 crosses of C57/BL6 mice strain that shows many similarities with X-linked myopathy with excessive autophagy (X-MEA) in humans. X-MEA is a congenital lysosomal myopathy characterized by progressive vacuolation and atrophy of skeletal muscle. In humans, X-MEA has been associated to a VMA21 gene hypomorphic alleles (1,2), that encodes for an important constituent of V-ATPase proton pump complex. Vma21p deficiency impairs autophagic acidification and consequently its degradative functions.

Skeletal muscle biopsies from 50 mice crosses of C57/BL6 strain and 10 control mice of different strains were collected and snap frozen in liquid nitrogen. Cryosection were subjected to a panel of different histological and histoenzymatic stains; immunohistochemical (HRP method) or immunofluorescence detection of sarcolemmal (β spectrin, β dystroglycan, dystrophin) and autophagic (LAMP2, LC3, Beclin1, p62, BAG3) markers was carried out. Immunofluorescence analysis for Calsequestrin and RT-PCR for VMA21 mRNA were performed as well. Part of the samples was fixed in glutaraldehyde for ultrastructural examination.

Hematoxylin-eosin stained cryo-sections from only male C57/BL6 crosses mice showed marked increase in fiber size variation, and massive presence of large intracellular vacuoles containing basophilic material which was reddish at Engel's trichrome stain. The vacuoles were positive for Esterase and Alizarin Red stain. Immunostaining with antibodies directed against β spectrin, β dystroglycan, and dystrophin showed strong vacuolar membrane positivity. Some vacuoles were positive for the LAMP-2 lysosomal membrane, for autophagic markers LC3, Beclin1 and P62. Aggregates resulted negative at immunofluorescence staining for the tubular associated protein Calsequestrin, allowing us to rule out tubular myopathy. Immunofluorescence examination showed also a co-localization of LC3 and BAG3, suggesting that also chaperone-mediated autophagy could be implicated in the worsening of the myopathy, especially in oldest animals. At the ultrastructural investigation two types of vacuoles were found, corresponding to pre-autophagosomes and autophagolysosomes accumulations. Lastly, Q-RT-PCR analysis revealed a significant decrease of VMA21 mRNA rate in muscle tissue from old male mice compared to younger male mice and in controls from different strains.

Our data suggest that this mouse could be a novel spontaneous mouse model for X-MEA.

1-Nishino I. Lysosomal myopathies. Handbook of Clinical Neurology, 2007; 86: 205-214.

2-Ramachandran N, Munteanu I, Wang P, Ruggieri A, et al. VMA21 deficiency prevents vacuolar ATPase assembly and causes autophagic vacuolar myopathy. Acta Neuropathol. 2013, 125(3):439-57

Lab animal Veterinary Pathology
myopathy, autophagy, mouse

GENETIC AND PATHOLOGICAL FOLLOW-UP STUDY IN GOATS EXPERIMENTALLY AND NATURALLY EXPOSED TO A SHEEP SCRAPIE ISOLATE

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Scrapie is a slowly progressive and fatal neurodegenerative disease affecting sheep, goat and mouflon and is one of the family of transmissible spongiform encephalopathies (TSE)[1]. Transmission of scrapie from sheep to goat has extensively proved[2], however an adequate knowledge on the genetic and pathological aspect characterizing this interspecies transmission lacks.

Here we studied the dynamic of scrapie occurrence, with particular emphasis on genetic and phenotypical aspects, throughout 2 generations (F1 and F2) in a goat herd in which the parental generation was experimentally challenged with a natural sheep scrapie isolate by oral infection.

Thirty-two goats were orally infected with a scrapie brain homogenate from ARQ/ARQwildtype. The goats were mated to obtain 2 additional generations of offspring which were kept in the same environment to be naturally exposed to scrapie agent.

Clinical monitoring of the experimental group was performed daily. At necropsy from all these animals included in the experiment the brain, the spinal cord and the gut-associated lymphoid tissue (GALT) were also promptly collected. One half was fixed in formaldehyde for immunohistochemical (IHC) investigations, while the other one was frozen at -20°C for immunobiochemical analysis.

All the wildtype or G37V, Q168Q-P240P, and P/S240P goats had neurological signs indicative of scrapie after an incubation time ranging from 678 to 796 days, while goats with R154H, H154H, R211Q or P168Q-P240P dimorphisms became sick after a longer average incubation time (1,271 days). At 1,912 and 2,066 days post infection, the goats with D145D or Q222K dimorphisms were clinically healthy and without pathological prion protein in nervous and lymphoid tissues, respectively.

Interestingly, all but 2 of the wildtype, G37V and P/S240P goats belonging to the F1 offspring developed clinical scrapie at an average age of 746 days.

Our results demonstrate that 222K genetic variant is associated with scrapie resistance in goat even against scrapie of ovine source. Differently, 211Q and 154H did not provide a similar level of resistance as following infection with specie homologous isolates[3]. In addition, we observed for the first time a protective effect of the 145D goat variant against scrapie. Finally, our preliminary results demonstrate that immunohistochemical and molecular phenotypes of our natural ARQ/ARQwildtype scrapie ovine source was substantially preserved in goat carrying different susceptible PRNP variants.

1. Prusiner S.B. Science 1991, 252:1515-1522.

2. Foster J.D. et al. Vet Rec. 2001 Feb 10;148(6):165-71.

3. Lacroux C. et al. J Virol. 2014 Mar;88(5):2406-13.

PATHOLOGY

Scrapie, Goat, Genetic

ELLIS-VAN CREVELD (EVC-2) SYNDROME IN TYROLEAN GREY CATTLE: MORPHOLOGICAL STUDY OF A TYPE OF CHONDRODYSPLASTIC DWARFISM.

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Ellis-van Creveld (EVC2) syndrome has been first described in children and is a complex clinical syndrome that presents with short limbs, retarded growth, polydactyly, ectodermal and heart defects and ciliopathies. This syndrome is due to a mutation of EVC2 gene (1). The aim of this study was to describe the gross and histological features of bovine chondrodysplastic dwarfism (CD) with deletion of EVC2 gene.

Four calves (3 female and one male) aged 2 to 5 months, with a clinical diagnosis of CD, were subjected to necropsy. The same cases were included in a whole genomic re-sequencing study that confirmed the deletion of the EVC2 gene (2). Bones, ligaments, heart and genital tract were routinely processed. Sections were stained with H&E.

At necropsy, the limbs of all the subjects were disproportionately short and bulky, rotated and arched in a “dumbbell-like” position. The long bones were severely reduced in length, with a very short diaphysis. In the 3 female calves, despite the young age, the genital tract was fully developed. In one case endocardiosis of the atrioventricular valves was observed. Histologically, the growth plates were irregular and closed prematurely. The reserve zone was variably thickened at the expense of proliferative and hypertrophic zones. Chondrocytes in the latter zones were disorganized, had multifocal loss of normal columnar arrangement, and were haphazardly arranged individually or in nests. The metaphysis was reduced in length, and the trabeculae in the primary spongiosa were shortened. Ovaries had follicles and corpora lutea; ligaments occasionally had multifocal lymphoplasmacytic inflammation.

CD due to an autosomal recessive mutation of the Limbin gene was described for the first time in the Japanese brown breed (3). A genetic study on inherited chondrodysplasia due to EVC2 deletion was recently reported in Tyrolean grey cattle (2), and now we describe the pathological aspects of EVC2 in this breed. As in humans, where EVC2 involves multiple organs, one of our cases showed endocardiosis. No genital lesions have been reported so far in human EVC2. EVC2 syndrome in Tyrolean Grey cattle is characterized by CD, genital and heart defects and could be a useful model for human medicine.

1. Kamal et al., J Oral Maxillofac Pathol. 2013, 17:132-5.
2. Murgiano et al., Plos one 2014, In press
3. Takeda et al., Proc Natl Acad Sci U S A. 2002, 6;99:10549-54

ANATOMIA PATOLOGICA

Calf , EVC2 syndrome , chondrodysplasia

GROWTH PROMOTERS REGULATION OF REGUCALCIN GENE EXPRESSION IN SEX ACCESSORY GLANDS OF BEEF CATTLE

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Regucalcin (RGN) is a calcium (Ca²⁺)-binding protein regulating the intracellular Ca²⁺ homeostasis and the activity of several enzymes and proteins involved in intracellular signalling pathways (1). Recently, RGN was identified as a sex steroid-regulated gene in prostate and testis of rat and human (2, 3) and in sex accessory glands of veal calves (4).

This study investigated RGN expression in the sex accessory glands of beef cattle experimentally treated with growth promoters (GPs) to establish whether the RGN gene can be considered as a novel biomarker for the detection of GPs abuse in beef cattle.

In trial 1, 18 Charolaise beef cattle, 17-22 months old, were divided as follows: group A (n=6) treated weekly with 20 mg/animal of 17 β -estradiol for 5 weeks; group B (n=6) treated daily with 0.7 mg/animal of dexamethasone 21-phosphate disodium salt for 40 days; group K1 (n=6) was the control. The animals were euthanized six days after the last treatment. In trial 2, 24 Friesian beef cattle, 13-20 months old, were divided as follows: group C (n=8) was administered 200 mg of trenbolone acetate and 20 mg of 17 β -estradiol (Revalor-200[®]) as a subcutaneous implant for 89 days; group D (n=8) was administered 200 mg of trenbolone acetate (Finaplix[®]-H) as a subcutaneous implant for 89 days; group K2 (n=8) was the control. The implants remained in place until slaughtering. Samples of the testis, prostate and bulbo-urethral glands were collected from each animal and preserved for further analyses. Quantitative PCR (qPCR) of RGN mRNA, immunohistochemistry (IHC) and western blotting (WB) of RGN protein were performed. Statistical differences were determined by ANOVA, followed by Dunnett's post test.

In trial 1, high doses of 17 β -estradiol (group A) significantly down-regulated the RGN mRNA expression in testis (P<0.01), whereas no effect has been detected in bulbo-urethral glands and prostate. In trial 2, low doses of 17 β -estradiol in combination with trenbolone acetate (group C) significantly down-regulated RGN mRNA expression in prostate (P<0.05) and testis (P<0.05), whereas trenbolone acetate alone (group D) caused a decrease of RGN expression only in testis (P<0.05). The IHC and WB analyses confirm the results obtained in qPCR.

Contrary to data reported in rat (2) and veal calves (4), the estrogen regulation of RGN expression in prostate and bulbo-urethral glands of beef cattle was less pronounced, maybe due to beginning of puberty. On the other hand, testis response was similar to the data previously reported in veal calves (4).

1) Laurentino et al, 2012. Mol Hum Reprod 18 2) Maia et al, 2007. Mol Cell Biochem 311 3) Laurentino et al, 2011. Reproduction 142 4) Starvaggi Cucuzza et al, SISVet 2013

Patologia Animale

regucalcin, growth promotes, beef cattle

NECROPSY FINDINGS OF ANIMAL CRUELTY IN WATER BUFFALO CALVES

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Rearing of male water buffalo (Italian Mediterranean Buffalo) calves has never concerned farmers because of the lack of economic profits; in order to reduce the cost of management, male buffalo calves are usually sent to the slaughter-house too early and the animal welfare is too often a minor detail for the farmers.

The aim of this work is to describe the macroscopic and microscopic findings in young buffaloes died during transportation or after their arrive at the slaughterhouse and, contextually, provide the veterinarians an useful guide for monitoring animal welfare.

Fifty water buffalo calves, farmed in Campania region (Southern Italy), aged between 7 and 20 days underwent necropsy and samples from all the major organs (lung, liver, heart, thymus, kidney, spleen, adrenal glands, gut, abomasum and skeletal muscle) were collected for histopathological evaluation in order to determine the cause of death and check for the presence of lesions suggestive of animal cruelty and stress.

The cause of death was suggestive for respiratory or heart failure or neurogenic shock occurring for: severe enteritis (55%), acute pneumonia (30%) and trauma (15%). Moreover, in every cases stress induced lesions were observed such as: secondary thymic atrophy, serous fat atrophy, atrophy or hypertrophy of the zona fasciculata of the adrenal cortex^{1,2,3}. Severe muscle atrophy was observed in 30% percent of cases caused by malnutrition.

The crime of animal cruelty is underreported and often goes without investigation especially in farm animals. Necropsy is the unique valuable tool for defining the cause of death of animals and to highlight stress-induced lesions and cruelty⁴.

1 Pearse G. Histopathology of the thymus. *Toxicologic Pathology*, 34:515–547, 2006

2 Marcato P.S. *Patologia Sistemática Veterinaria*. Edagricole 2002

3 Koko V, Djordjeviae J, Cvijiaie G, Davidoviaie V. Effect of acute heat stress on rat adrenal glands: a morphological and stereological study. *J Exp Biol*. 2004 Nov;207(Pt 24):4225-30

4. Merck,M.D. (2007) *Veterinary Forensics. Animal Cruelty Investigations*. Blackwell

Veterinary Pathology and Animal Welfare
animal cruelty, necropsy, water buffalo

EXPRESSION OF AUTOPHAGY MARKERS IN AGED BOVINE BRAINS

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Autophagy is a highly regulated process involving the bulk degradation of cytoplasmic macromolecules and organelles in mammalian cells via the lysosomal system. The mechanisms for delivery of cargo to lysosomes label three different autophagic pathways: macroautophagy (herein referred as autophagy), microautophagy and chaperone mediated autophagy (CMA). Dysregulation of autophagy is implicated in the pathogenesis of many neurodegenerative diseases (Fields et al.) and integrity of the autophagosomal-lysosomal network appears to be critical in the progression of aging (Cuervo et al.). Here, we survey autophagy markers in aged bovine brains describing its possible role in neurodegeneration.

Samples of frontal cortex and hippocampus were collected from the brain of 20 aged cows (10-15 years old) and 5 normal controls (1-3 years old). Formalin-fixed and paraffin embedded 4µm sections were stained with Hematoxylin and Eosin and Periodic Acid-Schiff (PAS). Immunohistochemical stains were performed on selected sections using primary antibodies such as Beclin 1, LC3, LAMP-2, Ubiquitin and beta-amyloid 1-16. Antigen-antibody binding were detected by a horseradish-peroxidase (HRP) method and the slides were counterstained with hematoxylin.

We observed a mild to severe satellitosis, neuronal necrosis and a diffuse accumulation of PAS positive, granular deposits within neurons attributable to lipofuscin storage. An intraneuronal strongly positive immunoreaction was detected for Beclin-1, LC3, Ubiquitin and beta-amyloid 1-16 while immunoreaction for LAMP2 was weak or absent.

An increased expression of Beclin 1 and LC3 proves an excessive activation of autophagy and immunoreactivity for Ubiquitin indicates an intraneuronal accumulation of ubiquitinated proteins. A decrease of LAMP-2 expression suggests a primary defect in CMA activity that is related to the accumulation of pathogenic proteins such as beta-amyloid in the cytoplasm of the neurons (Rajawat et al.). Our data have showed an increase of autophagy in aged bovine brains that may be responsible for neurodegenerative conditions, including aging and neuronal death. Furthermore, progressive increase in intralysosomal concentration of lipofuscin may be responsible for a diminished efficiency of lysosomal degradation of proteins promoting their accumulation.

Jerel Fields et al., Age-dependent molecular alterations in the autophagy pathway in HIVE patients and in a gp120 tg mouse model: reversal with beclin-1 gene transfer, *J. Neurovirol.* (2013) 19:89–101

Cuervo et al, Impaired Degradation of Mutant alpha-Synuclein by Chaperone-Mediated Autophagy *Science* (2004) 305: 1292–5

Rajawat YS, Hilioti Z, Bossis I Aging: central role for autophagy and the lysosomal degradative system. *Ageing Res Rev* (2009) 8: 199–213

neuropatology

autophagy, aging, neurodegeneration

OXYTOCIN AND OXYTOCIN RECEPTOR EXPRESSION IN C2C12 MYOBLASTS TREATED WITH 17-BETA-ESTRADIOL: PRELIMINARY STUDY

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Sex steroid administration induces muscular hypertrophy by enhanced protein synthesis and satellite cells recruitment (Kadi 2008; Rhoads 2009). Recently, skeletal muscle has been identified as a secretory organ (Bertoluzzi et al., 2006) and our preliminary results confirm the hypothesis that skeletal muscle is able to synthesize and secrete oxytocin (Oxt) in cattle treated with 17-beta-estradiol (E2), inducing an increase of muscle mass (Divari et al., 2013). Moreover it is known that estrogen treatment induces murine myoblasts proliferation (Kahlert et al., 1997).

Aim of our study is to investigate the relationship between E2 treatment and Oxt pathway in murine C2C12 culture and to detect and quantify cell proliferation.

Murine C2C12 myoblasts were seeded in DMEM high-glucose medium supplemented with 10% FCS, 1% glutamine and 1% antibiotic-antimycotic. Twenty-four hours before the treatments, cells were starved in a serum-free medium. Then, 10-8M E2 was administered for further 24 hours (pretreatment). Finally, myoblasts were challenged with 10-8M E2, alone or in association with 10-5M oxytocin, for different short period of time: 30 min, 1 hour, 2 hours, 4 hours. Real-Time PCR to evaluate the expression of Oxt prepropeptide and its receptor (OxtR) and Western blot to confirm the relative protein expression were performed. Flow cytometry immunodetection of bromodeoxyuridine to detect and quantify cells proliferation on 24 h 10-8M E2 treated myoblasts was carried out.

In myoblasts treated 24h+1h, 24h+2h with E2, Oxt gene expression was significantly ($p < 0.01$) higher than in controls. A similar but slighter increase in Oxt expression was recorded in cells without pretreatment of E2. OxtR gene expression was strongly up-regulated after 24 hours of E2 treatment ($p = 0,0016$). The presence of Oxt in medium did not induce any gene regulation.

Proliferation assay did not show any significant difference between treated and untreated cultures.

Results show the role of E2 in gene regulation of C2C12 myoblasts, in particular on the oxytocin pathway. These data, obtained in a murine model, confirm previous results achieved in cattle treated with E2 (De Jager 2011, Divari 2013), but further investigations are needed to better understand the oxytocin regulation of distinct skeletal muscle processes.

Bertoluzzi et al. (2006). *Proteins* 62:776-792

De Jager et al. (2011). *Physiological Genomics*, 43, 467–78.

Divari, et al. (2013). *Food Chemistry*, 141, 4358–66.

Kadi F. (2008). *British Journal of Pharmacology*, 154, 522–8.

Kahlert, et al., (1997). *Biochemical and Biophysical Research Communications*, 232, 373–8.

Rhoads et al., (2009). *Domestic Animal Endocrinology*, 36, 111–26.

pathology

murine myoblasts, oxytocin, estrogen

NON-DIAGNOSTIC FINE NEEDLE ASPIRATION BIOPSY (FNAB) OF CUTANEOUS MASSES IN DOGS AND CATS**Forlani A.*^[1], Caruso M.^[1], Ghisleni G.^[1], Roccabianca P.^[1], Caniatti M.^[1]**^[1]*DIVET, Facoltà di Medicina Veterinaria, Università degli studi di Milano, Italy ~ Milano*

The management of cutaneous masses in which cytology is non-diagnostic remains controversial and studies addressing this problem seem not available. In this report we review the causes of non-diagnostic FNAB cytologic samples in a 12 year operational span.

Cytological records of all non-diagnostic FNAB from cutaneous masses in dogs and cats (January 2002-October 2013) were retrospectively reviewed. Only cases with concurrent histology (considered as the goldstandard) were included. Cytological diagnostic samples of 100 cutaneous masses served as controls. Signalment, site, size and type of lesion (neoplastic vs non-neoplastic and benign vs malignant in case of neoplasia) were included and the number of slides submitted per case was recorded. A chi-squared test was used and p values ≤ 0.05 were considered significant.

A total of 138 cases was included. On histology 19 were classified as inflammation, 95 as neoplastic and 24 were non-neoplastic/non-inflammatory lesions. Non-diagnostic results were associated with the exclusive presence of erythrocytes (96 cases), by poor cellularity (39 cases) and by artifacts (3 cases). Site of lesions was trunk (52 cases), limbs (38 cases), head and neck (33 cases), mammary gland (14 cases) and non-specified in 1 case. Although not statistically significant, lesions on the head provided more non-diagnostic samples. Size of lesions was less than 2 cm in 39 cases, between 2-5 cm in 33 cases and above 5 cm in 19 cases. Lesions larger than 5 cm were significantly less diagnostic. Cytology was a good technique for round cell tumor diagnosis whereas, mesenchymal tumors were a frequent cause of a non-diagnostic results. Cytology diagnosed more easily benign than malignant neoplasms when lipomas were included. Compared to the control group, the number of slides obtained was higher in inconclusive cases for the category "4-6 slides".

Size and type of tumor were statistically correlated with reduced diagnostic power of FNAB cytology. Tumor type (mesenchymal versus epithelial or round) confirmed to have a role in the adequacy of samples.¹ The larger size of lesions typical of mesenchymal tumors and their poor cell yield represented one of the major causes of non-diagnostic results. Lesions on the head may provide more non-diagnostic cytology compared to other sites probably due to the patient compliance. Blood contamination is a common cause of non-diagnostic samples since poorly cellular samples not contaminated by blood can be easily esteemed grossly. The higher number of slides collected in non-diagnostic cases suggests that the operator is aware of the poor quality of the sampling. Despite the limitations of this retrospective study, the evaluation of non-diagnostic cytological samples may provide valuable information and improve the understanding of the limitations of diagnostic cytology.

1- Raskin RE. The acquisition and management of cytology specimens. In: Canine and feline cytology – A Color Atlas and Interpretation Guide. Raskin RE, Meyer DJ. 2nd Elsevier Saunders, St Louis, Missouri pp 1-14, 2010.

CITOLOGIA

Cytology, non-diagnostic FNAB, Skin

TP53 EXPRESSION AND ANALYSIS OF SINGLE NUCLEOTIDE POLYMORPHISM (SNPS) IN FELINE SQUAMOUS CARCINOMAS

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Feline cutaneous squamous cell carcinoma (FC-SCC) is the most common skin malignant tumour in cats (15–50% of all skin neoplasms) and the white-coated cats have a 5–13 times higher incidence. Development of FC-SCC has been associated with chronic sunlight exposure and TP53 protein represents one of the most important tumor suppressor over-expressed and mutated in response to UV light exposure. In FC-SCC TP53 expression has been demonstrated but mutation analysis in hot spot of TP53 gene have not been investigated yet. The aim of the present study was to evaluate the expression and presence of mutations of TP53 in FC-SCC.

Immunohistochemistry against feline TP53 was performed on formalin-fixed paraffin-embedded FC-SCC from 25 white-coated domestic shorthair. IHC expression score was evaluated considering the percentage of positive nuclei in keratinocytes as follows: 0 ($\leq 10\%$), 1+ ($>10-30\%$), 2+ ($>30-60\%$), 3+ ($>60\%$). Eight samples overexpressing TP53 (score +3) were submitted to genomic DNA extraction and mutation analysis by direct sequencing was performed from exon 4 to exon 8 of feline TP53 gene.

Immunohistochemical results for TP53 showed that 16 FC-SCC (64%) were positive (32% score +3, 16% score +2 and 16% score +1) while 9 cases (36%) were negative. DNA sequencing analysis revealed two Single Nucleotide Polymorphism (SNP) in the intron 8 in 6 samples and a missense mutation (C>T) in the exon 5 in one sample codifying for aminoacid tyrosine

In this study we found that TP53 is expressed in 64% of FC-SCC and this expression can be correlated to UV injury since TP53 is one of the first tumor suppressor activated by UV. The presence of SNPs in intron 8 has been already described as a feline genotypic variation and cannot be considered correlated to the disease. The identification of a missense mutation in exon 5 has not been previously reported in FC-SCC. If confirmed in a wider number of cases, the C>T nucleotide change (substitution UV-dependent) may indicate a possible role of UV in causing this genomic injury in FC-SCC.

- J Feline Med Surg. 2010 Oct;12(10):760-8. doi: 10.1016/j.jfms.2010.04.010. Epub 2010 Jul 31. Epidermal growth factor receptor expression is predictive of poor prognosis in feline cutaneous squamous cell carcinoma. Sabattini S1, Marconato L, Zoff A, Morini M, Scarpa F, Capitani O, Bettini G.

- J Radiat Res. 2011;52(2):115-25. Review The mechanisms of UV mutagenesis. Ikehata H, Ono T

anatomia patologica - oncologia

TP53, feline SCC, oncology

SOX9: IMMUNOHISTOCHEMICAL STUDY OF NORMAL AND NEOPLASTIC CANINE SERTOLI CELLS**Banco B.*^[1], Sironi G.^[1], Martignoni B.^[1], Giudice C.^[1], Grieco V.^[1]**^[1]*Dipartimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di Milano ~ Milano*

SOX9 protein plays a pivotal role for male sexual development. Among its numerous functions, it regulates the transcription of the anti-Müllerian hormone (AMH) gene and interacts with other genes to promote the development of testis cords, the multiplication and maturation of Sertoli cells (SCs) and the maintenance of spermatogenesis in the adult testis. The expression of SOX9 in normal testes has been demonstrated in humans, mice and rats. In canine species SOX9 expression in normal SCs has never been investigated and no data are available about neoplastic canine SCs (Sertoli Cell Tumours, SCTs). The present study aimed to investigate the expression of SOX9 in canine SCs during testicular maturation and neoplastic transformation.

Testicular samples derived from 1 foetus, 4 newborns, 2 prepuberal puppies, 3 adult dogs, 28 SCTs (1 of them metastasizing) and 3 Leydig cell tumors (LCTs) were selected from the archive and tested immunohistochemically with a polyclonal antibody against SOX9 (1:150).

Histologically, 18/28 SCTs were typical SCTs, 10/28 were classified as “lipid rich” SCTs, and 6/28, including the metastasizing one, characterized by capsular invasion, solid growth, severe anisocytosis and anisocytosis, large areas of necrosis and hemorrhages were considered undifferentiated SCTs.

Immunohistochemically, all SCs from foetal, neonatal and adult testes had a strong, diffuse and exclusively nuclear labelling for SOX9. In prepuberal testes, SOX9 stained exclusively SCs nucleus in one puppy and both nucleus and cytoplasm in the other one. Leydig cells (LCs) were constantly negative in all samples. Similarly negative were all the LCTs.

Concerning the 28 Sertoli cell tumors, 2 were negative for SOX9 and were reclassified as LCTs, while in the remaining 26 SCTs, SOX9 was diffusely expressed confirming the diagnosis of SCTs. In these tumours, the expression of SOX 9 was nuclear, nuclear and cytoplasmic or exclusively cytoplasmic in 14/26, 10/26 and 2/26 SCTs respectively. Moreover, all the undifferentiated SCTs and “lipid rich” cases were characterized by less intense staining.

This is the first report on immunohistochemical SOX9 expression in canine testes and demonstrates that in normal SCs from foetal, neonatal and adult testes, SOX9 labelled the nucleus as in human species. On the other hand, the cytoplasmic labelling observed in one puppy might parallel with prepuberal rat testes staining. Considering this datum, the cytoplasmic expression of SOX9 described in numerous canine SCTs could reflect cellular immaturity/dedifferentiation. In addition, the expression of SOX9 in SCTs and its absence in LCTs suggests that SOX9 is a reliable marker in the differential diagnosis between canine SCTs and LCTs.

Capel B (2000) “The battle of the sexes”. *Mechanism of Development*, 92: 89-103.

Jiang T., Hou C-C., She Z-Y., Yang W-X (2013) “The SOX gene family: function and regulation in testis determination and male fertility maintenance”. *Molecular Biology reports*, 40:2187-2194.

Lan K-C., Chen Y-T., Chang C., Chang Y-C., Hsin-Jung Lin H-J., Huang K-E., Kang H-Y (2013) “Up-Regulation of SOX9 in Sertoli Cells from Testiculopathic Patients Accounts for Increasing Anti-Mullerian Hormone Expression via Impaired Androgen Receptor Signaling”. *PLoS*, 10: 1-11.

Pelliniemi L.J., Fröjdman K (2001) “Structural and Regulatory Macromolecules in Sex Differentiation of

Gonads". *Journal of Experimental Zoology*, 290: 523–528.

Canine testes- oncology- pathology
canine Sertoli cells, immunohistochemistry, SOX9

GENETIC HETEROGENEITY OF CANINE DLBCL BY OLIGONUCLEOTIDE ARRAY CGH

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The role of genomic alterations (GA) at high resolution by oligonucleotide Array CGH (oACGH) in Diffuse Large B-cell Lymphoma (DLBCL) has been scarcely investigated in dogs. The first aim of this work was to analyse canine DLBCL in order to find genomic regions or even gene-specific GA at a higher resolution than conventional cytogenetic. We also compared genomic imbalances by oACGH in a reduced number of biopsy specimens of DLBCL at diagnosis and at their clonally related relapses.

We analysed 12 newly-diagnosed multicentric DLBCLs using an 180,000 oACGH on lymph nodes (LN) samples. oACGH was also repeated in LNs of 3 relapsing dogs and 4 dogs in remission after chemotherapy. All LNs were matched with corresponding skin biopsies. Recurrent aberrations were defined as gains or losses occurred at a frequency greater than 30%. Data analysis was performed using bioinformatic resources: Ensemble Genome Browser, Functional Annotation Tool Database for Annotation, Visualization, and Integrated Discovery (DAVID) Bioinformatics Resources and CGHTools.

In pre-treatment DLBCLs, the pattern of GA consisted of 90 different genomic imbalances (mean per tumour, 17), 46 gains and 44 losses. Two gains in Chr13 were significantly correlated to stage III-IV of the disease ($p=0.002$). Statistical analysis identified gains ($n=6$) and losses ($n=8$) significantly associated with time of remission ($FDR<0.001$). Functional annotation obtained by DAVID showed enriched pathways related to nucleotide biosynthesis, ascorbate and aldarate metabolism considering gained regions; in loss intervals immune response was the most enriched pathway. In the LNs of 4 dogs in remission after chemotherapy, 4 new GA were found, whereas 3 new GA were observed in relapsing dogs, compared with the sample at diagnosis.

In pre-treatment DLBCLs, individual variability in the number of GA was found, however 14 recurrent aberrations were identified. Recurrent losses involving IGK, IGL and IGH were observed in all tumours and in 2 dogs in clinical remission. The recurrent gains along the length of Chr13 were found in more than 50% of cases and were associated with a longer time of remission. In these segments, some genes significantly involved in several human and canine tumours, such as MYC, LDHB, HSF1, KIT and PDGFRA, were annotated. The recurrent gains along the length of Chr31 (>41% of cases) were associated with a shorter time of remission. One ex novo GA, involving TCR, was present in dogs in remission after chemotherapy. A reduced number of chromosomal rearrangements were found in relapsed ($n=17$) DLBCLs when compared with pre-treatment DLBCLs ($n=90$). To our knowledge this is the first time where regions of GA are associated with response to therapy and outcome of lymphoma in dogs. Further studies are needed with a larger number of cases and to identify correlations with gene expression and protein transduction.

oncologia

DLBCL, Dog, Oligo Array CGH

HYSTOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF FELINE RENAL CELL CARCINOMA: A CASE SERIES

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Renal cell carcinomas (RCCs) are rare neoplasms that occur mainly in middle-aged to old cats. To the authors' knowledge few studies are focused on the morphology, however a immunophenotypical characterization of RCC has never been performed.

In this study, 4 cases of RCCs were retrieved from the archive of the Histopathology Service of the Dept. of Comparative Biomedicine and Food Science in a spanning period of 9 years. Tumours were obtained by nephrectomy. Specimens were classified by predominant histological pattern according to WHO criteria. The presence or absence of necrosis, nuclear pleomorphism and mitotic index (MI) were also noted. Furthermore, a panel of antibodies including cytokeratin (CK), vimentin (VIM), E-cadherin (E-CAD), β -catenin (β -CAT), CD10 and c-KIT was selected to characterize the tumors. The angiogenic activity was analysed by VEGF and VEGF-R2. A semiquantitative analysis was assessed for all antibodies. Normal renal tissue was used as control.

The cats were all male, 3 domestic shorthair and 1 Persian, averaging 10.5 years in age. The tumors were classified as tubular RCCs (3) and papillary RCC (1). The MI was <1 per HPF associated with a slight nuclear pleomorphism.

Epithelial cells (ECs) in the tumors were CK, VIM, E-CAD, VEGF-R2 positive and c-KIT negative; 3 cases were β -CAT positive whereas only 2 tumors were CD10 and VEGF positive. All the markers were detected both at cytoplasmatic and membranous level, except for VEGF, VEGF-R2 and VIM that were only cytoplasmatic.

In humans, CK and VIM co-expression is used to confirm the diagnosis of RCC. Despite the low number of cases, feline RCCs seem to have a similar profile for CK and VIM. This observation might be helpful to exclude possible form of carcinoma metastasis in kidney and the panel of the two antibodies included in a routine diagnostic assay for suspects of RCCs. The absence of c-KIT expression in ECs suggests that this tyrosine kinase receptor is not involved in the pathogenesis of feline RCCs. This data is different from previous results in human and canine RCCs where specific histotypes are associated with c-KIT expression.

In human, E-CAD and CD10 are useful markers to differentiate distal or proximal origin of RCCs. E-CAD expression in ECs associated to absence of CD10 immunoreactivity suggests the distal origin in two tumors in our study. So far, the double positive expression of both markers in ECs might be related either proximal or distal tubular origin in the other 2 tumors, but aberrant expressions cannot be rule out.

VEGF-R2 expression in the neoplastic ECs didn't differ from control ECs whereas VEGF results might be compatible with a variable angiogenic activity intrinsic to the tumour.

Our results confirm the low frequency of RCCs in cats and also reveal a peculiar immunohistochemical profile different from the canine RCCs.

oncologia

cat, immunohistochemistry, renal cell carcinoma

AN IMMUNOHISTOCHEMICAL STUDY OF THE PTEN/AKT PATHWAY INVOLVEMENT IN CANINE AND FELINE MAMMARY TUMOURS

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The PTEN/AKT pathway is strongly involved in cell metabolism, proliferation and survival. Its dysregulation is implicated in several human cancers. The aim of this study was to investigate the role of this pathway in canine (CMTs) and feline mammary tumors (FMTs) by immunohistochemistry (IHC).

Fifty CMTs (10 adenomas and 40 carcinomas) and 30 FMTs (30 carcinomas) were submitted to IHC to evaluate PTEN, phospho-AKT and Rictor expression.

All the canine adenomas (100%), 25 of 40 (63%) canine carcinomas, and 7 of 30 (23%) FMTs were PTEN-positive. In dogs, no adenomas and 15 of 25 (37%) carcinomas expressed phospho-AKT, while 24 of 30 FMTs (82%) were phospho-AKT-positive. One of 10 (10%) canine adenomas (100%), 24 of 40 (60%) canine carcinomas, and 20 of 30 (67%) FMTs were Rictor-positive. In dogs, PTEN correlated with complex carcinomas, lower mitotic index, absence of lymphatic invasion and longer survival; phospho-AKT with simple carcinomas, lymphatic invasion and poorer survival and Rictor with lymphatic invasion. In cats, PTEN correlated with tubulopapillary carcinomas, lower mitotic index, absence of lymphatic invasion and better survival, while phospho-AKT and Rictor with poorer survival. In CMTs, phospho-AKT inversely correlated with PTEN expression and positively with Rictor. In FMTs, PTEN inversely correlated with phospho-AKT and Rictor while phospho-AKT positively correlated with Rictor expression.

Our data show a strong PTEN/AKT pathway involvement in behavior worsening of CMT and FMTs. This data could provide a rationale for further studies of this system in veterinary oncology to due prognostic and therapeutic implications.

Jiang BH, Liu LZ. PI3K/PTEN signaling in angiogenesis and tumorigenesis. *Adv Cancer Res.* 2009;102:19-65.

Maniscalco L, Iussich S, de Las Mulas JM, et al. Activation of AKT in feline mammary carcinoma: a new prognostic factor for feline mammary tumours. *Vet J.* 2012;191(1):65-71.

Ressel L, Millanta F, Caleri E, Innocenti VM, Poli A. Reduced PTEN protein expression and its prognostic implications in canine and feline mammary tumors. *Vet Pathol.* 2009;46(5):860-868.

Patologia veterinaria/Oncologia comparata

Dog and cat, Mammary tumours, PTEN/AKT pathway

EXPRESSION OF BECLIN-1 IN CANINE MAMMARY TUMOURS. PRELIMINARY INVESTIGATIONS

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Autophagy is a self-catabolic process consisting in the degradation of intracellular structures and organelles by lysosomal enzymes and it is involved in development, homeostasis, and cell survival. Its dysfunction is related to several pathologic processes, such as infections, metabolic disorders, neurodegeneration, and tumorigenesis. Autophagy is a multistep process characterized by nucleation, elongation, and autolysosome formation and it is carried out by a group of proteins called autophagy-related proteins (Atg). Among these, Beclin-1 is required for the nucleation of the phagophore and maturation of the autolysosome representing key moments in the autophagy process; disruption of this protein has been associated with cancerogenesis in many human neoplasms. Since relatively little is known in veterinary medicine on the subject the aim of this study was to evaluate by immunohistochemistry the Beclin-1 expression in normal and neoplastic canine mammary glands and to correlate the results with the histopathologic features of these tissues.

5 normal and 25 neoplastic canine mammary glands were examined. Neoplastic samples were classified according to WHO criteria and graded into grades I to III tumours, applying Elston Ellis parameters, immunohistochemistry was performed by using rabbit polyclonal human anti-Beclin 1 antibody (Santa Cruz Biotechnology); immunoreactivity was evaluated by counting immunostained cells

In normal mammary glands strong cytoplasmic expression of Beclin 1 in many epithelial ductal cells was observed. In malignant tumours Beclin-1 expression was significantly weaker than in normal mammary glands and the number of immunolabelled neoplastic cells was decreased. Low expression was associated with all parameters of malignancy such as lower degree tubule formation, nuclear pleomorphism, number of mitoses and presence of necrosis. In contrast to results previously reported by others¹, we did not observe Beclin-1 nuclear positivity in any canine mammary gland sample, neither in normal tissues nor in malignant tumours

The decrease of Beclin-1 expression in malignant tumours and its correlation with all parameters of malignancy, supports the hypothesis that Beclin-1 functions as a tumor suppressor protein and its disruption may be involved in neoplastic transformation and progression. This suggests a potential involvement of dysfunction or suppression of autophagy in canine mammary gland cancerogenesis.

¹Liu J-L. et al. BMC Veterinary Research 9:75-84, 2013.

VETERINARY ONCOLOGY

AUTOPHAGY, BECLIN1, MAMMARY TUMOURS

PAPILLARY MENINGIOMA IN THE DOG: A CLINICOPATHOLOGICAL CASE SERIES STUDY.

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Papillary meningioma (PM) is considered one of the most aggressive variants of meningioma in humans and classified as grade III by WHO classification system. To date, the biological behavior of this tumour is still unclear in dogs. In this study we investigated the clinicopathological correlations of canine PMs in order to define if PM might be considered of grade III in dogs as in humans, despite its benign histological pattern. To provide more information regarding the biological behavior of this meningioma subtype in dogs, we also investigated doublecortin (DCX), E-cadherin, and N-cadherin expression by IHC.

FFPE tissue from 16 canine PMs, obtained by surgery and necropsy, was included in the present retrospective study. Clinical and follow up data were obtained from medical records. 5 µm sections of the selected PMs were stained with H&E, and the tumors were graded according to the criteria of the human WHO international histological classification of CNS tumors. To investigate the expression of DCX, E-cadherin and N-cadherin, additional 4 µm sections were used for IHC, performed with avidin-biotin-peroxidase complex method (ABC, Dako, Milan, Italy).

PMs accounted for 18% of meningiomas archived in our lab. PM tended to occur in animals older than 7 years with a male/female ratio of 1.7, and the supratentorial compartment was the most common affected site. Based on human WHO classification system, seven tumors (43,8%) and nine tumors (56,2%) were classified as grade I and as grade II, respectively. Grade III was not identified. As for surgical cases recurrence was observed in 87.5% and the mean survival time was 10.8 months. Five recurrent surgical meningiomas showed necrosis up to 50% of the tumor. In the non-surgical cases a mean survival time of 24 days was observed. The IHC results were not related neither with histological grade of malignancy, nor clinical behavior. However, an apparent negative correlation between E-cadherin and N-cadherin expression was found in tumors showing a low survival time.

Despite benign histological findings, we confirmed the aggressive behavior of PM also in dogs. This was especially true for untreated animals that showed a significantly lower survival time if compared with that reported for canine meningiomas (Bilderback et al., 2006). As for surgical cases, the post-surgery survival time was higher than that observed in canine meningioma (Axlund et al., 2002), probably due to the improvement of surgical techniques occurred in the last ten years. As in humans, the biological malignancy of canine PM seems to be correlated to intratumoral necrosis more than effective brain invasion. Although the absence of conclusive data, we might suppose that a "cadherin-switch" is involved in the biological progression of canine PM.

Bilderback A, Faissler D, Sato AF, et al. (2006) Transfrontal craniectomy, radiation therapy, and/or chemotherapy in the treatment of canine meningiomas. Proc 24th ACVIM;

Axlund TW, McGlasson ML, Smith AN (2002) Surgery alone or in combination with radiation therapy for treatment of intracranial meningiomas in dogs: 31 cases (1989-2002). J Am Vet Med Assoc 221:1597-600

Oncologia

Papillary meningioma, Biological behaviour, Dog

A BIOCOMPATIBLE SYSTEM AS CARRIER OF ANTINEOPLASTIC DRUGS IN GLIOBLASTOMA TREATMENT: PRELIMINARY DATA

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Aim of the present study is to report the in vitro and in vivo effects of Solid Lipid Nanoparticles (SLN), free drug and SLN-loaded drug in glioblastoma treatment. SLN are biocompatible systems made of physiological lipids proposed to be used as antineoplastic drug carriers (Battaglia et al., 2010, 2012). The difficulty of classical cytotoxic drugs to overcome the blood brain barrier (BBB), which have high in vitro efficacy against glioblastoma, is a major limitation to drug therapy. Anticancer drug delivery through SLN to the brain parenchyma for the treatment of glioblastoma may represent a valid strategy: aiming to overcome the BBB, SLN should be modified on the surface and functionalised with ligands to the receptors, variously expressed on endothelial cells, according to central nervous system metabolic requirements. Different lipophilic and hydrophilic antineoplastic drugs can be entrapped in SLN.

Blank and Paclitaxel-loaded SLN were tested. Glioblastoma cell lines were used to test the effects in vitro. The cytotoxicity's evaluation of drug-loaded SLN was performed by Trypan blue exclusion test and MTT method at different times and concentrations. Ultrastructural investigations were also made.

In vivo biocompatibility was evaluated on male Wistar rats (n = 8) after i.v. administration of blank and Paclitaxel-loaded SLN. Rats were sacrificed and submitted to a complete necropsy, in order to evaluate signs of toxicity in different organs. Liver, kidney, heart, lung and brain samples were collected to perform histological investigations. Selected tissue portions were paraffin embedded, sectioned at 3µm and stained with haematoxylin and eosin, Masson's trichrome and Sudan stains.

In vitro Paclitaxel loaded SLN produced a significant cell death on cell lines similar to or slightly increased in comparison with the drug alone. Ultrastructurally the main lesions were vacuoles in mitochondria, lysis and glycogen accumulation.

In vivo rats treated with blank SLN didn't show signs of systemic toxicity. In rats treated with Paclitaxel loaded SLN not significant lesions were detected in liver, kidney, heart and brain. Pulmonary granulomatous and adaptative changes, whose aetiopathogenetic mechanism remains to be clarified, were observed.

Despite the encouraging data regarding the in vitro cytological studies, further in vivo biodistribution studies on male Wistar rats are needed to confirm the suitability of SLN as drug delivery systems to the brain.

Further investigations will lead to better understand the mechanism of the pulmonary reported changes associated with the administration of SLN loaded paclitaxel drug.

Battaglia L e Gallarate M 2012 Expert Opinion on Drug Delivery 2012, 9(5): 497-508

Battaglia L et al., 2010. J Microencaps 27, 78–85

Neuropathology

Glioblastoma, Animal model, Nanoparticles

CONTRIBUTION OF CELL MARKERS TO THE STUDY OF RMS PATHOGENESIS

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Red mark syndrome (RMS) is a chronic non-lethal skin disease affecting farmed rainbow trout (*O. mykiss*) in U.K., Austria, Germany, Italy (Galeotti et al., 2011), Serbia and U.S.A. Histology shows a lymphocyte/macrophage infiltration in scale pockets, dermis and ipodermis. Aiming at the full comprehension of RMS aetio-pathogenesis, we focused on the mechanisms of cell recruitment/activation in the skin lesions, in order to elucidate if and how an hypothetical microbial agent might trigger the host inflammatory response.

Samples of skin from infected fish were evaluated by histology, immunohistochemistry and electron microscopy. The following markers were used: rabbit to human CD3 (A-0452, Dako); rabbit to rainbow trout IgT/IgM (Prof. Sunyer); rabbit to salmonid HSP70 (AS05061A, Agrisera); rabbit to human GM-CSFR α (sc-690, Santa Cruz Biotech.); mouse to PCNA (2586, Cell Signaling Technology); mouse to AE1/AE3 Cytokeratin (M3515, Dako); mouse to E Cadherin (M3612, Dako).

Anti trout IgT and IgM marked a limited number of scattered cells in the dermis and ipodermis. Anti CD3 marked a relevant number of cells composing the skin infiltrate. HSP70 marked monocyte/macrophages, dendritic like-cells and endothelial cells, within the scale pockets involved by inflammation. GM-CSFR α positive monocyte-macrophage were scattered in the derma, surrounding the scales. Anti-Cytokeratin and E Cadherin marked the epithelial cells. PCNA positive cells have been detected in epidermis, dermis and hypodermis, as well among infiltrating lymphocytes, stromal fibroblasts and vascular endothelial cells.

HSP70 are considered not only as acute phase proteins, but also as molecules able to mediate immunity and inflammation (Pockley, 2003); they can be released by several cell populations in response to various stimuli. Briefly HSP70 could act as an “antigen” inducing a severe T lymphocyte response, leading to an auto-immune like reaction. Dendritic cells and APCs are stimulated by HSP70 to release TNF- α , IL1- β and GM-csf. We might speculate that a microbial agent promotes HSP70 expression by macrophages/endothelial cells, within scale pockets. HSP70 might be also internalized by skin APCs. The pro-inflammatory cytokines released could then trigger the local inflammatory process. The GM-csf stimulates the development of osteoclasts. Skin APCs could express HSP70 and therefore stimulate T cell proliferation. These findings might justify the severe cell infiltration detectable in the skin of RMS affected fish.

Pockley, 2003. The Lancet, 362.

Galeotti et al., 2011. Proceedings 15th EAAP Conference, Split, Croatia.

ANATOMIA PATOLOGICA

Red mark syndrome, rainbow trout, cell markers

AN OUTBREAK OF EQUINE GRASS SICKNESS IN ITALY

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Equine grass sickness (EGS) is a debilitating, often fatal, neurodegenerative disease, that affects almost exclusively grazing horses (1,2). As the name suggests, EGS has a strong association with grazing, with only a few cases reported with no exposure to pasture (1). The precise aetiology of the disease is unknown, although several causes and risk factors have been proposed. Clinical signs result from neurogenic obstruction of the alimentary tract due to neuronal degeneration in the autonomic and enteric nervous systems (1,3). Although cases of EGS have been occasionally described in other countries, the disease is almost exclusively reported in Great Britain (1). The current study describes an outbreak of EGS in Italy, including gross, histological, bacteriological and immunohistochemical findings.

The outbreak occurred in a horse farm, located in the North of Italy, with approximately 20 horses living on the pasture. Most of them showed severe weight loss, progressive weakness, anorexia, poor body condition, decreased appetite, and dysphagia.

Three horses were found dead and submitted for post-mortem examination. The horses were in extremely poor body condition with severe diffuse muscular atrophy and moderate ventral oedema. All the three horses had distension of the stomach and small intestine with impaction of the large colon and caecum and hard dry faeces in colon and rectum. In addition, all displayed severe diffuse oesophageal muscular hypertrophy, mainly in the distal third of the organ. Histological findings were restricted to intestinal plexuses and ganglia that revealed marked neuronal degeneration, neuronal vacuolations, and decreased number of neurons. Immunohistochemistry demonstrated intracytoplasmic accumulation of synaptophysin within neurons of the enteric plexuses and cervical and coeliac ganglia. Bacteriology from several organs and enteric content was negative. Grass clippings, gastrointestinal contents and faeces were negative for *C. botulinum* toxins.

Gross, histological and immunohistochemical examinations were suggestive of a chronic form of EGS. Equine grass sickness is an extremely rare condition in Italy, however it should be considered as a differential diagnosis for horses with severe weight loss and anorexia.

1. Pirie RS, Jago RC, Hudson NPH. Equine grass sickness. *Equine Vet J.* 2014. [Epub ahead of print]
2. Wylie CE, Proudman CJ, McGorum BC, Newton JR. A nationwide surveillance scheme for equine grass sickness in Great Britain: Results for the period 2000-2009. *Equine Vet J.* 2011;43:571-579.
3. Maxie MG, Youssef S. Chapter 3: Nervous system. In: Maxie MG, ed. Jubb, Kennedy, Palmer's Pathology of Domestic Animals. Vol 1. 5th ed. St Louis: Mosby Elsevier, 2007;281-457.

Patologia equina

Grass sickness, ganglia, intestinal plexuses

STUDY OF THE PTX3 ACTIVITY IN THE PULMONARY INFLAMMATORY RESPONSE IN A MURINE MODEL

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Innate immunity plays key roles in activation and orientation of adaptive immunity and consists of a cellular and a humoral arm. The humoral arm is composed by a heterogeneous collection of weird molecules that represent functional ancestors of antibodies and form an integrated system of diverse molecules, including collectins, ficolins, and pentraxins (1). Pentraxins constitute a superfamily of multifunctional multimeric proteins and Pentraxin-3 (PTX3) is the first member of the long pentraxin subfamily. It is released from dendritic cells, mononuclear phagocytes, fibroblasts, endothelial and epithelial cells upon exposure to inflammatory signals such as cytokines (e.g. IL-1 β , TNF- α), TLR agonists, microbial moieties (e.g. LPS, OmpA) or microorganisms (1,2). PTX3 acts as an opsonin, binding the bacteria to facilitate their phagocytosis by the DCs and macrophages. On these bases, we investigated the role of PTX3 during a pulmonary infection by *Shigella flexneri*, in mouse.

For this purpose, C57Bl/6 mice were inoculated intranasally with 20 μ l of 0.9% NaCl suspensions containing 3×10^8 CFU of the strain. Control mice were similarly inoculated with 20 μ l of PBS. At the same time, PTX3 were administered via ip route once per day for three consecutive days following challenge. We monitored animals for mortality and clinical signs. After 72 h post-infection, mice were euthanatized and lungs and BALF were collected and processed for histopathological studies, and macrophage analysis. We analyzed the inflammatory response and tissue damage in lungs through histological evaluation and immunohistochemistry analysis. In order to characterize the cell-mediated response in T-cell population, tissue were tested with different primary antibody by immunohistochemistry staining. Moreover we performed in vitro macrophage activity assays, following primary culture by BALF, evaluating phagocytosis and respiratory burst.

The intranasal infection resulted in bacterial invasion of bronchial and alveolar epithelia with concomitant development of acute suppurative bronchiolitis and lethal pneumonia. Infected mice lungs showed acute bronchiolitis with diffuse alveolar damage, exuberant neutrophilic exudate and peribronchiolar and interstitial inflammatory infiltrate. Mice treated with PTX3 showed particularly activation of BALT and increased macrophage activity.

Our results show that in the mouse, PTX3 modulates the inflammatory response by reducing the acute phase, stimulating activation of the BALT and then leading a cell-mediated response.

1- Bottazzi et al., 2009.

2- Garlanda et al. 2005.

general pathology e immunopathology

PTX3, Pneumonitis, Immunomodulation

THE ROLE OF GROWTH FACTORS IN CANINE X-LINKED HEREDITARY NEPHROPATHY: AN ANIMAL MODEL OF PROGRESSIVE RENAL FAILURE

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To investigate the role of several growth factors involved in tubulointerstitial (TI) damage progression, extracellular matrix remodeling and Epithelial Mesenchymal Transition in a dog model of progressive renal failure.

10 male dogs with X-Linked Hereditary Nephropathy (XLHN) and 5 unaffected male littermates (controls) were studied. Clinical data and renal samples were obtained at 4 (T0), 6 (T1), 9 (T2) months of age and at necropsy (T3) performed at a standardized clinical endpoint. Glomerular and TI lesions were scored by light microscopy, and 21 genes known to be involved in TI damage were selected and investigated by real-time quantitative reverse transcription PCR. Morphologic, clinic pathologic and molecular data were analyzed with GLM procedure (SAS institute).

XLHN dogs had proteinuria at all time-points; controls had none. No histological lesions were identified in controls at any time-point or in XLHN dogs at T0. At T1, XLHN dogs had a significant ($p < 0.05$) increase of mesangial cellularity and matrix deposition, cystic glomerular atrophy (CGA), tubular necrosis and interstitial fibrosis. At T2, up to 88% of glomeruli were nonfunctional and CGA was prevalent (50%). Multifocal TI inflammation, fibrosis, tubular atrophy and dilation were present. At T3, CGA was even more prevalent (60% of all glomeruli), and TI inflammation and fibrosis were severe.

Glomerular obsolescence, CGA, mesangial hypercellularity, matrix increase, fibrosis, tubular dilation-atrophy, and inflammation were positively correlated ($p < 0.01$).

At T0, Transforming Growth Factor- β (TGF- β), Connective Tissue Growth Factor (CTGF), Matrix Metalloproteinase (MMP)-2, Platelet-Derived Growth Factor-D (PDGF-D) and PDGF receptor α (PDGFR α) were overexpressed in XLHN dogs compared with controls ($p < 0.05$). At T1 and T2, Clusterin (CLUST) and Tissue inhibitor of MMP-1 (TIMP-1) expression was increased; however, Epidermal Growth Factor Receptor (EGFR) mRNA was reduced ($p < 0.05$).

TGF- β and CTGF mRNA levels were positively correlated at all time-points. Expression of CTGF was positively correlated with PDGFR α , MMP-2 and MMP-9. TIMP-1, CLUST and MMP-2 mRNA levels were positively correlated with fibrosis, tubular atrophy and dilation ($p < 0.05$).

Histologic and clinic pathologic data showed canine XLHN to be a good model of chronic progressive renal failure demonstrating progressive and irreversible glomerular and TI lesions including fibrosis. Onset of proteinuria before histological changes occur (ie, at T0) suggests a key role for proteinuria in initiating progressive TI damage. TGF- β , CTGF, MMP-2, PDGF-D and PDGFRA were identified as potential key players in the progression of chronic renal damage. Increased expression of CLUST and TIMP-1 might function as a protective mechanism against progressive injury. The study investigates the expression of several molecules related with tissue injury and repair in dogs with an hereditary nephropathy that is frequently used as a model of chronic renal failure. Examined molecules are potentially involved in the pathogenesis of this condition that may be future possible target of drugs or biomarkers of early renal injury.

Malattie degenerative
renal failure, dog, gene expression

Poster Aipvet

A CASE OF CANINE THYROID CARCINOMA WITH HETEROTOPIC OSSIFICATION AND EXTRAMEDULLARY HEMOPOIESIS

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The present report describes a case of canine thyroid carcinoma with heterotopic ossification and extramedullary hemopoiesis in a 10 years old mongrel dog. Few anamnestic data were available: an apparently healthy dog was referred with the only symptom of polyphagia. A solid mass, not adherent to surrounding tissue, was palpable on the left side of the neck. The mass was surgically removed, fixed in 10% buffered formalin and send to the pathology division of our Department to be histologically examined. Grossly neoplastic mass was about 3x5 cm, well circumscribed, defined by a thin fibrous capsule. On cross section the mass had a brown-tan colour with a greyish, firm to hard, central area, grossly consistent with bone tissue.

Before trimming, the mass was therefore immersed for 1 week in a decalcifying acid. Then, samples were collected, passed through graded alcohols, clarified in xylene and paraffin embedded. From paraffin blocks, sections were obtained and stained with Haematoxylin and Eosin.

Histologically, the tumor was composed of irregular, small follicular structures, nests and solid lobules of polygonal cells sustained by a variable amount of collagenous stroma. Neoplastic cells had poorly defined cell borders, moderate amount of eosinophilic cytoplasm and round to oval vesiculous nucleus with prominent nucleolus. Anisocytosis and anisokaryosis were moderate and mitotic figures ranged from 0 to 1 X HPF. Small multifocal necrotic foci and focal haemorrhages were also present scattered throughout the tumor. The central area of the tumor was composed of mature trabecular bone. Intertrabecular spaces were filled by both adipose cells and hemopoietic cells. A histological diagnosis of thyroid carcinoma with heterotopic bone formation and extramedullary hemopoiesis was posed.

Heterotopic ossification is a well recognized phenomenon involving organs and tissues affected by various pathologic processes, i.e. ischemia, hematomas, degenerative changes, chronic inflammation and, less frequently, tumors. Few cases of thyroid tumors with heterotopic ossification and extramedullary hemopoiesis have been described in human species. In the canine species, the presence of focal mineralization or scattered bone formation within thyroid carcinomas has been reported but poorly documented. Scattered calcifications have been described in normal thyroid gland while ossification has been reported in a single case of thyroidal carcinosarcoma. The present report represents the first description of a canine thyroid carcinoma with wide areas of mature bone formation (heterotopic ossification) and extramedullary hemopoiesis. Mechanisms of heterotopic ossification are still unknown, however the presence of inducible osteoprogenitor cells, of heterotopic environment conducive to osteogenesis and of inductive signalling such as bone morphogenetic proteins has all been evocated in the pathogenesis of this condition.

Capen CC. Tumors of the endocrine glands. In: Meuten DJ, Tumors in domestic animals, IV eds. Meuten Donald J 2002; pp. 642

Grubor B, Haynes JS. Thyroid carcinosarcoma in a dog. Vet Pathol 2005; 42(1):84-7

Liu K, Tripp S, Layfield LJ. Heterotopic ossification: review of histologic findings and tissue distribution in a

10-year experience. *Pathol Res Pract* 2007;203(9):633-40

Na KY, Kim HS, Lee SK, Jung WW, Sung JY, Kim YW, Park YK. Papillary thyroid carcinoma with bone formation. *Pathol Res Pract* 2013 15;209(1):14-8

Pontikides N, Botsios D, Kariki E, Vassiliadis K, Krassas GE. Extramedullary hemopoiesis in a thyroid nodule with extensive bone metaplasia and mature bone formation. *Thyroid* 2003; 13(9):877-80

Westhoff CC, Karakas E, Dietz C, Barth PJ. Intrathyroidal hemopoiesis: a rare histological finding in an otherwise healthy patient and review of the literature. *Langenbecks Arch Surg* 2008; 393(5):745-9

Pathology oncology canine
thyroid carcinoma, dog, heterotopic

A CASE OF PAPILOMAVIRUS-ASSOCIATED TONGUE ADENOCARCINOMA IN A CAPTIVE WHITE RHINOCEROS

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Papillomaviruses induce hyperplastic and tumoral lesions in different animal species. We describe a case of Papillomavirus-Associated adenocarcinoma which occurred in a 41-year-old male white rhinoceros, maintained at the Zoological Garden of Pistoia, Tuscany.

The rhinoceros showed weight loss and difficulty in chewing the hay for three months before death. At necroscopy, the most relevant finding was a lesion that involved the tongue, characterized by erosion of the mucosa with increased involvement of the dorsal surface. The cut surface showed lardaceous appearance with escape of purulent material.

Histologically, the mass predominantly involved the submucosa and muscular portion of the tongue, resulting in closely packed large, medium, and small glands consisted of columnar cells without mucous cells. Tumor cells exhibited amphophilic to pale eosinophilic cytoplasm, high nucleo-cytoplasmic ratio and medium mitotic rate. No squamous differentiation was noted, and the squamous epithelium immediately adjacent to the adenocarcinoma showed slight perinuclear halos suggestive of PV-related changes.

The tumor cells showed diffuse staining for A1-A3 pan-keratins, but staining for keratin 7 (CK7) clearly separated the adenocarcinoma from the adjacent non-neoplastic squamous epithelium. Keratin 19, keratin 20 and MUC-5AC were negative. Immunohistochemical staining performed with a monoclonal antibody against papillomaviruses evidenced a strong nuclear immunoreactivity only in glandular epithelium similarly to CK7. DNA extracted from paraffin-embedded tissue was tested by PCR using degenerated primers (FAP 59-64), amplifying common gene region of papillomaviral L1. The PCR result was an amplicon of 480 bp. Sequence similarity analysis with the BLAST tool of the National Center for Biotechnology Information and Papillomavirus Episteme tool showed that this fragments belongs to new putative PVs, not yet characterized for rhinoceros.

To our knowledge this is the first case of Papillomavirus-Associated non-salivary gland-type adenocarcinoma arising in the base of the tongue in a captive white rhinoceros.

1) Batista et al. Entropy-based approach for selecting informative regions in the L1 gene of bovine papillomavirus for phylogenetic inference and primer design. *Genet Mol Res.* 2013;12(1):400-7. 2) Goodman et al., Successful treatment of a squamous cell carcinoma in a white rhinoceros, *Ceratotherium simum*. *Vet Dermatol.* 2007;18(6):460-3. 3) Naik et al., Squamous cell carcinoma in an Indian rhinoceros, *Vet Rec.* 1986;118(21):590-1. 4) Roperto et al., Bovine papillomavirus type 2 (BPV-2) E5 oncoprotein binds to the subunit D of the V₁-ATPase proton pump in naturally occurring urothelial tumors of the urinary bladder of cattle. *PLoS One.* 2014 Feb 24;9(2):e88860. eCollection 2014. 5) Suzuki et al. Carcinosarcoma in a white Rhinoceros (*ceratotherium simum simum*). *J Vet Med Sci.* 2013;75(12):1657-60.

ONCOLOGIA ANIMALI ESOTICI

PAPILLOMAVIRUS, WHITE RHINOCEROS, ADENOCARCINOMA

A CASE OF SYSTEMIC CORONAVIRUS-ASSOCIATED DISEASE IN A DOMESTIC FERRET IN ITALY.

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Since 2006 a Coronavirus-associated visceral disease with clinicopathologic features resembling the “dry form” of Feline Infectious Peritonitis, has been recognized in ferrets (1,2). Confirmed cases have been reported in Spain, USA, UK and Japan (3,4,5). The present work describe the first confirmed case of Systemic Coronavirus-associated disease in a domestic ferret in Italy, with description of clinicopathologic findings.

An 8 months-old, entire male ferret (*Mustela putorius furo*) was referred for weakness and coughing. Clinical examinations showed fever, enlarged retropharyngeal lymph nodes and heart murmur. Hemato-biochemical analysis demonstrated anemia, leucocytosis, hyperproteinemia and hyperglobulinaemia (polyclonal gammopathy). Imaging revealed generalized lymphadenomegaly, severe splenomegaly and multifocal nodular renal lesions. Kidney cytological smears were compatible with granulomatous disease. Histology from spleen, mesenteric lymph node and kidney biopsies was consistent with pyogranulomatous inflammation. Due to deterioration of the condition, the ferret was euthanized five months after the first presentation and post-mortem examination revealed disseminated nodular lesions, mainly localized in kidney, spleen, mesenteric and mediastinal lymph nodes, diaphragm and lung. Histology confirmed a systemic pyogranulomatous disease. Immunohistochemistry was performed using anti-FCoV monoclonal antibody and positive staining for Coronavirus antigen was detected in the cytoplasm of macrophages in the pyogranulomas, providing a definitive diagnosis of ferret systemic coronavirus-associated disease. In conclusion ferret systemic Coronavirus-associated disease should be considered in the differential diagnosis of young ferrets presenting with enlarged lymph nodes, hyperproteinemia and hyperglobulinaemia and histology and immunohistochemistry represents the gold standard for a definitive diagnosis.

1. Martinez J, Reinacher M, Perpignan D, Ramis A. Identification of Group 1 Coronavirus Antigen in Multisystemic granulomatous lesions in ferrets (*Mustela putorius furo*). *J Comp Pathol.*2008;138:54-58.
2. Perpignan D, Lopez C. Clinical aspects of systemic granulomatous inflammatory syndrome in ferrets (*Mustela putorius furo*). *Vet Rec.*2008,162:180-184.
3. Garner MM, Ramsell K, Morera N, et al. Clinicopathologic features of a systemic coronavirus-associated disease resembling feline infectious peritonitis in the domestic ferret (*Mustela putorius*). *Vet Pathol.*2008,45:236-246.
4. Michimae Y, Mikami S, Okimoto K, et al. The First Case of Feline Infectious Peritonitis-like Pyogranuloma in a Ferret Infected by Coronavirus in Japan. *J Toxicol Pathol.*2010,23:99-101.
5. Graham E, Lamm C, Denk D, et al. Systemic coronavirus-associated disease resembling feline infectious peritonitis in ferrets in the UK. *Vet Rec.*2012,171:200-201

Patologia del furetto

Ferret, Coronavirus, granulomatous diseases

COMPARISON OF DIFFERENT PROCEDURES TO ISOLATE FELINE PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS) FROM SMALL VOLUMES OF BLOOD

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Studies on leukocytes isolated from feline blood do not provide details on the performances of isolation techniques.¹ From the few numerical data available, however, it can be assumed that the purity of isolated cell populations is high but their recovery rate is low.² Therefore, large volumes of blood (difficult to collect from cats with spontaneous diseases) are required to obtain enough leukocytes for in vitro studies. The aim of this study was to assess the performances of isolation techniques on small volumes of feline blood.

Blood samples (1 to 5 mls) were drawn from clinically healthy cats and placed in EDTA-coated tubes. Fifteen sessions of tests (10 using Ficoll, 5 using Percoll) were performed. In 9 cases cells were further separated by adherence on Petri dishes (PD) and in 5 cases using iron-labelled monoclonal antibodies against leukocyte antigens followed by magnetic sorting (MS). Cell purity (i.e. the percentage of each population) and recovery (i.e. the percentage of cells of each population recorded after isolation compared with blood) were then calculated.

The purity of lymphocytes was significantly higher ($P=0.015$) with Ficoll (79.6 ± 3.3) than with Percoll (61.0 ± 12.0); the purity of monocytes was low, and significantly higher ($P=0.015$) with Percoll (32.7 ± 13.5) than with Ficoll (9.0 ± 1.6). The recovery rate of lymphocytes was low, and significantly higher ($P<0.001$) with Ficoll (54.7 ± 27.5) than with Percoll (12.0 ± 4.2). The recovery rates of monocytes recorded with Ficoll (59.4 ± 34.7) or Percoll (38.5 ± 9.38) were not significantly different. The purity of cell types in PD was not morphologically determinable. However, assuming that at least 90% of adherent and non adherent cells were monocytes and lymphocytes, respectively, the recovery rate for both the populations was lower than 10% in most cats. The purity after MS was generally high, but the recovery rate was variable and the number of yielded cells very low.

In conclusion, none of the techniques applied in this study provides good performances in terms of number of cells, purity and recovery rate, when applied to small volumes of blood. This suggests that the isolated cells could not be representative of the population in blood, and limits the use of these 3 techniques in cats with spontaneous diseases. Therefore, cell functions in spontaneous feline disease should be investigated in whole blood rather than on isolated cell populations.

1. Kipar A et al. *Vet Immunol Immunopathol* 98:215-221; 2004
2. Roberts RL and Gallin JI. *Blood* 65:433-440; 1985

Veterinary clinical pathology
Leukocyte isolation, Feline, in vitro studies

COMPREHENSIVE MANAGEMENT OF A PITUITARY ADENOMA WITH MALIGNANT FEATURES IN A MALTESE DOG

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The aim of the study is to describe the long-term survival of a dog affected by an ACTH producing-pituitary adenoma treated by surgery, medication and radiation therapy.

A 7-yr-old old male Maltese dog was referred to DIMEVET for epileptic seizures, polyuria, polydipsia, polyphagia and symmetrical alopecia. Pituitary-dependent hypercortisolism was diagnosed by endocrine tests. Computed tomography (CT) revealed a pituitary mass with a P/B ratio 0.75, reference <0.311 that was treated with transsphenoidal hypophysectomy; Immunohistochemistry of the surgical specimen confirmed an ACTH-producing adenoma. Hypercortisolism went into remission for three years but then polyuria/polydipsia and alopecia reappeared and CT scan revealed a small sellar abnormal structure (interpreted as pituitary tissue regrowth). Hypercortisolism was confirmed by endocrine tests and medical treatment with trilostane was started, with good control of the clinical signs for one year, until the occurrence of obtundation, seizures and stupor. CT scan showed a sellar mass and radiation therapy was performed with a protocol of 20 fractions of 2.25 Gy. Neurological signs regressed and trilostane treatment was continued with good control of the disease. Six years after the first admission, the dog developed lumbosacral pain and inability to walk and was euthanatized for animal welfare reasons. Histological sections were stained with H&E and PAS and immunostained with antibodies against ACTH, MSH, GH, C-erb-B2 and Ki67.

Macroscopically the formalin fixed brain revealed grey tissue in the pituitary region extending caudally to the mesencephalon. Histologically, unencapsulated neoplastic tissue in contiguity with the meninges and infiltrating the neuropil was detected; neoplastic cells arranged in islands and cords were embedded in a rich amorphous eosinophilic extracellular matrix; neoplastic cells showed an intensely eosinophilic cytoplasm. The nucleus was vesicular, often two or three nuclei were seen, with chromatin margination and a prominent nucleolus. Anisocytosis and anisokaryosis were moderate. Mitotic figures were three in ten random selected fields at 400x magnification. A diagnosis of a recurring infiltrative ACTH-adenoma was made; Ki67 labelling index was 4,8%.

Pituitary tumors that recur or progress despite resection and radiotherapy are often termed "atypical" adenomas, as they do not appear overtly malignant by histological criteria, but exhibit aggressive biologic behavior, have a Ki67 labelling index >3%, p53 immunoreactivity.² Reports of invasive canine pituitary tumors are rare; this is the first report of a long-term survival of a dog affected by this type of tumor that was treated by surgery and subsequently by radiation therapy. Comprehensive management of pituitary adenomas using the various treatment modalities may significantly prolong the dog's life.

1Kooistra et al., 1997; 2Mamelak et al., 2011.

neuropathology, endocrine pathology

pituitary adenoma, dog, survival

COORDINATED IMMUNE RESPONSE OF MEMORY AND CYTOTOXIC T CELLS TOGETHER WITH IFN- γ SECRETING CELLS AFTER PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) NATURAL INFECTION IN CONVENTIONAL PIGS.

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Porcine reproductive and respiratory syndrome virus (PRRSV) infection in the field usually dampens the pig immune system both as innate and acquired immunity. The extent of immune dysregulation and/or depression depends on the virulence of the PRRSV isolate, the intrinsic ability to interact with the immune system and on the age of the animals. (1)

The present study aims at evaluating the antibody and cellular immune response in pigs naturally infected by PRRSV in order to highlight the immune modulation.

Twenty conventional pigs were selected from a herd with a history of PRRSV infection and monitored for 22 weeks from weaning (4 weeks of age) through the fattening phase (up to 26 weeks of age). The pigs were divided in two groups: a group was naturally exposed to PRRSV infection (N=10, PRRSV-exposed) by cominglement with infected resident animals and the unexposed group was used as control (N=10, C).

Blood samples were collected 2 weeks apart and PRRSV infection was detected by quantitative PCR in serum. The humoral immune response was quantified as total serum PRRSV-specific antibodies by ELISA while the cellular response was characterized by flow cytometry and IFN- γ ELISPOT to enumerate circulating T cell subsets and PRRSV-specific IFN- γ secreting cells (SC) in PBMC. (2, 3) In this study, the distribution of cells involved in cell mediated response and IFN- γ producing cells were investigated through innovative methods (flow Cytometry and ELISpot assay) in pigs naturally infected by the PRRSV through exposure to infected animals. Clinical signs were recorded throughout the study.

The results showed that all exposed pigs became infected at 16 weeks of age and viremia lasted until 20 weeks in almost 50% of the exposed animals, whereas the C group remained negative. The PRRSV-exposed group developed an antibody response since 18 weeks of age.

In infected pigs, total CD4⁺ and CD8 α ⁺ T cells increased from 18 weeks onwards, due to a significant increase of cytotoxic T CD8 β ⁺ and memory T helper CD4⁺CD8 α ⁺low lymphocytes. An early and transient increase was observed for naïve T helper CD4⁺CD8 α ⁻ cells. Also virus-specific IFN- γ SC were significantly recalled from 18 weeks, peaking at 22 weeks. Control animals showed non-significant fluctuations in cell percentages and negligible SC levels. In this study, the IFN- γ SC response was strongly induced in parallel with the positive modulation of cytotoxic and memory T cells suggesting the potential activation of these subsets to secrete the immune cytokine.

This approach demonstrated a strong IFN- γ response but also some peculiar aspect of the immune response, likely depending on the delayed infection time of animals exposed to the virus. Overall, taking into account that PRRSV infection was more delayed compared to what is generally observed in the field, the age of pigs may have favoured a more pronounced immune response.

References: 1) Martelli P. et al. Vaccine Volume 27, Issue 28, 8 June 2009, 3788-3799; 2) Ferrari L. et al. Vet Immunol Immunopathol 151 2013, 193- 206. 3) Burgara-Estrella A. et al. Viruses 2013, 5, 663-677

Patologia generale

PRRSV, PIG, IMMUNITY

CUTANEOUS NEOSPOROSIS IN A GOLDEN RETRIEVER

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This report describes cutaneous cytological and histological lesions caused by *Neospora caninum*.

A 10 years old, intact female Golden Retriever under treatment with cyclosporine for an autoimmune disorder had sudden development of multifocal dorsal cutaneous nodules. Skin cytological specimens obtained by imprint and fine-needle aspiration were stained with May-Grünwald Giemsa. Skin punch biopsy specimens were obtained. Biopsies were fixed in 10% buffered formalin, routinely processed and stained with haematoxylin and eosin. Unstained smears and deparaffinized sections of skin were immunochemically stained with polyclonal anti-*Toxoplasma gondii* and anti-*Neospora caninum* primary antibodies.

Cytology demonstrated a prevalence of degenerated neutrophils admixed with fewer macrophages, rare neutrophils associated with adipocytes and fibroblasts. On the background and within macrophages numerous crescent shaped, 4-6 µm microorganisms, with a light basophilic cytoplasm and a central nucleus (tachyzoites) were visible. Histology revealed diffuse and severe neutrophilic, histiocytic, eosinophilic dermatitis and panniculitis associated with necrotizing vasculitis. Elevated numbers of free and cytoplasmic tachyzoites within macrophages and keratinocytes of the epidermis and follicular infundibula were present. Immunocytochemistry and immunohistochemistry warranted a diagnosis of cutaneous Neosporosis (CN). Clindamycin administration (11 mg/kg PO every 12 hours) and withdrawal of immunosuppressive medication resulted in prolonged clinical remission.

Cutaneous nodules are a rare manifestation of Neosporosis.¹⁻⁴ Age-related immunodeficiency and immunosuppressive therapies seem to predispose to CN.¹⁻⁴ Information on cause and prognosis are fragmentary in this instance. The current dog was alive 1 month after diagnosis.

1 – La Perle K.M.D., Del Piero F., Carr R.F. et al.: 2001, Cutaneous neosporosis in Two Adults Dogs on Chronic Immunosuppressive Therapy. *J Vet Diagn Invest* 13:252-255.

2 – Ordeix L., Lloret A., Fondevila D. et al.: Cutaneous Neosporosis During Treatment of Pemphigus Foliaceus in a Dog. *J Am Anim Hosp Assoc* 38:415-9.

3 - Poli A., Mancianti F., Carli M.A. et al.: 1998, *Neospora Caninum* infection in a Bernese cattle dog from Italy. *Vet Parasitol* 78:79-85.

4 – Perl S., Harrus S., Satuchne (Goldvaser) C. et al.: 1998, Cutaneous Neosporosis in a dog in Israel. *Vet Parasitol* 79:257-261.

DERMATOPATOLOGIA

Dog, *Neospora caninum*, Skin

EFFECT OF DIFFERENT ENVIRONMENTAL CONDITIONS ON SOME HAEMATOLOGICAL PARAMETERS IN COWS.

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The aim of this study was to evaluate physiopathological responses to different environmental conditions (ambient temperature, relative humidity and temperature-humidity index) on haematological parameters. Blood samples were collected at 4 time points under different environmental conditions (T1, T2, T3 and T4) by 43 Piemontese cows aged 2–12 years and analysed for haematological parameters. For each period, ambient temperature and relative humidity were recorded by means of a data logger and the temperature-humidity index (THI) was calculated as indicator of thermal comfort for cattle. Data were then measured by one way analysis of variance (ANOVA).

The obtained results showed a statistical significant effect of time on the following parameters: RBC (P<0.0001), Hb (P<0.0001), Hct (P<0.0001), MCV (P<0.0001), MCH (P<0.0001), MCHC (P<0.0001), Plt(P<0.0001), WBC (P<0.0001), neutrophils (P<0.0001), lymphocytes (P<0.0001), monocytes (P<0.0001) and eosinophils (P<0.0001).

The majority of haematological values obtained in the present study, even though within the physiological range for cattle, showed that variations in haematological parameters are related to changes in ambient temperature, relative humidity and temperature-humidity index. These results provide insight into the physiological responses of Piemontese cow to different environmental conditions, allowing to better evaluate its ability to adapt and cope with environmental stress (1-3).

1. Grünwaldt EG, Guevara JC, Estévez OR, Vicente A, Rousselle H, Alcuten N, Aguerregaray D, Stasi CR. (2005) Biochemical and haematological measurements in beef cattle in Mendoza plain rangelands (Argentina). *Trop Anim Health Prod.* Aug;37(6):527-40.

2. Casella S., Scianò S., Zumbo A., Monteverde V., Fazio F., Piccione G. (2013). Effect of seasonal variations in Mediterranean area on haematological profile in dairy cow. *Comparative Clinical Pathology* 22(4): 691-695.

3. Koubkova M., Knizkova I., Kunc P., Hartlova H., Flusser J., Dolezal O. (2002). Influence of high environmental temperatures and evaporative cooling on some physiological, hematological and biochemical parameters in high-yielding dairy cows. *Czech Journal of Animal Science.* 47: 309-318.

Patologia Clinica

cows, haematology, environment

EFFECTIVENESS OF ENDOMETRIAL CYTOLOGY OBTAINED BY LOW-VOLUME UTERINE FLUSH TECHNIQUE IN POSTPARTUM ENDOMETRITIS OF DAIRY COWS

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Aim of this study was to evaluate the effectiveness of endometrial cytology obtained by low-volume uterine flush technique to identify endometritis in postpartum period of dairy cows, correlating data with bacteriological examination and clinical findings.

Endometritis, responsible of great economical losses in dairy industry, is difficult to diagnose, due to the lack of gold standard criteria accepted by practitioners (1). Subclinical endometritis (SEND), defined as uterine inflammation in the absence of clinical signs, is frequently associated with significant reduction of reproductive performance and often diagnosed through endometrial cytology (2).

The study was performed in 40 multiparous Holsteins. From October to December 2013, cows underwent clinical evaluations from the day of parturition until 40 days postpartum. Intrauterine samples were collected between 30 and 40 d postpartum by infusing 100-150 ml of sodium chloride 0.9% sterile solution and then recovering the fluid; samples were delivered to the laboratory within 12 hours. After centrifugation of the fluid, an aliquot was used for bacteriological examinations and another for cytology. According to the published literature, a cut off of 10% of polymorphonuclear leucocytes (PMNs), was used to identify cows with endometritis (3).

Between 30 and 40 d postpartum, all cows were flushed and examined: 17 cows (42%) showed a positive cytology; 6 of these cows were diagnosed with a clinical endometritis, whereas the remaining 11 were clinically healthy (SEND cows). Of the 23 remaining cows with a negative cytology (58%), 20 were healthy, whereas 3 cows showed clinical endometritis (false negative). Bacteriology allowed the isolation of several bovine uterine pathogens both in clinical endometritis cases and in clinically healthy cows. Six different groups of bacteria were isolated such as *Trueperella pyogenes* (n=6), *Escherichia coli* (n=5), *Enterococcus faecalis* (n=7), coagulase-negative staphylococci (n=8), *Bacillus* spp. (n=1), *Pseudomonas* spp. (n=3) and *Enterobacter agglomerans* (n=1). Bacteriology was negative in 9 cows.

Low-volume uterine flush cytology between 30 and 40 days postpartum has proved to be a useful technique to reveal SEND especially in cases associated with bacteria considered only potentially pathogenic for the uterus, such as *E. faecalis* or *B. licheniformis*. However, we underline the limits of the technique that can lead to obtain false negative. The results of our study emphasize the need for integration of the two diagnostic tools, namely cytology and bacteriology.

1)Kasimanickam R, et al; A comparison of the cytobrush and uterine lavage techniques to evaluate endometrial cytology in clinically normal post-partum dairy cows. *Can Vet J* 2005; 46:255-259.

2)Madoz LV, et al; Endometrial cytology, biopsy, and bacteriology for the diagnosis of subclinical endometritis in grazing dairy cows. *J Dairy Sci* 2014.; 97:195-201.

3)Sheldon I M, et al; Defining postpartum uterine disease in cattle. *Theriogenology*, 65 (2006) 1516-1530.

Patologia della riproduzione

endometrial cytology, cow, uterine bacteriology

ERYSIPELOTRIX RHUSIOPATHIAE ENDOCARDITIS IN A SHEEP

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Erysipelotrix rhusiopathiae is a pathogen of a wide variety of animals. Chronic polyarthritis are reported in sheep and lambs. The authors describe one systemic erysipelas infection in an adult sheep in Sicily.

A female, cross-breed, 18 months old sheep showing clinical signs of phosphate esters suspected poisoning, was sent for diagnostic investigations to the Department of Veterinary Sciences of Messina University. A complete necropsy was made and samples of affected organs were partially fixed in 10% neutral buffered formalin to perform histological investigations and partially stored at -80°C for biomolecular researches. Total DNA was extracted from heart tissue and employed in PCR test targeted to the 23S ribosomal region. The DNA sequences were amplified by primers annealing at 23S of ribosomal genes as reported in the literature (Takeshi et al., 1999). PCR products were sequenced and the obtained data were analyzed by Wu Blast 2 sequence alignment software for strain identification.

At necropsy abundant foamy exudates in the trachea and bronchi and pleural hemorrhages were observed. Moreover, hypertrophy of the left heart showing multiple grayish white foci were present. The mitral valve showed a severe acute endocarditis characterized by irregular friable vegetations. In the kidney a voluminous necrotic area with hemorrhagic limits between cortical and medullary was detected. Histology confirmed the presence of multiple myocardial and renal septic infarcts. The flaps of the mitral valve were fibrotic, infiltrated by polymorphonuclear cells, with adherent multiple thrombi. DNA extracted and the sequence analysis showed a complete overlapping with the 23S rDNA of Erysipelothrix rhusiopathiae sequence.

Erysipelothrix rhusiopathiae is a bacterium commonly isolated in swine. In author's opinion this report describes an unusual cardiac localization in sheep previously only rarely described (Chineme et al., 1973; Maclachlan, 1978). The occurrence of this unusual localization imposes the necessity do not neglect common lesions using always regularly laboratory investigations.

Chineme et al. (1973). Cardiovascular lesions associated with erysipelas in a sheep. J Am Vet Med Assoc. 162(4):278-9. Maclachlan GK. (1978). Ovine endocarditis from Erysipelothrix insidiosa. Vet Rec. 102(7):150. Takeshi K. et al. (1999). Direct and Rapid Detection by PCR of Erysipelothrix sp. DNAs Prepared from Bacterial Strains and Animal Tissues. J. Clin. Microbiol. 37 (12), 4093-4098

Systemic erysipelas infection

Sheep, E. rhusiopathiae, endocarditis

GLIOBLASTOMA IN AN EWE

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Tumors of neuroepithelial origin (gliomas) include astrocytomas, oligodendrogliomas, and ependymomas. In veterinary medicine, primary neoplasms of the central nervous system (CNS) are most frequently reported in dogs and less commonly in cats, whereas the same tumors are considered distinctly uncommon within the other domestic species [1].

The present report aims at describing the pathological and immunohistochemical findings observed in a Sarda breed sheep with glioblastoma.

A 6 years old Sarda breed sheep was euthanized and necropsied after showing severe and worsening neurological signs, which started 8 months before and mainly consisted of depression and head pressing. At necropsy a wide range of tissues were sampled, promptly fixed in 10% neutral buffered formalin and routinely processed for histopathological investigations (hematoxylin and eosin stain). Furthermore, selected tissue sections of the cerebral mass were subject to immunohistochemistry (IHC) using the following primary antibodies: anti-gliial fibrillary acidic protein, anti-vimentin, anti-synaptophysin and olig2.

At necropsy, a large (about 5 cm in diameter) whitish to grayish mass was seen partially replacing the left cerebral hemisphere.

Microscopically, the tumor consisted of polymorphic cells, with a high number of mitotic figures and large areas of necrosis and dystrophic calcification surrounded by pseudo-palisading of cells. The neoplasm was highly vascular with glomeruloid vascular proliferation.

Foci of "oligodendroglioma-like" differentiation and few multinucleated giant cells were also occasionally seen. Mitotic figures were very frequent, up to 5 mitoses per high magnification power field (Ob. x40), and often atypical. At IHC, neoplastic cells proved to be immunoreactive (IR) for vimentin and olig2 but constantly negative for synaptophysin. Only few scattered neoplastic cells were GFAP-IR. However, IHC for GFAP demonstrated the presence of few reactive astrocytes, mainly surrounding the foci of necrosis.

Few cases of primary brain tumors have been described in sheep: medulloblastoma in lambs [2], a case of ependymoma in a Suffolk sheep aged less than 1 year [3], and more recently a case of oligodendroglioma in a 1 year old male Iranian fat-tailed sheep [4]. To the best of our knowledge, the present is the first report of glioblastoma in sheep. Even in sheep, glioblastoma is similar to anaplastic astrocytoma with the additional features of necrosis and vascular proliferation and share many of the histopathological and immunohistochemical features observed in dogs [5].

1. Mandara M.T et al. (2011) Poletto Editore, Vermezzo, 119 Milano, Italy, pp. 221-287.

2. Cotchin E. (1975) *Pro R Soc Med* 99 68, 653-655.

3. Elsinghorst A.M. (2003) *Vet Quart* , 103 25(4), 165-169

4. Derakhshanfar A. and Mozaffari A.A. (2010) *J S Afr Vet Assoc* 81(2), 114-115.

5. Lipsitz D. et al (2003) *Vet Pathol* Nov;40(6):659-69.

PATHOLOGY

Glioblastoma, Sheep, Immunohistochemistry

GLYCOGEN RICH CARCINOMA OF CANINE MALE AND FEMALE MAMMARY GLAND

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Glycogen rich clear cell carcinoma (GRCC) is a rare subtype of human invasive mammary gland (MG) carcinoma, in which at least 90% of the neoplastic cells have clear cytoplasm containing glycogen(1). The aim of this study was to describe the histological, histochemical and immunohistochemical (IHC) features of GRCC of canine MG.

Serial formalin fixed paraffin embedded tissue sections of two GRCC canine mammary carcinoma, one female (case I) and one male (case II) were stained with H&E, PAS, PAS diastase (dPAS), and Alcian Blue (AB). IHC was performed with anti-ER, -PR, -cerbB2, -CK19, -CK14, -CK5/6, -p63, -vimentin, -SMA, -calponin, -S100, -EGFR, -c-KIT, -E-cad and -ki67 antibodies. Sudan III was carried out on formalin-fixed frozen tissue in case II.

Histologically, 90% of neoplastic cells showed sharply distinct borders, clear or finely granular cytoplasm and low N:C ratio. In case II, residual 10% of the neoplastic cells had lipid-like vacuolated cytoplasm. In both tumors, the cytoplasm resulted strongly positive with PAS. Treatment with diastase abolished PAS reactivity. Case I showed PAS+ and dPAS- staining also in lymph node metastasis. In case II, 10% of neoplastic cells were positive to Sudan III. No stain with AB was obtained. Case I showed positivity for CK19 and CK5/6, negativity for ER, PR and c-erbB2, resulting a basal-like phenotype in primary tumor and lymph node metastasis. They were both positive for EGFR, E-cad, c-KIT, and weakly for calponin. Case II was a basal-like phenotype, presenting CK 19, E-cad, c-KIT, weak CK14 and strong vimentin positivity. The proliferative ki67 index was 26.75% in case I and 8.2% in case II.

Based on the morphology, typical features of human MG GRCC are the “fried eggs appearance”, clear cytoplasm and small dark punctate nuclei (2). On the best of our knowledge, this is the first report regarding GRCC in canine MG. The diagnosis was confirmed by PAS+ of intracytoplasmic glycogen granules and lack of stain with dPAS. Case II was considered a GRCC with lipid rich differentiation. Both cases had a basal-like phenotype and the expression of EGFR and c-KIT was suggestive of an association of cell proliferation with signal transduction of surface molecules (3). GRCC can be considered a new rare histological subtype of canine mammary tumors, with clear cytoplasm, PAS+ and dPAS-, expressing the triple negative phenotype, a tumor with clinical aggressive behavior that should be differentiated from lipid rich carcinoma.

1. Tavassoli, Deville. Lyon: IARC Press, 2003
2. Rosen, Philadelphia. LWW press, 2001
3. Kim et al., Yonsei Med J 53:1142-1146, 2012

ANATOMIA PATOLOGICA

Canine, Mammary carcinoma, Glycogen rich

GRANULOMATOUS DERMATITIS OF THE AURICULAR PINNA IN A HEIFER

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This study is aimed to describe the morpho-pathological and etiopathogenic aspects of a unique case of granulomatous dermatitis.

A 2 y.o. Italian Friesian heifer showed a papillomatous-like lesion at the edge of the right auricular pinna. The lesion sized 9 cm x 11.5 cm, was exophytic, globular in shape, firm and with the outer surface uneven, depigmented and ulcerated. Cytological examination revealed the presence of an inflammatory population mainly composed by neutrophils and macrophages. On the basis of the cytological observation, the mass was excised and subjected both to histological staining (H&E, PAS, Gram) and molecular biology examination (PCR).

The histological examination allowed the diagnosis of a pyogranulomatous inflammation characterized by a epithelioid and giant cells, neutrophilic and macrophagic infiltrate organized around focal areas of eosinophilic homogeneous material consistent with the Splendore-Hoeppli phenomenon. PAS staining highlighted numerous coccoid formations within the piogranulomatous reaction or freely standing in the tissue. Gram stain revealed Gram-positive bacterial colonies confirmed by PCR as *Corynebacterium mucifaciens*.

Granulomatous dermatites are caused by agents against whom the body is sensitized and react through an immunomediated response(1). Sometimes the above reactions are histologically characterized by the Splendor-Hoeppli phenomenon that may represent the deposition of antigen-antibody complexes (immunoglobulins and major basic proteins) and debris from the host inflammatory cells(2). The lesion described in this work seemed worth of description because of the etiologic agent. In fact, *Corynebacterium mucifaciens* is a newly-described species belonging to the the largest genus in the group of coryneform bacteria(3). Moreover, the seat of development was extremely atypical for the bovine species. *Corynebacterium mucifaciens* is better differentiated from closely-related species by molecular biology techniques, such as sequencing of the 16S rRNA gene and is isolated from human blood or other normally-sterile body fluids, often considered as part of the normal skin flora or contaminants. To date, literature data report the occurrence of disease due to *Corynebacterium mucifaciens* only in humans where it has been related to severe infections, lethal bacteremia (4), cavitory pneumonia (5), corneal ulcer (6), otitis and nasal polyps (7). To the author's knowledge, this is the first report of a lesion induced by *C. mucifaciens* in veterinary medicine.

1. MARCATO PS (2002) - Patologia sistematica veterinaria, Edagricole, Bologna.
2. Hussein MR (2008) - Mucocutaneous Splendore-Hoeppli phenomenon. *Cutan Pathol* 35:979-988.
3. Funke G, Lawson PA, Collins MD (1997) - *Corynebacterium mucifaciens* sp. nov., an Unusual Species from Human Clinical Material. *Int J Syst Bacteriol.* 47(4):952-7.
4. Cantarelli V, Z.Brodt T, Secchi C, Inamine E, De Souza Pereira F, Pilger DA (2006) - Fatal case of bacteremia caused by an atypical strain of *Corynebacterium mucifaciens*. *Braz J Infect Dis.* 10(6):416-8.
5. Djossou F, Bezian MC, Moynet D, Le Fleche-Mateos A, Malvy D (2010) - *Corynebacterium mucifaciens* in an immunocompetent patient with cavitory pneumonia; *BMC Infectious Diseases* 10:355.
6. Sanz-Rodríguez N, Almagro-Moltó M, Vozmediano-Serrano MT, Gómez-Garcés JL (2014) - First case of a corneal ulcer associated with *Corynebacterium mucifaciens*. *Enferm Infecc Microbiol Clin* [Epub ahead of print; available on line at <http://www.sciencedirect.com/science/article/pii/S0213005X14000081>]
7. Morinaka S, Kurokawa M, Nukina M, Nakamura H (2006) - Unusual *Corynebacterium mucifaciens* isolated from ear and nasal specimens. *Otolaryngol Head Neck Surg* 135(3):392-6.

Patologia Veterinaria

C. mucifaciens, Skin granulomas, Splendore-Hoeppli

GRANULOMATOUS MIOSITIS DUE TO CORYNEBACTERIUM PSEUDOTUBERCULOSIS IN A HORSE

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This study is aimed to describe the morpho-pathological and etiopathogenic aspects of a case of granulomatous myositis in a horse.

A 12 years old Quarter horse mare was evaluated because of the presence of different subcutaneous nodules and masses. The lesions were cytologically diagnosed as pyogranulomas and were resistant to the given pharmacological treatment. The persistence of the lesions along with the deterioration of the clinical status suggested to surgically remove three of them. Macroscopically they were fixed to the muscles, painless and firm in consistency. On cut section, they showed a purulent exudate. Tissue samples from the lesions were fixed in 10% buffered formalin and paraffin wax embedded. Histological sections were stained with H&E, PAS, Masson trichrome, Grocott and Gram. Molecular biology assay (PCR) was performed too.

Histological examination revealed, in all samples, a diffuse mixed inflammatory infiltrate characterised by polymorphonuclear granulocytes (neutrophils and mainly eosinophils), macrophages, lymphocytes, plasma cells, epithelioid and multinucleated giant cells invading the endomysium. Different sized round foci of caseous necrosis with calcification and areas of collagenolytic degeneration were present in all sections. PAS and Grocott stains didn't show fungi whereas Gram stain revealed the presence of blue pleomorphic Gram + microorganisms free or inside macrophages.

The described results indicated a severe diffuse granulomatous myositis. PCR analysis revealed the etiologic agent as *Corynebacterium pseudotuberculosis*.

Corynebacterium pseudotuberculosis infection occurs world wide as caseous lymphadenitis in small ruminants and granulomatous infection in horse and cattle.

The bacterium can survive for extended periods in the environment and in the soil. Disease transmission is thought to occur thorough a contaminated environment by direct contact between animals and insects such as house flies (*Musca domestica*), stable flies (*Stomoxys calcitrans*), and other arthropods serving as mechanical vectors (1). Clinically, the infection in horses most commonly causes external abscesses or "pigeon fever" (90% of cases), however, internal abscesses (8%) or ulcerative lymphangitis (1%) may also occur (2).

In our case, none of the known pathogenetic pathways reported in the literature seems to be completely comparable being some aspects common to the so called pigeon fever and others to the chronic form of ulcerative lymphangitis. The presented case is, therefore, very rare and interesting for the pathological findings and, overall, from the epidemiological point of view.

1. Spier SJ, Leutenegger CM, Carroll SP, Loye JE, Pusterla JB, Carpenter TE, et al. (2004) - Use of a real-time polymerase chain reaction based fluorogenic 5' nuclease assay to evaluate insect vectors of *Corynebacterium pseudotuberculosis* infections in horses. *Am J Vet* 65:829–34.

2. Szonyi B, Swinford A, Clavijo A, Ivanek R (2013) - Re-emergence of Pigeon Fever (*Corynebacterium pseudotuberculosis*). Infection in Texas Horses: Epidemiologic Investigation of Laboratory-Diagnosed Cases. *Journal of Equine Veterinary Science* XX:1–7.

Patologia Veterinaria

horse, muscle granulomas, pigeon fever

HISTOLOGICAL ASPECTS OF AN UNUSUAL COLONIC DUPLICATION CYST IN A CONSTIPATED CAT

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In animals as in humans the main anomalies of canalization of enterocolic tract are represented by the persistence of Meckel's diverticulum, intestinal duplication cyst, vitelline or omphalic duct cyst or finally, diverticula losing a direct connection with the colonic lumen^{1,3}. In these condition histology may be characterized by the indistinguishable mucosal type between normal and accessories tract, or by the presence of metaplastic epithelial types. We describe a case of 5-year-old domestic short-haired spayed female cat presenting abdominal pain and suspended defecation, in which abdominal ultrasonography revealed the presence of an oval formation connected to the colon, whose wall was similar to the colonic one. Ultrasonographically the content appeared inhomogeneous. Surgery and related histopathology allowed a most likely diagnosis of a colonic duplication cyst.

For histology analysis, samples were routinely processed and stained with hematoxylin-eosin and Alcian-PAS using different types of mucins, then characterized also immunohistochemically.

Mucosal histology and histochemistry revealed particular heterotopic tissues represented by some spotted areas of gastric metaplasia, antral/pyloric in type, and a very particular area of mucosa that resemble respiratory mucosa in type. No heterotopic pancreatic tissue, Brunner glands or biliary epithelium, frequently reported in other cases¹, were observed in this cat. Two nonreactive lymphoid aggregates were observed in the lamina propria of the cyst, in absence of elements evidencing inflammation and/or subsequent bacterial superinfection of the cystic mucosa. After surgery the cat returned to defecate normally.

Duplication cyst is an uncommon congenital abnormality of the alimentary tract. Most often the patients are asymptomatic and colonic duplication cysts remain undiagnosed for years. In this case report we present a fourth description of intestinal duplication cyst case in a cat^{1, 2, 3, 4} with a descending colon location and a mucosal characterization by histology/immunohistochemistry. The importance of description of this rare congenital malformation is finalized to the inclusion in the differential diagnosis of cystic masses of the gastrointestinal tract.

1) Kook PH, Hagen R, Willi B, Ruetten M, Venzin C. Rectal duplication cyst in a cat. *J Feline Med Surg* 2010;12:978-981.

2) Kramer A, Kyles AE, Labelle P. Surgical Correction of Colonic Duplication in a Cat. *J Am Anim Hosp Assoc* 2007;43:128-131.

3) Parry-Smith P, Czerwinska M, Krudewig C. Duodenal duplication cyst in a young cat. *Vet Rec* 2008;162:826-827.

4) Radlinsky MAG, Biller DS, Nietfeld J, Enwiller T. Subclinical intestinal duplication in a cat. *J Feline Med Surg* 2005;7:223-226.

General and Special Pathology

disontogenic cyst , histochemistry, immunohistochemistry

HSP32 AND HSP90 IMMUNOEXPRESSION, IN RELATION TO GRADING AND OTHER HISTOPATHOLOGICAL FEATURES, IN CANINE CUTANEOUS MAST CELL TUMOURS

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Literature data indicate Hsp32 and Hsp90 as interesting molecular targets in canine neoplastic mast cells. However, their immunoeexpression patterns in canine mast cell tumours (MCT) have not been investigated so far. Thus, the aim of the present study was to evaluate the immunohistochemical expression of Hsp32 and Hsp90 in 22 samples of canine cutaneous MCT, in relation to histological grade and other pathological variables, such as absence/presence of epidermal ulceration or tumour necrosis, growth pattern and mitotic index

Grading was established on the basis of the systems proposed by Patnaik et al. (1984) and Kiupel et al. (2011) as follows: 13 grade II (10 low-grade and 3 high-grade) MCT and 9 grade III (all high-grade) MCT. A semi-quantitative method was used to analyse immunoreactivity. Fisher's exact test and Cramer's V were used to evaluate the associations between the examined parameters.

Hsp32 showed a variably intense and distributed cytoplasmic immunostaining, not associated with histological grade. However, the low Hsp32 immunoeexpression (<50% of positive cells) detected in the majority of grade III/high-grade MCT samples suggests the need for further investigating the efficacy of the pharmacologic Hsp32-inhibitors on aggressive canine MCT. Reduced Hsp32 immunoreactivity was associated with presence of tumour necrosis ($p=0.035$; Cramer's $V=0.542$), a finding in agreement with the pro-survival functions of Hsp32 in neoplastic canine MC.

Hsp90 cytoplasmic immunosignal was variably associated with nuclear and/or membranous staining. Proportion of Hsp90 immunoeexpression was not associated with histopathological features and grade.

Noteworthy was the detection of a membranous expression of Hsp90, in light of its molecular chaperone functions towards the receptor tyrosine kinase Kit. Further studies are currently underway in order to investigate the possible relationship between Hsp90 and Kit immunoeexpressions in canine MCT.

- Hadzijusufovic et al. (2008) Targeting of heat-shock protein 32/heme oxygenase-1 in canine mastocytoma cells is associated with reduced growth and induction of apoptosis. *Exp Hematol* 36:1461-70.
- Kiupel et al. (2011) Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behaviour. *Vet Pathol* 48:147-55.
- Lin et al. (2008) The novel HSP90 inhibitor STA-9090 exhibits activity against Kit-dependent and - independent malignant mast cell tumors. *Exp Hematol* 36:1266-77.
- Patnaik et al. (1984) Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. *Vet Pathol* 21:469-74.

disciplinare

HSPs, Mast cell tumor, Dog

IDENTIFICATION OF REGULATORY T CELLS IN CANINE MAMMARY GLAND TUMORS

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In the present study we evaluated foxp3 expression in benign and malignant canine mammary gland tumors by immunohistochemistry and we investigated its prognostic significance. Foxp3 is a member of the forkhead /widge-helix family of transcription regulators involved in regulating immune system development and function (2). This gene plays a crucial role in the generation of CD4+/CD25+ regulatory T cells. Tregs seem to enable tumors to elude host antitumoral immune response (1, 3).

Animals: a total of 33 samples of canine mammary gland tumors were selected from the archives of Veterinary Pathology of the Department of Veterinary Medical Sciences over a period between 2006 and 2013. 15 neoplasias were diagnosed as benign and 18 as malignant, based on the WHO (1999) classification. Among the benign tumors 10 were complex adenomas, 3 papillary adenomas and 1 mixed benign tumor. Among the malignant tumors 5 were solid carcinomas, 3 complex carcinomas, 9 tubulo-papillary carcinomas and 1 in situ carcinoma.

All the samples were immunohistochemically tested with an anti Foxp3 primary antibody (purified Anti-Mouse/Rat Foxp3 clone FJK - 16s of the firm eBioscience), at a 1:200 dilution in PBS overnight 4°C.

Each slide was evaluated microscopically on 5, 20X fields and all the positive cells were counted in peritumoral and intratumoral infiltrates. Benign tumors showed fewer infiltrates than malignant neoplasias. Foxp3 expression was observed in 9 benign tumors out of 15. In 6 complex adenomas the number of tregs observed was between 1 and 12, only one showed more than 13 tregs, 2 papillary adenomas showed less than 12 tregs.

Foxp3 expression was observed in 14 malignant tumors out of 18. In solid carcinomas foxp3 was highly expressed (more than 40 tregs) while in complex carcinomas, expression was less (between 1 and 12) and in 3 tubulo-papillary carcinoma between 1 and 12 cells were positive and in 4 between 13 and 40. In the in situ carcinoma foxp3 was very highly expressed (more than 40)

In the present study the number of tregs was counted and compared with the histological grade of canine mammary tumors and we observed that foxp3 (tregs transcription factor) was mainly expressed in intratumoral and peritumoral infiltrating areas of neoplasias with poor prognosis.

These results suggest that the increase number of tregs lymphocytes in mammary neoplasias may play a role in tumor progression as they suppress the immune response against it, and allow the tumor progression.

1)Oh S.Y., 2012,Vet and Compar. Oncology DOI:10.1111/j1476-5829.201.00327x,

2)Kim J.H., 2012, The Vet, Journal 193 22-227

3)Horiuchi Y., 2009, Microbiol. and Immunol.53:468-474

IMMUNOREGULATION

T LYMPHOCYTES, MAMMARY GLAND TUMORS, FOXP3

MOLECULAR DETECTION OF OVIS ARIES PAPILLOMAVIRUS TYPE 3 IN FORMALIN FIXED, PARAFFIN EMBEDDED (FFPE) SHEEP SQUAMOUS CELL CARCINOMA SAMPLES.

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Recently (Alberti et al., 2010) the prototype of a novel papillomavirus genus (OaPV3) was detected in normal skin and squamous cell carcinoma (SCC) lesions of Sardinian sheep. OaPV3 belongs to the Dyokappa genus and its genome significantly differs from the 2 species of ovine papillomaviruses previously reported in Australia that instead group with the artiodactyl Deltapapillomavirus species, and have been isolated from benign cutaneous lesions. Here we investigate the relevance of OaPV3 in SCC by screening a panel of FFPE sheep SCC samples

Based on the sequence of OaPV3 L1 and E6 two DNA probes and a set of 4 primers were designed and respectively used to develop an In situ Hybridisation test (ISH) and two RT-PCR assays. These assays were applied to a collection of 41 FFPE sheep SCCs samples obtained from cutaneous tumours (5 nasal, 6 ear, 10 periocular, 3 dorsal, and 17 mammary tumours).

Diagnosis of SCC was confirmed by histopathological examination of the 41 samples. Molecular tests, summarised in the table, revealed that 26 out of 41 (63%) samples were positive to at least one test. Different tests showed different sensitivities. Also, tumours localised in different parts of the sheep body seemed to show variable degrees of positivity to OaPV3.

Results demonstrate a high prevalence (63%) of OaPV3 in sheep SCCs. This level of prevalence is particularly important and comparable to the prevalence of HPVs in human SCC (50 to 69%, Meyer et al., 2000), and suggests that OaPV3 represent an important risk factor for the development of sheep SCC. The level of positivity of nasal and periocular lesions was greater respect to other tumour locations. This can be explained by the greater level of solar exposition and/or to traumas of these area respect to other locations. As expected, RT-PCR has a greater sensitivity than ISH. However, only combining these two tests the total number of positives can be detected, and both the presence/expression and localisation in the tumour can be investigated. Concluding, we cannot rule out the presence of unknown viral types in negative tissues. Further investigation is needed to investigate the presence of viral variants associated to different tumour locations and the presence of uncovered papillomaviruses in negative samples.

1. Alberti et al., 2010. *Virology* 25:352-9.
2. Meyer et al., 2000. *Dermatology* 201:204-11

Anatomia Patologica e Malattie Infettive
OaPV3, squamous carcinoma, RT-PCR and ISH

Tumour location	N° samples	N° of positive	positive to all tests	ISH positive	RT-PCR positive	ISHE6 positive	ISHL1 positive	RT-PCR positive E6	RT-PCR positive L1
Nose	5	5	2	4	5	4	4	4	3
Ear	6	6	1	4	5	4	3	4	4
Eye	10	4	0	4	4	3	2	2	2
Flank	3	2	0	2	2	1	2	1	1
Udder	18	9	1	5	8	3	2	4	6
Tot.	42	26	4	19	24	15	13	15	16

MYOGENIC REGULATORY FACTORS EXPRESSION IN MURINE C2C12 CELLS TREATED WITH 17BETA ESTRADIOL: PRELIMINARY DATA

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Myogenesis is controlled by a family of transcription factors known as Myogenic Regulatory Factors (MRFs) including Myogenic Differentiation 1 (MyoD1), Myogenic Factor 5 (Myf 5), Myogenin (Myog) and Myogenic Regulatory Factor 4 (MRF4), which are differently expressed throughout the myogenic process. MyoD1 and Myf5 are mainly involved in the satellite cells commitment into proliferating myoblasts, whereas Myog and MRF4 are expressed during middle and late differentiation stages. MRF4 is also involved in fibers maturation (Dedieu 2002, Ferri 2009). These factors are regulated by extrinsic signals such as extracellular matrix substances, growth factors and hormones (Rhoads, 2009).

It is known that estrogens control myogenesis and skeletal muscle fibers changes. However the hormones mechanism of action are not yet completely understood (Boland 2008, De Jager 2011). In this preliminary study we evaluated the 17 β estradiol (E2) influence on MRFs expression in C2C12 cells.

C2C12 cells were seeded and, at about 80% confluence, cells were switched into a 10% horse serum medium to induce differentiation.

Cells were treated 5 times with 10⁻⁵ M or 10⁻⁸ M E2 every 48 hours and were harvested every 48 hours to evaluate expression of MyoD, Myf5, Myog, MRF4 by Real-time PCR and Western Blot. The evaluation of Myosin heavy chain I (MHCI) gene expression was carried out to confirm myotubes formation. Data were properly analyzed using GraphPad InStat (vers. 3.05).

No difference in myoblasts MRFs gene expression was revealed, while a difference in MRF4 gene expression was detected between treated myotubes and controls culture. In particular, 10⁻⁸ M E2 administration induced a greater MRF4 expression in myotubes than in untreated culture and 10⁻⁵ M E2 administration. Western Blot analysis confirmed these data.

The study confirmed that MRFs expression varie throughout the myogenic process, as previously described by Dedieu, 2002 and Ferri, 2009. In particular MRF4 expression, which is physiologically up-regulated during the differentiation phase, is further increased on myotubes by E2 administration.

Results confirm the over expression of MRF4, already described in adult bovine skeletal muscle cultures treated with E2 (Divari, 2013), and could help to understand the estrogen pathway regulation in muscle hypertrophy

Boland, R., et al. 2008. *Steroids* 73, 859–863.

Dedieu, S., et al. 2002. *Int J Dev Biol*, 46, 235-241

De Jager, N. et al. 2011. *Physiol. Genomics* 43, 467–478.

Divari S., et al., 2013. *Food Chem.* 141, 4358-4366

Ferri, P., et al. 2009. *J. Cell. Biochem.* 108, 1302–1317

Rhoads, R. P., et al. 2009. *Domest. Anim. Endocrinol.* 36, 111–126.

Wyzykowski, J. C. et al. 2002. *Mol. Cell. Biol.*, 22, 6199-6208

Pathology

myogenic factors, estrogen, mouse cell culture

NECROSCOPIC FINDINGS ASSOCIATED WITH EARLY REPLACEMENT IN BREEDING DOES

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In commercial rabbit farms, mortality and culling are of great relevance from the financial viewpoint.¹ Lesions in breeding rabbits are recorded mainly on animals at the end of their reproductive life and the replacement is usually performed based on reproductive parameters without further diagnostic investigations. The aim of this study was to assess the main necroscopic lesions in does replaced from reproduction and not slaughtered.

Sixty-seven dead does were collected from a commercial rabbit farm (Centro genetico Martini s.p.a., S. Maria Codifiume, FE) housing 5000 does, and submitted for postmortem examination. During necropsy, samples for histological and microbiological examinations were also collected. Information about age, number of deliveries and number of mating were recorded. To evaluate the effect of age, does were arbitrarily divided into two categories based on the median of number of deliveries (if available).

The age of does ranged from 115 to 587 days (median 286), the number of deliveries ranged from 0 to 13 (median 2). On postmortem examination, respiratory and genital tracts were the most frequently affected (n = 40; 59.7% each), the digestive system was involved in 30 does (44.77%) and other concomitant lesions, such as traumatic fractures, abdominal effusion, splenomegaly or cardiac injuries were seen in 36 cases (53.73%). Presence of pododermatitis was also observed in 42 animals (62.69%). Genital lesions included 16 uterine torsion (40%), 12 endometritis (30%), 12 uterine retention (30%) and others, such as dystocia or rupture (n= 6; 15%). Uterine torsion was significantly more frequent in does with more than 2 deliveries (P< 0.05). No other significant differences were observed in the 2 sets of animals grouped according to the number of deliveries. Bacteriological examinations performed on randomly selected pleural effusions were positive for *Pasteurella* spp. (n = 2); *Staphylococcus* spp. were isolated from endometritis (n = 2), macerated fetus (n = 1) and pododermatitis (n = 1).

Mortality in does was highest during the first two deliveries, similar results of highest mortality risks in the first three deliveries are reported also in literature.² Respiratory and genital lesions were the most relevant, in contrast with other authors,² who reported a higher prevalence of respiratory and digestive pathologies. Moreover, in our study, uterine torsion resulted the most important lesion associated with increased number of deliveries, although the related pathogenesis is still unclear.

1 Gómez EA (2006): Producción industrial de gazapos: algun puntos críticos. Boletín de cunicultura 145:12-23.

2 Rossel JM & de la Fuente LF (2009): Culling and mortality in breeding rabbits. Preventive Veterinary Medicine, 88:120-127.

Pathology

Necropsy, Rabbit reproduction, Rabbit Pathology

PARASITIC DISEASES IN MEDITERRANEAN JOHN DORY (ZEUS FABER)

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The aim of the present paper was to identify, describe and compare tissue changes due to parasites occasionally found in different subjects belonging to a wild teleost species, Zeus faber.

A total of 28 John dory (*Z. faber*) were examined. 4 fish had been collected during an experimental trawl survey carried out along the southern Tyrrhenian coasts in May 2012. 24 fish were collected from the fish market of Porto Empedocle and were coming from southern Mediterranean sea (Strait of Sicily). Scientific investigations were performed following routine histopathology methods. For parasitology investigation, parasite specimens were fixed in 70% ethanol, clarified by lactophenol and finally observed under the stereoscope. The molecular investigation was performed for anisakid larvae identifications by RFLP-PCR, amplifying ITS1, ITS2 and the 5.8S subunit.

Two fish, out of a total of four coming from Tyrrhenian sea, showed macroscopic parasites on the skin. Particularly, on the external surface of one fish a marine leech (*Hirudinea*) belonging to the genus *Calliobdella* was found. In the second specimen an adult taenia, 24 cm long, was found. The parasite was identified at genus level as *Alloptychobothrium* sp. At macroscopical evaluation of internal organs, within coelomic cavity of the same fish few anisakid larvae encysted in the stomach serosa were seen.

Only a single fish, out of a total of 24 subjects coming from the Strait of Sicily, at macroscopical external examination, showed grossly evident tissue changes. This fish showed a nodular bulge, 1 cm in size, in the left jaw. Histologically, such newborn tissue, was characterized by a granulomatous reaction containing pieces of parasitic bodies surrounded by an external chitinous cuticle; these parasites were identified as copepods, likely belonging to the genus *Chondrachanthus*. At the level of the caudal and the left pectoral fin, several whitish nodules (more than 50), about 1-2 mm sized, were seen as seriated or grouped growths; histologically, such nodules resemble granulomas found in the jaw. Within the coelomic cavity of all the fish a large number of anisakid larvae, identified by PCR as *Anisakis pegreffii*, were detected. Only two fish were negative for parasites. Moreover, in two fish, adult cestoda, belonging to the same genus described above, were detected within the intestinal lumen. Finally, slight fatty liver degeneration was demonstrated in all the examined fish.

By the results here obtained and according to the literature on teleost parasitoses, it can be assumed that parasites are fully integrated in all the different aquatic ecosystem and can be found in both wild and farmed teleost fish, although generally in wild conditions parasites have a low impact and only rarely can cause mortality. Although copepods and leeches are a common finding in the John dory, no data are available on intestinal cestoda. Finally, according to Angelucci et al. (2011), the presence of anisakid larvae in a so high percentage of specimens is in contrast with data recently reported on *Z. faber* fished in Mediterranean sea (Costa et al., 2010).

1) Angelucci G., Meloni M., Merella P., Sardu F., Madeddu S., Marrosu R., Petza F., Salati F. Prevalence of *Anisakis* spp. and *Hysterothylacium* spp. larvae in teleosts and cephalopods sampled from waters off Sardinia. *J Food Prot.* 2011 Oct;74(10):1769-75. doi: 10.4315/0362-028X.JFP-10-482.

2) Costa A., Sciortino S., Reale S., Alio V., Cusimano M., Caracappa S. 2008. Indagine sulla presenza di Nematodi Anisakidae in specie ittiche marine. *Atti X Congresso Nazionale S.I.Di.L.V.* Available at: http://www.sidilv.org/index.php?option~com_docman&task~cat_view&gid~4&Itemid~6. Accessed 10 September 2010.

fish pathology

ZEUS FABER, MEDITERRANEAN SEA, PARASITE

PATHOLOGICAL AND IMMUNOHISTOCHEMICAL EVALUATIONS OF EQUINE INFECTIOUS ANEMIA INFECTION IN MULES

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Following the implementation of a National surveillance program for equine infectious anaemia (EIA), a high seroprevalence was detected among Italian mules population. EIA infection in mules has been studied limitedly. To increase the knowledge about the pathogenesis EIA virus (EIAV) in mules and to better understand their role in the epidemiology of the infection, a study was conducted.

Naturally EIA-infected mules were immunosuppressed. At the end of the observation period, the mules were euthanized, and their organs were examined for gross and microscopic lesions. Furthermore, immunohistochemistry (IHC) was conducted to investigate the distribution of the virus.

At necropsy, no relevant gross lesions were observed. Microscopic examination of the different animals revealed mild multifocal haemorrhages in several tissues, a moderate to severe hemosiderosis in spleen and lymph nodes and mild to moderate lymphoid infiltrates, mainly in kidney, and lesser in liver and in the adrenal glands. Membranous glomerulonephritis was detectable in about 40% of the kidney. In the lungs, a mild interstitial pneumonia characterized by slight hypercellularity and thickening of the alveolar septa was evident. Interestingly, mild lymphocytic myocarditis with fibres degeneration, together with a multifocal, mild to moderate, lymphocytic meningoencephalitis, with perivascular cuffings thicker in the brain stem, were observed.

IHC, performed with a monoclonal antibody anti-p26 protein, detected EIAV infected cells in different tissues, both in sites as well as in the absence of lesions; this is particularly true in the adrenal glands, where a high number of positive reacting macrophages were detected in the medulla, and in the heart, with a lower positivity in the interstitial space. In contrast to what described in horses, the amount of positive cells in spleen seem to be lower. A positive signal was revealed mainly in the periportal areas of the liver, the alveolar septa of lungs and interstitium of the kidney cortex, and generally limited to cells morphologically resembling macrophages.

Microscopic lesions and EIAV localization in mules, highly resembles the infection described in horses. However, differently from what is reported for certain strains of EIAV in horses, no virus was detected in the endothelia. The present description is a preliminary contribution for the study of pathological alterations and EIAV localization in non-horse species.

T. C. McGuire et al. Immunofluorescent localization of equine infectious anemia virus in tissue. *Am J Pathol.* Feb 1971; 62(2): 283–294. Spyrou V et al. Equine infectious anemia in mules: virus isolation and pathogenicity studies. *Vet Microbiol.* 2003 Aug 29;95(1-2):49-59. J. Lindsay Oaks et al. Equine Infectious Anemia Virus Is Found in Tissue Macrophages during Subclinical Infection. *J Virol.* Sep 1998; 72(9): 7263–7269.

Sanità animale, virologia, anatomoistopatologia
EIA virus, Mules, Immunohistochemistry

PRIMARY “AMYLOID TUMOR” OF THE MAMMARY GLAND IN A DOG

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The present report describes an unusual mammary lesion in a dog, with gross and histological features similar to the lesion known as “amyloid tumor of the breast” in the woman.

A fourteen year-old female spayed Dalmatian dog was presented to the practitioner with a large, bilobated lump involving left and right IV mammary gland. The mass was surgically excised and submitted for histopathological examination.

Grossly, two large subcutaneous nodular, firm masses, merging on the median line, expanded left and right IV mammary glands. Histologically, masses were encapsulated and mainly composed of large, coalescing lakes of pale eosinophilic, homogenous hyaline amorphous material, that stained positive with Congo Red (amyloid). Numerous plasma cells with moderate anisocytosis and anisokariosis and rare mitoses were multifocally recognizable. Multinucleated giant cells (MNGCs) with nuclear features similar to plasma cells were occasionally present. Moreover, MNGCs with scant eosinophilic cytoplasm and up to 25 dense nuclei were visible closely associated with amyloid deposits. Moderately atrophic mammary parenchyma was present at periphery. Immunohistochemistry (ABC method) was performed to characterize MNGCs and amyloid, applying anti-Lysozyme; Lambda-light-immunoglobulin-chains and HLA antibodies. Plasma cells and MNGCs were Lysozyme and HLA negative. Lambda-chains stained plasma cells, the majority of MNGCs and amyloid (AL amyloid).

Bilateral mammary extramedullary plasmacytoma with abundant AL amyloid deposition was diagnosed. Clinical workup did not reveal systemic amyloidosis or multiple myeloma. Sixteen months later the dog was humanely killed because of cardiac failure. No signs of multiple myeloma or recurrence of the tumor were reported.

Primary amyloidosis of the breast not associated with mammary carcinoma (amyloidoma), is a rare entity that has been documented in women since 1973, affecting mostly elderly patients, with bilateral involvement and related neither to systemic amyloidosis nor to multiple myeloma. Despite similar gross and histological appearance, some cases have been described as primary amyloidosis, while others as plasmacytoma with massive amyloid deposition

In the canine species, mammary gland amyloid deposition was described in association with mammary carcinoma whereas mammary primary amyloidosis or plasmacytoma with massive amyloid deposition have never been reported so far. To the best of author's knowledge this is the first report in the dog of a bilateral mammary extramedullary plasmacytoma with features consistent with primary amyloidoma of the breast in the woman.

1. Balbino BF, Hernandez FJ. Amyloid tumor of the breast. Arch pathol 1973; 95, 102-105
2. Taniyama H, Kitamura A, Kagawa Y, Hirayama K, Yoshino T, Kamiya S. Localized amyloidosis in canine mammary tumors. Vet Pathol. 2000 Jan; 37 (1): 104-107

Veterinary and comparative pathology
amyloid, dog, mammary gland

SKELETAL MUSCLE EXPRESSION OF MYOSTATIN, IGF-1 AND GATA-2 IN CATTLE TREATED WITH GROWTH PROMOTERS

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Hormones are key regulators of mammalian muscle metabolism both in health and disease. Myostatin (MSTN), a member of transforming growth factor-beta family is a negative regulator of skeletal muscle mass. Several authors reported a direct relationship between myostatin expression and hormones treatment (Santos et al., 2012). Moreover, insulin-like growth- factor 1 (IGF-1) as well as GATA-2 have been broadly implicated in skeletal muscle growth, hypertrophy and regeneration (Musarò et al., 1999). This study aimed to determine the effects of different growth promoters on myostatin, IGF-1 and GATA-2 expression in skeletal muscle of veal calves and beef cattle.

Trial 1: 24 Charolaise beef cattle, randomly divided into 3 groups received: group A (n=6) 5 doses of 17beta-estradiol (20 mg/week/animal, IM), group B (n=6) DEX per os (0.7 mg/day/animal) for 40 days, group C (n=6) prednisolone per os (15 mg/day/animal) for 30 days; and group K1 (n=6) was untreated. Trial 2: 24 Friesian beef cattle, divided in 4 groups were treated as follows: group D (n=8) Revalor-200 subcutaneous implant for 89 days; group E (n=8) Revalor-200 subcutaneous implant for 89 days and dexamethasone per os (0.7 mg/day/ animal) for 40 days; group F (n=8) Finaplix-H subcutaneous implant for 89 days; group K2 (n=8) was the control.

Trial 3: 18 Friesian veal calves, randomly allotted in 3 groups received: group G (n=6) 35 mg/week of 17beta-estradiol, for 6 times; group H (n=6) 175 mg/week of testosterone, for 6 times; and group K3 (n=6) was untreated.

Trial 4: 30 Friesian veal calves, divided in 4 groups received: group L (n=8) was 17beta-estradiol (5 mg/week/animal) for 6 times and brotizolam (0.25 mg/day/animal) for 31 days; group M (n=8) 17beta-estradiol (5 mg/week/animal) for 6 times and dexamethasone (0.4 mg/day/animal) for 31 days; group N (n=8) nandrosol (150 mg/biweekly/animal) for 4 times and ractopamine (80 mg/day/animal) for 31 days; and group K4 (n=6) was untreated.

Samples of the sternocleidomaistodeo muscle were collected from each animal and subjected to quantitative PCR for MSTN, IGF-1 and GATA-2 genes.

No statistical difference was observed in skeletal muscle for MSTN and GATA-2 genes examined in all experimental groups compared to controls.

The IGF-1 expression was significantly up-regulated in the group A ($P<0.01$) as well as in the group F beef cattle ($P<0.01$). Also in veal calves IGF-1 gene expression was significantly up-regulated. In particular, group L and group N gene expression were respectively 2.18-fold increase ($P<0.05$) and 2.48-fold increase ($P<0.01$) compared to controls.

The growth-promoting effects of sex steroids administration in livestock are well known.

IGF-1 is a potent anabolic factor that can activate myocyte proliferation and differentiation, leading to muscle hypertrophy. This study demonstrate the up-regulation of IGF-1 in skeletal muscle of cattle treated with estrogenic and androgenic molecules, as reported in humans and rats (Gentile et al., 2010; Pöllänen et al., 2010). However, in our study the increase of IGF-1 did not induce the up-regulation of mRNA GATA-2, as previously reported by Musarò and colleagues (1999). Further studies are needed to understand the role of sex steroids in the regulation of skeletal muscle hypertrophy.

Gentile et al., (2010). *J. Mol. Endocrinol.* 44, 55–73.
Musarò et al., (1999). *Nature.* 400, 581-585
Pöllänen et al., (2010) *Growth Horm. IGF Res.* 20, 372–379.
Santos et al., (2012). *Neuromuscular Disorders.* 22, 864.

pathology

skeletal muscle, growth promoters, cattle

SPONGY POLIOENCEPHALOPATHY IN TWO BELGIAN MALINOIS PUPPIES

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Spongy degeneration is seen in prion diseases and in a variety of progressive, invariably lethal neurodegenerative disorders. In various dog breeds it can involve the white or the grey matter. Amongst those involving the gray matter, cases are described in Bull Mastiff, Rottweiler, Saluki, Cocker Spaniel and Australian Cattle dog. A hereditary spongy degeneration has been reported in Malinois puppies, which suffer from congenital tremors with ataxia. In this report two cases of spongy degeneration in Malinois puppies are described, emphasizing the morphological and immunohistochemical features of the CNS lesions.

Two unkindred Belgian Malinois puppies were presented for mild depression, tremors of the head, cerebellar ataxia, and progressive ascending paraparesis. Complete blood count and serum biochemistry were normal. Serology for canine distemper virus, Toxoplasma spp. and Neospora spp. was negative. Cerebrospinal fluid showed no abnormalities. Because of the progressive ataxia and development of proprioceptive deficits on all limbs, and poor prognosis, the owners elected for humane euthanasia.

Brain, spinal cord, and samples of major organ were fixed in 4% buffered formaldehyde. Transverse sections of brain and spinal cord were embedded in paraffin, sectioned at 5 µm, and stained with haematoxylin and eosin, Luxol fast blue. Immunohistochemistry for 2',3'-cyclic nucleotide-3'-phosphohydrolase (CNPase), Olig2, glial fibrillary acidic protein (GFAP), phosphorylated neurofilaments, heat shock protein (HSP)70 and ubiquitin were performed on selected sections.

No lesions were detected at gross examination.

Histological lesions were restricted to the brain and were characterized by the presence of bilateral and symmetric large interneuronal vacuoles in the area of cerebellar nuclei, granular cell layer and foliate white matter of the cerebellum. Phosphorylated neurofilaments revealed numerous axonal spheroids; no ubiquitin-positive intracellular deposits were detected and a normal myelin pattern was evident with Luxol Fast Blue and CNPase immunohistochemistry. Oligodendrocytes showed a normal nuclear reaction for Olig2.

Lesions detected in these puppies are similar to previously described cases (1,2); the predominant lesions were found in cerebellar nuclei and flocculonodular cerebellar cortex, whereas spongy degeneration was not detected in the cerebral cortex. These are the first cases of spongy polioencephalopathy of Malinois dogs described in Italy, and the first time that immunohistochemical features are reported.

1.Cachin et al., 1991, 2.Kleiter et al., 2011

neuropathology

neuropathology, malinois, immunohistochemistry

STEM CELL MARKERS IN BENIGN AND MALIGNANT CANINE PROSTATE TISSUES: AN IMMUNOHISTOCHEMICAL INVESTIGATION

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Canine prostatic carcinoma (PCa) is considered a relevant model for studying advanced, hormone refractory PCa in men. It has been proposed that cancer contains a minor population of cells that can self-renew while simultaneously giving rise to tumour cells (cancer stem cells). Survivin is an acknowledged cancer therapy-resistance factor overexpressed in several tumour types, proposed as a valid cancer biomarker for human prostatic cancer for an early screening for malignancy. Sox9 is a stem cell marker expressed in several adult tissues, required for prostate development. Accumulating evidence indicates that it contributes to the development of human PCa. No studies have been published concerning the immunolocalization of survivin and Sox9 in canine prostatic hyperplasia (BPH) and neoplasia. AIM: to evaluate the patterns and levels of expression of survivin and Sox9 in canine BPH and PCa, in order to correlate their expression with malignant histological features.

immunohistochemistry with specific antibodies in a set of canine BPH and PCa.

Survivin nuclear and rare cytoplasmic immunostaining were present among the basal/reserve cell layer of normal and hyperplastic prostatic lobules. An increase of survivin expression was observed in PCas compared with BPHs. 6/11 PCas showed rare positive nuclei, mainly among the "basaloid" neoplastic cells in the areas with a tubular-papillary arrangement. In the areas with a solid pattern the cytoplasmic immunostaining was more diffuse. Sox9 expression was absent in normal prostatic glands and in all BPHs. 6/9 cases of PCa were highly positive.

Based on the role of survivin as a stem cell marker and the main role in proliferation of nuclear survivin, the positive cells among basal cell layer in normal and HBP cases could represent transit amplifying cells maintaining some stem cell proprieties. The increased survivin expression in Pcas would indicate the molecule as a valid prognostic marker. The absence of expression of Sox9 in the normal gland and all the BPHs suggests that Sox9 is not a stem cell marker of canine adult prostatic stem cells. The high expression observed in PCas clearly suggests an important role of the molecule in canine prostatic carcinogenesis and malignant progression. Further studies should be done in order to confirm this hypothesis.

Argyle DJ. Prostate cancer in dogs and men: a unique opportunity to study the disease. *Vet J.* 2009; 180: 137-138.

Liao CP, et al. Cancer stem cells and microenvironment in prostate cancer progression. *Horm Cancer.* 2010;1(6):297-305.

Li XL, et al. On-chip selective capture of cancer cells and ultrasensitive fluorescence detection of survivin mRNA in a single living cell. *Lab Chip.* 2013 Oct 7;13(19):3868-75.

Huang Z, et al. Sox9 is required for prostate development and prostate cancer initiation. *Oncotarget.* 2012;3(6):651-63.

Disciplinare

dog, stem cell, prostate cancer

STUDY OF THE HIPPO-TRANSDUCER TAZ IN CANINE AND FELINE MAMMARY TUMORS: PRELIMINARY DATA

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Among others, HIPPO signaling was recently indicated as a key regulator in both physiological and neoplastic cell growth, differentiation, and survival. One of the major output of HIPPO pathway is the inhibition of the coactivator transducer TAZ which controls cell growth, proliferation and survival but also cell differentiation, stem cell renewal, reprogramming, and patterning. TAZ activity correlates with high grade, metastasis, and Cancer Stem Cells (CSCs) content in human breast cancer. Particularly, TAZ is required to sustain self-renewal and tumor-initiation capacities in breast CSCs, moreover its biological activity is associated with epithelial-mesenchymal transition which disrupts TAZ inhibition, increasing its activity. The aim of our study was to evaluate TAZ expression in canine (CMCs) and feline mammary carcinomas (FMCs) particularly investigating correlation to grading and histopathological morphology.

We investigated TAZ expression in 60 CMCs and FMCs and surrounding non-neoplastic mammary gland by immunohistochemistry. The selected mammary tumors equally belonged to the following histopathological groups: simple tubular carcinomas grade I, simple carcinomas grade III, ductal-associated tumors grade I (cats), complex carcinoma grade I (dog), simple-carcinoma-and-myoepithelioma (SCMM) grade I (dog). Three different scoring systems were applied and compared.

TAZ was found to be diffusely overexpressed in grade III simple CMCs and FMCs. In addition, basal cells were TAZ positive both in neoplastic and hyperplastic canine glandular tissue. Interestingly, a moderate positivity was detected also in basal undifferentiated cells of ductal-associated FMCs. Canine SCMMs showed higher TAZ expression compared to complex CMCs.

These preliminary results indicate that also in CMCs and FMCs TAZ may be an important pathway transducer for aggressiveness and might regulate basal cells proliferation and fate. Further samples collection and analyses are required to give insights into its role and regulation in mammary cancers and eventually its additional prognostic information.

Liu S, Wicha MS. Targeting breast cancer stem cells. *J Clin Oncol*, 2010 1;28(25):4006-12.

Cordenonsi M, et al. The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. *Cell*, 2011 11;147(4):759-72.

Ferletta M, et al. Canine mammary tumors contain cancer stem-like cells and form spheroids with an embryonic stem cell signature. *Int J Dev Biol* 2011;55(7-9):791-9.

Blacking TM, et al. CD44 is associated with proliferation, rather than a specific cancer stem cell population, in cultured canine cancer cells. *Vet Immunol Immunopathol*, 2011 15;141(1-2):46-57.

Pang LY, et al. Feline mammary carcinoma stem cells are tumorigenic, radioresistant, chemoresistant and defective in activation of the ATM/p53 DNA damage pathway. *Vet J*, 2013;196(3):414-23.

Anatomia patologica veterinaria - Tumori mammari

Hippo, TAZ, Mammary tumor

THE ASSESSMENT OF FEED EFFECTS ON LIVER AND INTESTINE OF REARED FLATFISH: WHAT IS THE BEST STANDARD ANATOMOPATHOLOGIC PROTOCOL?

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This presentation highlights the importance of using standardized methods by the fish pathologists to assess the effects of feed on reared flatfish. The liver and intestine are the most important organs for the digestion and absorption of nutrients from feed, and monitoring their histological structure is of fundamental importance in assessing the effects of nutrient mixtures. A critical analysis of the histological methods on feed effects will be presented, aiming to suggest a standardized protocol that should be adopted, on the basis of the advantages and disadvantages that have been encountered in the course of our recent research trials.

Six feeding trials have been carried out on juvenile common sole (*Solea solea*), Senegalese sole (*Solea senegalensis*) and turbot (*Psetta maxima*) (fed diets with increasing dietary protein levels; 2, 3 increasing dietary plant protein in replacement of fishmeal; 4 increasing lipid levels 5 and increasing mussel meal 6, respectively).

Routine histology (haematoxylin-eosin stain on formalin-fixed and paraffin-embedded sections) was carried out to check degenerative and/or inflammatory changes. In the liver, as well as the graduation of the lipid content, further histochemical techniques have been applied in order to characterize the type of intracellular accumulation (OilRedO, Toluidine blue, PAS) and transmission electron microscopy (TEM), while in intestine in situ techniques to assess the cellular turnover and trophism of epithelial mucosa (anti-PCNA immunohistochemistry and TUNEL method for apoptosis) evaluated by image analysis and TEM were employed.

The routine histological evaluation is helpful only to demonstrate degenerative-inflammatory changes and its usefulness increases when combined with a grading system for the lipidic content of the liver that revealed the most frequent degeneration assessed. To confirm the lipidic content, Toluidine Blue on semithin sections revealed better than OilRedO which run on frozen sections that require a more difficult management of the samples.

IHC on intestinal sections and the evaluation of cellular kinetics and mucosal turnover are necessary tools to assess the influence of a diet on mucosal trophism.

For both tissues TEM is the elective method to further characterize the type of intracellular accumulation and the early signs of cell damage.

On the basis of our experience it is necessary to adopt a standardized approach to evaluate the effects of experimental diets on liver and intestine; in particular, investigations in liver require Toluidine Blue on semithin sections and TEM, while for intestine IHC together with digital image analysis and morphometry are suggested in addition to routine histology.

1Raskovic et al, 2011; 2Gatta et al., 2011; 3Bonaldo et al., 2011; 4Mandrioli et al., 2012; 5Bonvini et al., 2014 (Proceedings of ISFNF); 6Bonaldo et al., 2014 (Proceedings of ISFNF).

fish pathology

flatfish, liver and intestine, histopathology

UTERINE LEIOMYOMA ASSOCIATED WITH CYSTIC ENDOMETRIAL HYPERPLASIA-PYOMETRA COMPLEX IN ASIAN ELEPHANT (*ELEPHAS MAXIMUS*).

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Leiomyoma is the most common uterine tumor in women, occurring also in many domestic animals³. Most captive female elephants are nulliparous and aged and many have endometrial diseases, factors that may hinder fertility^{1,4}. We here describe a case of uterine leiomyoma associated with cystic endometrial hyperplasia—pyometra complex occurred in a 42-year-old female Asian Elephant (*Elephas maximus*), maintained at the Rome Zoological Garden.

The Elephant, nulliparous, occasionally expelled necrotic material from uro-genital opening for nine years before death. Histologically, this material was always identified as an exudate in the process of organization, resulting positive to *Escherichia coli*. Repeated cycles of antibiotic (moxifloxacin) were made. Two days before death, the animal showed systemic malaise, weight loss and ataxia.

At necropsy, the uterus was significantly increased in volume and abundant purulent material was present in the lumen; the wall was considerably thickened, with the presence of numerous cysts mixed with nodular lesions of variable size, whitish in color and with increased compactness. Masses of necrotic material could also be seen adhering to the wall or free in the lumen. Histologically, numerous nodular lesions that showed neoplastic proliferation of smooth muscle cells in diffusion-type arrangement were observed. The mitotic index was low and there was absence of nuclear atypia. In addition, diffuse hyperplasia of the endometrium with formation of numerous cysts containing liquid amorphous eosinophilic were seen. In some areas there were extensive purulent-necrotic foci. Histological sections stained with Masson's method emphasized the presence of smooth muscle cells and, immunohistochemically, neoplastic cells were constantly positive for vimentin and for α -smooth muscle actin, while they were negative for cytokeratin and S-100. In this case report, the features of the neoplastic cells and the positivity for Masson's method, vimentin and for α -smooth muscle actin clearly express the origin of the tumor from smooth muscle². In addition, *Candida* sp., *Aeromonas* sp. and *E. coli* were isolated from the uterus.

To our knowledge, this is the first case of uterine leiomyoma associated with cystic endometrial hyperplasia-pyometra complex described in an Asian Elephant.

1)Aupperle et al., Chronic endometritis in an Asian elephant (*Elephas maximus*). *J Zoo Wildl Med.* 2008;39(1):107-10. 2)Ilha et al., Uterine lesions in 32 female miniature pet pigs. *Vet Pathol.* 2010;47(6):1071-5. 3)Meuten DJ, *Tumors in Domestic Animals*, 4th Edition Iowa State Press, 2002 Ames, Iowa, USA, 4)Sapundzhiev et al., Fibroleiomyoma in elephant uterus. *J Vet Med A Physiol Pathol Clin Med.* 2007;54(9):499-500. 5)Schaudien et al., Omental leiomyoma in a male adult horse. *Vet Pathol.* 2007;44(5):722-6.

ONCOLOGIA ANIMALI ESOTICI

UTERINE LEIOMYOMA, CYSTIC ENDOMETRIAL H, *ELEPHAS MAXIMUS*

WINTER DISEASE IN ASSOCIATION WITH INTESTINAL NON-FORMING XENOMA MICROSPORIDIA IN GILTHEAD SEABREAM (SPARUS AURATA)

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Winter disease (WD) is a multifactorial disease found primarily in sea caged gilthead sea bream (*Sparus aurata*) along the Mediterranean coast.^{1,2} An emaciative syndrome has been recently observed in Spain and *Enterospira nucleophila*, a new microsporidian species of the family Enterocytozoonidae has been described; these intracellular, non-forming xenoma microsporidia, opportunistic in nature exploit a weakened host immune status, as it could happen in WD.⁶ Teleost intestine contains Mast Cells (MCs), whose functional properties are similar to those of mammalian mast cells;⁵ recruitment of MCs to sites of persistent inflammation is a general response in parasites-affected fish. An increased number of the MCs is also reported in WD-affected fish.^{3,4} In December 2013 a disease outbreak in sea caged gilthead seabream in Italy, affecting 0+ year fish occurred. The aim of this study was to evaluate the histopathological changes related to these two conditions, to characterize the MCs by immunohistochemistry and the microsporidia by PCR.

From twenty gilthead sea bream intestinal tracts were fixed in buffered formalin at the sample site and sent to DIMEVET. Routine histological sections were obtained; Luna stain was also performed. Immunohistochemistry with CD117 antibody (1:100, Dako) was also carried out. Intestinal tissue was also subjected to molecular analysis; a fragment of the 18S rDNA was amplified and then sequenced.

Hindgut showed a moderate dilatation of the lumen in association with whitish casts, similar to the milk-like mucous casts reported in WD outbreaks.^{1,2} A severe mucosal atrophy with total folds flattening was present; within lamina propria and submucosa a mild to moderate MCs hyperplasia and mild mucous cells hyperplasia were observed. Multifocally, within enterocytes and rodlet cells, the nucleus and/or cytoplasm contained microsporidian spores, more evident with Luna stain. The sequences obtained from intestines showed 99.9% identity with *E. nucleophila*. Within perivisceral exocrine pancreatic acini, focal necrosis and MCs infiltration have been observed, as reported by other authors during WD outbreaks.

MCs are interpreted as “standing force” in particular tissues consistently exposed to pathogens, in contrast to a “mobilization force” that has been an advantage in those being exposed to noxious agents only occasionally.⁷

The severe mucosal flattening could be interpreted as an effect of a chronic insult, not only related to the microsporidia infection; other concurrent predisposing factors as those reported in WD could be then considered into the development of an overt pathology.

¹Birincioglu et al, 2013; ²Colorni, 2011; ³Contessi et al,2006; ⁴Ibarz et al, 2010; ⁵Lauriano et al.,2012;⁶ Palenzuela et al.,2014; ⁷Reite and Evensen,2006.

fish pathology

winter disease, *sparus aurata*, microsporidia

DILATION OF THE VERTEBRAL CANAL IN A PARETIC BOA CONSTRICTOR (BOA CONSTRICTOR IMPERATOR) AFFECTED BY PROLIFERATIVE OSTEOARTHRITIS

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Proliferative vertebral lesions are a common finding in snakes and have been classified into three distinct categories: bacterial osteoarthritis, including active septic osteomyelitis or osteoarthritis with multiple inflammatory foci, and non-bacterial degenerative ankylosing osteoarthritis. Computed tomography (CT)-guided biopsy could be especially useful to rule out active bacterial infections.

AIM. To describe a case of proliferative osteoarthritis and dilation of the vertebral canal in a paretic boa constrictor (*Boa constrictor imperator*).

Radiography and CT-guided biopsy, haematoxylin-eosin staining and histopathological examination.

A 1-year-old, male boa constrictor presented with paresis of the trunk originating cranial to the cloaca. Radiographies were consistent with proliferative bone lesions involving several vertebrae. CT demonstrated the presence of lytic lesions, characterized by widening of the spinal canal. CT-guided biopsies of the lesions were performed. Histology was consistent with bacterial osteomyelitis and osteoarthritis. Gram-negative bacteria (*Salmonella* sp.) were isolated from cultures of the biopsies. A medical treatment was attempted for several weeks without clinical or radiographical improvements. The animal was euthanized and necropsy confirmed the findings observed upon CT. The main lesion comprised 3 vertebral bodies and the vertebral foramen appeared dilated and filled with friable gray/green to brown (purulent/necrotic) material, surrounded by abnormal vertebral bone. Another lesion, which was clinically undetected, consisted in accumulation of purulent material on the ventro-lateral aspect of the vertebra, involving the left rib. Histologically, the vertebral foramen was filled with degenerated heterophils and necrotic material, with bacterial colonies, completely replacing the spinal cord, surrounded by a rim of reactive macrophages and fewer multinucleated giant cells.

Dilation of the vertebral canal is an uncommon finding in snakes presenting proliferative osteoarthritis and possibly carries poor prognosis. Considering that proliferative vertebral lesions may be secondary to bacterial or other causes, and blood culture is not one hundred per cent sensitive, CT-guided bone biopsies may be useful to reach a definitive diagnosis.

Frye, F. L., & Carney, J. Osteitis deformans (Paget's disease) in a boa constrictor. *Veterinary medicine, small animal clinician* 1974; 69,186-188.

Isaza, R., et al. Proliferative osteoarthritis and osteoarthritis 208 in 15 snakes. *Journal of Zoo and Wildlife Medicine* 2000; 31, 20-27.

Kiel, J. L. Spinal osteoarthropathy in two southern copperheads. *The Journal of Zoo Animal Medicine* 1977; 8, 21-24.

Vignoli, M., et al. Computed tomography-guided fine-needle aspiration and tissue-core biopsy of bone lesions in small animals. *Veterinary Radiology & Ultrasound* 2004; 45, 125-130.

generale

snake, *Salmonella*, osteoarthritis

A.P 02 Aipvet Poster

A CASE OF CANINE THYROID CARCINOMA WITH HETEROTOPIC OSSIFICATION AND EXTRAMEDULLARY HEMOPOIESIS

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The present report describes a case of canine thyroid carcinoma with heterotopic ossification and extramedullary hemopoiesis in a 10 years old mongrel dog. Few anamnestic data were available: an apparently healthy dog was referred with the only symptom of polyphagia. A solid mass, not adherent to surrounding tissue, was palpable on the left side of the neck. The mass was surgically removed, fixed in 10% buffered formalin and send to the pathology division of our Department to be histologically examined. Grossly neoplastic mass was about 3x5 cm, well circumscribed, defined by a thin fibrous capsule. On cross section the mass had a brown-tan colour with a greyish, firm to hard, central area, grossly consistent with bone tissue.

Before trimming, the mass was therefore immersed for 1 week in a decalcifying acid. Then, samples were collected, passed through graded alcohols, clarified in xylene and paraffin embedded. From paraffin blocks, sections were obtained and stained with Haematoxylin and Eosin.

Histologically, the tumor was composed of irregular, small follicular structures, nests and solid lobules of polygonal cells sustained by a variable amount of collagenous stroma. Neoplastic cells had poorly defined cells borders, moderate amount of eosinophilic cytoplasm and round to oval vesiculous nucleus with prominent nucleolus. Anisocytosis and anisokaryosis were moderate and mitotic figures ranged from 0 to 1 X HPF. Small multifocal necrotic foci and focal haemorrhages were also present scattered throughout the tumor. The central area of the tumor was composed of mature trabecular bone. Intertrabecular spaces were filled by both adipose cells and hemopoietic cells. A histological diagnosis of thyroid carcinoma with heterotopic bone formation and extramedullary hemopoiesis was posed.

Heterotopic ossification is a well recognized phenomenon involving organs and tissues affected by various pathologic processes, i.e. ischemia, hematomas, degenerative changes, chronic inflammation and, less frequently, tumors. Few cases of thyroid tumors with heterotopic ossification and extramedullary hemopoiesis have been described in human species. In the canine species, the presence of focal mineralization or scattered bone formation within thyroid carcinomas has been reported but poorly documented. Scattered calcifications have been described in normal thyroid gland while ossification has been reported in a single case of thyroidal carcinosarcoma. The present report represents the first description of a canine thyroid carcinoma with wide areas of mature bone formation (heterotopic ossification) and extramedullary hemopoiesis. Mechanisms of heterotopic ossification are still unknown, however the presence of inducible osteoprogenitor cells, of heterotopic environment conducive to osteogenesis and of inductive signalling such as bone morphogenetic proteins has all been evocated in the pathogenesis of this condition.

Capen CC. Tumors of the endocrine glands. In: Meuten DJ, Tumors in domestic animals, IV eds. Meuten Donald J 2002; pp. 642

Grubor B, Haynes JS. Thyroid carcinosarcoma in a dog. *Vet Pathol* 2005; 42(1):84-7

Liu K, Tripp S, Layfield LJ. Heterotopic ossification: review of histologic findings and tissue distribution in a 10-year experience. *Pathol Res Pract* 2007;203(9):633-40

Na KY, Kim HS, Lee SK, Jung WW, Sung JY, Kim YW, Park YK. Papillary thyroid carcinoma with bone formation. *Pathol Res Pract* 2013 15;209(1):14-8

Pontikides N, Botsios D, Kariki E, Vassiliadis K, Krassas GE. Extramedullary hemopoiesis in a thyroid nodule with extensive bone metaplasia and mature bone formation. *Thyroid* 2003; 13(9):877-80

Westhoff CC, Karakas E, Dietz C, Barth PJ. Intrathyroidal hemopoiesis: a rare histological finding in an otherwise healthy patient and review of the literature. *Langenbecks Arch Surg* 2008; 393(5):745-9

Pathology oncology canine
thyroid carcinoma, dog, heterotopic

A CASE OF PAPILOMAVIRUS-ASSOCIATED TONGUE ADENOCARCINOMA IN A CAPTIVE WHITE RHINOCEROS

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Papillomaviruses induce hyperplastic and tumoral lesions in different animal species⁴. We describe a case of Papillomavirus-Associated adenocarcinoma which occurred in a 41-year-old male white rhinoceros, maintained at the Zoological Garden of Pistoia, Tuscany^{3,5}.

The rhinoceros showed weight loss and difficulty in chewing the hay for three months before death². At necroscopy, the most relevant finding was a lesion that involved the tongue, characterized by erosion of the mucosa with increased involvement of the dorsal surface. The cut surface showed lardaceous appearance with escape of purulent material.

Histologically, the mass predominantly involved the submucosa and muscular portion of the tongue, resulting in closely packed large, medium, and small glands consisted of columnar cells without mucous cells. Tumor cells exhibited amphophilic to pale eosinophilic cytoplasm, high nucleo-cytoplasmic ratio and medium mitotic rate. No squamous differentiation was noted, and the squamous epithelium immediately adjacent to the adenocarcinoma showed slight perinuclear halos suggestive of PV-related changes.

The tumor cells showed diffuse staining for A1-A3 pan-keratins, but staining for keratin 7 (CK7) clearly separated the adenocarcinoma from the adjacent non-neoplastic squamous epithelium. Keratin 19, keratin 20 and MUC-5AC were negative. Immunohistochemical staining performed with a monoclonal antibody against papillomaviruses evidenced a strong nuclear immunoreactivity only in glandular epithelium similarly to CK7. DNA extracted from paraffin-embedded tissue was tested by PCR using degenerated primers (FAP 59-64), amplifying common gene region of papillomaviral L1. The PCR result was an amplicon of 480 bp. Sequence similarity analysis with the BLAST tool of the National Center for Biotechnology Information and Papillomavirus Episteme tool showed that this fragments belongs to new putative PVs, not yet characterized for rhinoceros.

To our knowledge this is the first case of Papillomavirus-Associated non-salivary gland-type adenocarcinoma arising in the base of the tongue in a captive white rhinoceros.

1) Batista et al. Entropy-based approach for selecting informative regions in the L1 gene of bovine papillomavirus for phylogenetic inference and primer design. *Genet Mol Res.* 2013;12(1):400-7. 2) Goodman et al., Successful treatment of a squamous cell carcinoma in a white rhinoceros, *Ceratotherium simum*. *Vet Dermatol.* 2007;18(6):460-3. 3) Naik et al., Squamous cell carcinoma in an Indian rhinoceros, *Vet Rec.* 1986;118(21):590-1. 4) Roperto et al., Bovine papillomavirus type 2 (BPV-2) E5 oncoprotein binds to the subunit D of the V₁-ATPase proton pump in naturally occurring urothelial tumors of the urinary bladder of cattle. *PLoS One.* 2014 Feb 24;9(2):e88860. eCollection 2014. 5) Suzuki et al. Carcinosarcoma in a white Rhinoceros (*ceratotherium simum simum*). *J Vet Med Sci.* 2013;75(12):1657-60.

ONCOLOGIA ANIMALI ESOTICI

PAPILLOMAVIRUS, WHITE RHINOCEROS, ADENOCARCINOMA

A CASE OF SYSTEMIC CORONAVIRUS-ASSOCIATED DISEASE IN A DOMESTIC FERRET IN ITALY.

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Since 2006 a Coronavirus-associated visceral disease with clinicopathologic features resembling the “dry form” of Feline Infectious Peritonitis, has been recognized in ferrets (1,2). Confirmed cases have been reported in Spain, USA, UK and Japan (3,4,5). The present work describe the first confirmed case of Systemic Coronavirus-associated disease in a domestic ferret in Italy, with description of clinicopathologic findings.

An 8 months-old, entire male ferret (*Mustela putorius furo*) was referred for weakness and coughing. Clinical examinations showed fever, enlarged retropharyngeal lymph nodes and heart murmur. Hemato-biochemical analysis demonstrated anemia, leucocytosis, hyperproteinemia and hyperglobulinaemia (polyclonal gammopathy). Imaging revealed generalized lymphadenomegaly, severe splenomegaly and multifocal nodular renal lesions. Kidney cytological smears were compatible with granulomatous disease. Histology from spleen, mesenteric lymph node and kidney biopsies was consistent with pyogranulomatous inflammation. Due to deterioration of the condition, the ferret was euthanized five months after the first presentation and post-mortem examination revealed disseminated nodular lesions, mainly localized in kidney, spleen, mesenteric and mediastinal lymph nodes, diaphragm and lung. Histology confirmed a systemic pyogranulomatous disease. Immunohistochemistry was performed using anti-FCoV monoclonal antibody and positive staining for Coronavirus antigen was detected in the cytoplasm of macrophages in the pyogranulomas, providing a definitive diagnosis of ferret systemic coronavirus-associated disease. In conclusion ferret systemic Coronavirus-associated disease should be considered in the differential diagnosis of young ferrets presenting with enlarged lymph nodes, hyperproteinemia and hyperglobulinaemia and histology and immunohistochemistry represents the gold standard for a definitive diagnosis.

1. Martinez J, Reinacher M, Perpignan D, Ramis A. Identification of Group 1 Coronavirus Antigen in Multisystemic granulomatous lesions in ferrets (*Mustela putorius furo*). *J Comp Pathol.*2008;138:54-58.
2. Perpignan D, Lopez C. Clinical aspects of systemic granulomatous inflammatory syndrome in ferrets (*Mustela putorius furo*). *Vet Rec.*2008,162:180-184.
3. Garner MM, Ramsell K, Morera N, et al. Clinicopathologic features of a systemic coronavirus-associated disease resembling feline infectious peritonitis in the domestic ferret (*Mustela putorius*). *Vet Pathol.*2008,45:236-246.
4. Michimae Y, Mikami S, Okimoto K, et al. The First Case of Feline Infectious Peritonitis-like Pyogranuloma in a Ferret Infected by Coronavirus in Japan. *J Toxicol Pathol.*2010,23:99-101.
5. Graham E, Lamm C, Denk D, et al. Systemic coronavirus-associated disease resembling feline infectious peritonitis in ferrets in the UK. *Vet Rec.*2012,171:200-201

Patologia del furetto

Ferret, Coronavirus, granulomatous diseases

COMPARISON OF DIFFERENT PROCEDURES TO ISOLATE FELINE PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS) FROM SMALL VOLUMES OF BLOOD

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Studies on leukocytes isolated from feline blood do not provide details on the performances of isolation techniques.¹ From the few numerical data available, however, it can be assumed that the purity of isolated cell populations is high but their recovery rate is low.² Therefore, large volumes of blood (difficult to collect from cats with spontaneous diseases) are required to obtain enough leukocytes for in vitro studies. The aim of this study was to assess the performances of isolation techniques on small volumes of feline blood.

Blood samples (1 to 5 mls) were drawn from clinically healthy cats and placed in EDTA-coated tubes. Fifteen sessions of tests (10 using Ficoll, 5 using Percoll) were performed. In 9 cases cells were further separated by adherence on Petri dishes (PD) and in 5 cases using iron-labelled monoclonal antibodies against leukocyte antigens followed by magnetic sorting (MS). Cell purity (i.e. the percentage of each population) and recovery (i.e. the percentage of cells of each population recorded after isolation compared with blood) were then calculated.

The purity of lymphocytes was significantly higher ($P=0.015$) with Ficoll (79.6 ± 3.3) than with Percoll (61.0 ± 12.0); the purity of monocytes was low, and significantly higher ($P=0.015$) with Percoll (32.7 ± 13.5) than with Ficoll (9.0 ± 1.6). The recovery rate of lymphocytes was low, and significantly higher ($P<0.001$) with Ficoll (54.7 ± 27.5) than with Percoll (12.0 ± 4.2). The recovery rates of monocytes recorded with Ficoll (59.4 ± 34.7) or Percoll (38.5 ± 9.38) were not significantly different. The purity of cell types in PD was not morphologically determinable. However, assuming that at least 90% of adherent and non adherent cells were monocytes and lymphocytes, respectively, the recovery rate for both the populations was lower than 10% in most cats. The purity after MS was generally high, but the recovery rate was variable and the number of yielded cells very low.

In conclusion, none of the techniques applied in this study provides good performances in terms of number of cells, purity and recovery rate, when applied to small volumes of blood. This suggests that the isolated cells could not be representative of the population in blood, and limits the use of these 3 techniques in cats with spontaneous diseases. Therefore, cell functions in spontaneous feline disease should be investigated in whole blood rather than on isolated cell populations.

1. Kipar A et al. *Vet Immunol Immunopathol* 98:215-221; 2004
2. Roberts RL and Gallin JI. *Blood* 65:433-440; 1985

Veterinary clinical pathology
Leukocyte isolation, Feline, in vitro studies

COMPREHENSIVE MANAGEMENT OF A PITUITARY ADENOMA WITH MALIGNANT FEATURES IN A MALTESE DOG

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The aim of the study is to describe the long-term survival of a dog affected by an ACTH producing-pituitary adenoma treated by surgery, medication and radiation therapy.

A 7-yr-old old male Maltese dog was referred to DIMEVET for epileptic seizures, polyuria, polydipsia, polyphagia and symmetrical alopecia. Pituitary-dependent hypercortisolism was diagnosed by endocrine tests. Computed tomography (CT) revealed a pituitary mass with a P/B ratio 0.75, reference <0.311 that was treated with transsphenoidal hypophysectomy; Immunohistochemistry of the surgical specimen confirmed an ACTH-producing adenoma. Hypercortisolism went into remission for three years but then polyuria/polydipsia and alopecia reappeared and CT scan revealed a small sellar abnormal structure (interpreted as pituitary tissue regrowth). Hypercortisolism was confirmed by endocrine tests and medical treatment with trilostane was started, with good control of the clinical signs for one year, until the occurrence of obtundation, seizures and stupor. CT scan showed a sellar mass and radiation therapy was performed with a protocol of 20 fractions of 2.25 Gy. Neurological signs regressed and trilostane treatment was continued with good control of the disease. Six years after the first admission, the dog developed lumbosacral pain and inability to walk and was euthanatized for animal welfare reasons. Histological sections were stained with H&E and PAS and immunostained with antibodies against ACTH, MSH, GH, C-erb-B2 and Ki67.

Macroscopically the formalin fixed brain revealed grey tissue in the pituitary region extending caudally to the mesencephalon. Histologically, unencapsulated neoplastic tissue in contiguity with the meninges and infiltrating the neuropil was detected; neoplastic cells arranged in islands and cords were embedded in a rich amorphous eosinophilic extracellular matrix; neoplastic cells showed an intensely eosinophilic cytoplasm. The nucleus was vesicular, often two or three nuclei were seen, with chromatin margination and a prominent nucleolus. Anisocytosis and anisokaryosis were moderate. Mitotic figures were three in ten random selected fields at 400x magnification. A diagnosis of a recurring infiltrative ACTH-adenoma was made; Ki67 labelling index was 4,8%.

Pituitary tumors that recur or progress despite resection and radiotherapy are often termed "atypical" adenomas, as they do not appear overtly malignant by histological criteria, but exhibit aggressive biologic behavior, have a Ki67 labelling index >3%, p53 immunoreactivity.² Reports of invasive canine pituitary tumors are rare; this is the first report of a long-term survival of a dog affected by this type of tumor that was treated by surgery and subsequently by radiation therapy. Comprehensive management of pituitary adenomas using the various treatment modalities may significantly prolong the dog's life.

1Kooistra et al., 1997; 2Mamelak et al., 2011.

neuropathology, endocrine pathology

pituitary adenoma, dog, survival

COORDINATED IMMUNE RESPONSE OF MEMORY AND CYTOTOXIC T CELLS TOGETHER WITH IFN- γ SECRETING CELLS AFTER PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) NATURAL INFECTION IN CONVENTIONAL PIGS.

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Porcine reproductive and respiratory syndrome virus (PRRSV) infection in the field usually dampens the pig immune system both as innate and acquired immunity. The extent of immune dysregulation and/or depression depends on the virulence of the PRRSV isolate, the intrinsic ability to interact with the immune system and on the age of the animals. (1)

The present study aims at evaluating the antibody and cellular immune response in pigs naturally infected by PRRSV in order to highlight the immune modulation.

Twenty conventional pigs were selected from a herd with a history of PRRSV infection and monitored for 22 weeks from weaning (4 weeks of age) through the fattening phase (up to 26 weeks of age). The pigs were divided in two groups: a group was naturally exposed to PRRSV infection (N=10, PRRSV-exposed) by cominglement with infected resident animals and the unexposed group was used as control (N=10, C).

Blood samples were collected 2 weeks apart and PRRSV infection was detected by quantitative PCR in serum. The humoral immune response was quantified as total serum PRRSV-specific antibodies by ELISA while the cellular response was characterized by flow cytometry and IFN- γ ELISPOT to enumerate circulating T cell subsets and PRRSV-specific IFN- γ secreting cells (SC) in PBMC. (2, 3) In this study, the distribution of cells involved in cell mediated response and IFN- γ producing cells were investigated through innovative methods (flow Cytometry and ELISpot assay) in pigs naturally infected by the PRRSV through exposure to infected animals. Clinical signs were recorded throughout the study.

The results showed that all exposed pigs became infected at 16 weeks of age and viremia lasted until 20 weeks in almost 50% of the exposed animals, whereas the C group remained negative. The PRRSV-exposed group developed an antibody response since 18 weeks of age.

In infected pigs, total CD4⁺ and CD8 α ⁺ T cells increased from 18 weeks onwards, due to a significant increase of cytotoxic T CD8 β ⁺ and memory T helper CD4⁺CD8 α ⁺low lymphocytes. An early and transient increase was observed for naïve T helper CD4⁺CD8 α ⁻ cells. Also virus-specific IFN- γ SC were significantly recalled from 18 weeks, peaking at 22 weeks. Control animals showed non-significant fluctuations in cell percentages and negligible SC levels. In this study, the IFN- γ SC response was strongly induced in parallel with the positive modulation of cytotoxic and memory T cells suggesting the potential activation of these subsets to secrete the immune cytokine.

This approach demonstrated a strong IFN- γ response but also some peculiar aspect of the immune response, likely depending on the delayed infection time of animals exposed to the virus. Overall, taking into account that PRRSV infection was more delayed compared to what is generally observed in the field, the age of pigs may have favoured a more pronounced immune response.

References: 1) Martelli P. et al. Vaccine Volume 27, Issue 28, 8 June 2009, 3788-3799; 2) Ferrari L. et al. Vet Immunol Immunopathol 151 2013, 193- 206. 3) Burgara-Estrella A. et al. Viruses 2013, 5, 663-677

Patologia generale

PRRSV, PIG, IMMUNITY

CUTANEOUS NEOSPOROSIS IN A GOLDEN RETRIEVER

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This report describes cutaneous cytological and histological lesions caused by *Neospora caninum*.

A 10 years old, intact female Golden Retriever under treatment with cyclosporine for an autoimmune disorder had sudden development of multifocal dorsal cutaneous nodules. Skin cytological specimens obtained by imprint and fine-needle aspiration were stained with May-Grünwald Giemsa. Skin punch biopsy specimens were obtained. Biopsies were fixed in 10% buffered formalin, routinely processed and stained with haematoxylin and eosin. Unstained smears and deparaffinized sections of skin were immunochemically stained with polyclonal anti-*Toxoplasma gondii* and anti-*Neospora caninum* primary antibodies.

Cytology demonstrated a prevalence of degenerated neutrophils admixed with fewer macrophages, rare neutrophils associated with adipocytes and fibroblasts. On the background and within macrophages numerous crescent shaped, 4-6 µm microorganisms, with a light basophilic cytoplasm and a central nucleus (tachyzoites) were visible. Histology revealed diffuse and severe neutrophilic, histiocytic, eosinophilic dermatitis and panniculitis associated with necrotizing vasculitis. Elevated numbers of free and cytoplasmic tachyzoites within macrophages and keratinocytes of the epidermis and follicular infundibula were present. Immunocytochemistry and immunohistochemistry warranted a diagnosis of cutaneous Neosporosis (CN). Clindamycin administration (11 mg/kg PO every 12 hours) and withdrawal of immunosuppressive medication resulted in prolonged clinical remission.

Cutaneous nodules are a rare manifestation of Neosporosis.1-4 Age-related immunodeficiency and immunosuppressive therapies seem to predispose to CN.1-4 Information on cause and prognosis are fragmentary in this instance. The current dog was alive 1 month after diagnosis.

1 – La Perle K.M.D., Del Piero F., Carr R.F. et al.: 2001, Cutaneous neosporosis in Two Adults Dogs on Chronic Immunosuppressive Therapy. *J Vet Diagn Invest* 13:252-255.

2 – Ordeix L., Lloret A., Fondevila D. et al.: Cutaneous Neosporosis During Treatment of Pemphigus Foliaceus in a Dog. *J Am Anim Hosp Assoc* 38:415-9.

3 - Poli A., Mancianti F., Carli M.A. et al.: 1998, *Neospora Caninum* infection in a Bernese cattle dog from Italy. *Vet Parasitol* 78:79-85.

4 – Perl S., Harrus S., Satuchne (Goldvaser) C. et al.: 1998, Cutaneous Neosporosis in a dog in Israel. *Vet Parasitol* 79:257-261.

DERMATOPATOLOGIA

Dog, *Neospora caninum*, Skin

EFFECT OF DIFFERENT ENVIRONMENTAL CONDITIONS ON SOME HAEMATOLOGICAL PARAMETERS IN COWS.

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The aim of this study was to evaluate physiopathological responses to different environmental conditions (ambient temperature, relative humidity and temperature-humidity index) on haematological parameters. Blood samples were collected at 4 time points under different environmental conditions (T1, T2, T3 and T4) by 43 Piemontese cows aged 2–12 years and analysed for haematological parameters. For each period, ambient temperature and relative humidity were recorded by means of a data logger and the temperature-humidity index (THI) was calculated as indicator of thermal comfort for cattle. Data were then measured by one way analysis of variance (ANOVA).

The obtained results showed a statistical significant effect of time on the following parameters: RBC (P<0.0001), Hb (P<0.0001), Hct (P<0.0001), MCV (P<0.0001), MCH (P<0.0001), MCHC (P<0.0001), Plt(P<0.0001), WBC (P<0.0001), neutrophils (P<0.0001), lymphocytes (P<0.0001), monocytes (P<0.0001) and eosinophils (P<0.0001).

The majority of haematological values obtained in the present study, even though within the physiological range for cattle, showed that variations in haematological parameters are related to changes in ambient temperature, relative humidity and temperature-humidity index. These results provide insight into the physiological responses of Piemontese cow to different environmental conditions, allowing to better evaluate its ability to adapt and cope with environmental stress (1-3).

1. Grünwaldt EG, Guevara JC, Estévez OR, Vicente A, Rousselle H, Alcuten N, Aguerregaray D, Stasi CR. (2005) Biochemical and haematological measurements in beef cattle in Mendoza plain rangelands (Argentina). *Trop Anim Health Prod.* Aug;37(6):527-40.
2. Casella S., Scianò S., Zumbo A., Monteverde V., Fazio F., Piccione G. (2013). Effect of seasonal variations in Mediterranean area on haematological profile in dairy cow. *Comparative Clinical Pathology* 22(4): 691-695.
3. Koubkova M., Knizkova I., Kunc P., Hartlova H., Flusser J., Dolezal O. (2002). Influence of high environmental temperatures and evaporative cooling on some physiological, hematological and biochemical parameters in high-yielding dairy cows. *Czech Journal of Animal Science.* 47: 309-318.

Patologia Clinica

cows, haematology, environment

EFFECTIVENESS OF ENDOMETRIAL CYTOLOGY OBTAINED BY LOW-VOLUME UTERINE FLUSH TECHNIQUE IN POSTPARTUM ENDOMETRITIS OF DAIRY COWS

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Aim of this study was to evaluate the effectiveness of endometrial cytology obtained by low-volume uterine flush technique to identify endometritis in postpartum period of dairy cows, correlating data with bacteriological examination and clinical findings.

Endometritis, responsible of great economical losses in dairy industry, is difficult to diagnose, due to the lack of gold standard criteria accepted by practitioners (1). Subclinical endometritis (SEND), defined as uterine inflammation in the absence of clinical signs, is frequently associated with significant reduction of reproductive performance and often diagnosed through endometrial cytology (2).

The study was performed in 40 multiparous Holsteins. From October to December 2013, cows underwent clinical evaluations from the day of parturition until 40 days postpartum. Intrauterine samples were collected between 30 and 40 d postpartum by infusing 100-150 ml of sodium chloride 0.9% sterile solution and then recovering the fluid; samples were delivered to the laboratory within 12 hours. After centrifugation of the fluid, an aliquot was used for bacteriological examinations and another for cytology. According to the published literature, a cut off of 10% of polymorphonuclear leucocytes (PMNs), was used to identify cows with endometritis (3).

Between 30 and 40 d postpartum, all cows were flushed and examined: 17 cows (42%) showed a positive cytology; 6 of these cows were diagnosed with a clinical endometritis, whereas the remaining 11 were clinically healthy (SEND cows). Of the 23 remaining cows with a negative cytology (58%), 20 were healthy, whereas 3 cows showed clinical endometritis (false negative). Bacteriology allowed the isolation of several bovine uterine pathogens both in clinical endometritis cases and in clinically healthy cows. Six different groups of bacteria were isolated such as *Trueperella pyogenes* (n=6), *Escherichia coli* (n=5), *Enterococcus faecalis* (n=7), coagulase-negative staphylococci (n=8), *Bacillus* spp. (n=1), *Pseudomonas* spp. (n=3) and *Enterobacter agglomerans* (n=1). Bacteriology was negative in 9 cows.

Low-volume uterine flush cytology between 30 and 40 days postpartum has proved to be a useful technique to reveal SEND especially in cases associated with bacteria considered only potentially pathogenic for the uterus, such as *E. faecalis* or *B. licheniformis*. However, we underline the limits of the technique that can lead to obtain false negative. The results of our study emphasize the need for integration of the two diagnostic tools, namely cytology and bacteriology.

1)Kasimanickam R, et al; A comparison of the cytobrush and uterine lavage techniques to evaluate endometrial cytology in clinically normal post-partum dairy cows. *Can Vet J* 2005; 46:255-259.

2)Madoz LV, et al; Endometrial cytology, biopsy, and bacteriology for the diagnosis of subclinical endometritis in grazing dairy cows. *J Dairy Sci* 2014.; 97:195-201.

3)Sheldon I M, et al; Defining postpartum uterine disease in cattle. *Theriogenology*, 65 (2006) 1516-1530.

Patologia della riproduzione

endometrial cytology, cow, uterine bacteriology

ERYSIPELOTRIX RHUSIOPATHIAE ENDOCARDITIS IN A SHEEP

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Erysipelotrix rhusiopathiae is a pathogen of a wide variety of animals. Chronic polyarthritis are reported in sheep and lambs. The authors describe one systemic erysipelas infection in an adult sheep in Sicily.

A female, cross-breed, 18 months old sheep showing clinical signs of phosphate esters suspected poisoning, was sent for diagnostic investigations to the Department of Veterinary Sciences of Messina University. A complete necropsy was made and samples of affected organs were partially fixed in 10% neutral buffered formalin to perform histological investigations and partially stored at -80°C for biomolecular researches. Total DNA was extracted from heart tissue and employed in PCR test targeted to the 23S ribosomal region. The DNA sequences were amplified by primers annealing at 23S of ribosomal genes as reported in the literature (Takeshi et al., 1999). PCR products were sequenced and the obtained data were analyzed by Wu Blast 2 sequence alignment software for strain identification.

At necropsy abundant foamy exudates in the trachea and bronchi and pleural hemorrhages were observed. Moreover, hypertrophy of the left heart showing multiple grayish white foci were present. The mitral valve showed a severe acute endocarditis characterized by irregular friable vegetations. In the kidney a voluminous necrotic area with hemorrhagic limits between cortical and medullary was detected. Histology confirmed the presence of multiple myocardial and renal septic infarcts. The flaps of the mitral valve were fibrotic, infiltrated by polymorphonuclear cells, with adherent multiple thrombi. DNA extracted and the sequence analysis showed a complete overlapping with the 23S rDNA of Erysipelothrix rhusiopathiae sequence.

Erysipelothrix rhusiopathiae is a bacterium commonly isolated in swine. In author's opinion this report describe an unusual cardiac localization in sheep previously only rarely described (Chineme et al., 1973; Maclachlan, 1978). The occurrence of this unusual localization imposes the necessity do not neglect common lesions using always regularly laboratory investigations.

Chineme et al. (1973). Cardiovascular lesions associated with erysipelas in a sheep. J Am Vet Med Assoc. 162(4):278-9. Maclachlan GK. (1978). Ovine endocarditis from Erysipelothrix insidiosa. Vet Rec. 102(7):150. Takeshi K. et al. (1999). Direct and Rapid Detection by PCR of Erysipelothrix sp. DNAs Prepared from Bacterial Strains and Animal Tissues. J. Clin. Microbiol. 37 (12), 4093-4098

Systemic erysipelas infection

Sheep, E. rhusiopathiae, endocarditis

GLIOBLASTOMA IN AN EWE

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Tumors of neuroepithelial origin (gliomas) include astrocytomas, oligodendrogliomas, and ependymomas. In veterinary medicine, primary neoplasms of the central nervous system (CNS) are most frequently reported in dogs and less commonly in cats, whereas the same tumors are considered distinctly uncommon within the other domestic species [1].

The present report aims at describing the pathological and immunohistochemical findings observed in a Sarda breed sheep with glioblastoma.

A 6 years old Sarda breed sheep was euthanized and necropsied after showing severe and worsening neurological signs, which started 8 months before and mainly consisted of depression and head pressing. At necropsy a wide range of tissues were sampled, promptly fixed in 10% neutral buffered formalin and routinely processed for histopathological investigations (hematoxylin and eosin stain). Furthermore, selected tissue sections of the cerebral mass were subject to immunohistochemistry (IHC) using the following primary antibodies: anti-glial fibrillary acidic protein, anti-vimentin, anti-synaptophysin and olig2.

At necropsy, a large (about 5 cm in diameter) whitish to grayish mass was seen partially replacing the left cerebral hemisphere.

Microscopically, the tumor consisted of polymorphic cells, with a high number of mitotic figures and large areas of necrosis and dystrophic calcification surrounded by pseudo-palisading of cells. The neoplasm was highly vascular with glomeruloid vascular proliferation.

Foci of "oligodendroglioma-like" differentiation and few multinucleated giant cells were also occasionally seen. Mitotic figures were very frequent, up to 5 mitoses per high magnification power field (Ob. x40), and often atypical. At IHC, neoplastic cells proved to be immunoreactive (IR) for vimentin and olig2 but constantly negative for synaptophysin. Only few scattered neoplastic cells were GFAP-IR. However, IHC for GFAP demonstrated the presence of few reactive astrocytes, mainly surrounding the foci of necrosis.

Few cases of primary brain tumors have been described in sheep: medulloblastoma in lambs [2], a case of ependymoma in a Suffolk sheep aged less than 1 year [3], and more recently a case of oligodendroglioma in a 1 year old male Iranian fat-tailed sheep [4]. To the best of our knowledge, the present is the first report of glioblastoma in sheep. Even in sheep, glioblastoma is similar to anaplastic astrocytoma with the additional features of necrosis and vascular proliferation and share many of the histopathological and immunohistochemical features observed in dogs [5].

1. Mandara M.T et al. (2011) Poletto Editore, Vermezzo, 119 Milano, Italy, pp. 221-287.

2. Cotchin E. (1975) *Pro R Soc Med* 99 68, 653-655.

3. Elsinghorst A.M. (2003) *Vet Quart* , 103 25(4), 165-169

4. Derakhshanfar A. and Mozaffari A.A. (2010) *J S Afr Vet Assoc* 81(2), 114-115.

5. Lipsitz D. et al (2003) *Vet Pathol* Nov;40(6):659-69.

PATHOLOGY

Glioblastoma, Sheep, Immunohistochemistry

GLYCOGEN RICH CARCINOMA OF CANINE MALE AND FEMALE MAMMARY GLAND

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Glycogen rich clear cell carcinoma (GRCC) is a rare subtype of human invasive mammary gland (MG) carcinoma, in which at least 90% of the neoplastic cells have clear cytoplasm containing glycogen(1). The aim of this study was to describe the histological, histochemical and immunohistochemical (IHC) features of GRCC of canine MG.

Serial formalin fixed paraffin embedded tissue sections of two GRCC canine mammary carcinoma, one female (case I) and one male (case II) were stained with H&E, PAS, PAS diastase (dPAS), and Alcian Blue (AB). IHC was performed with anti-ER, -PR, -cerbB2, -CK19, -CK14, -CK5/6, -p63, -vimentin, -SMA, -calponin, -S100, -EGFR, -c-KIT, -E-cad and -ki67 antibodies. Sudan III was carried out on formalin-fixed frozen tissue in case II.

Histologically, 90% of neoplastic cells showed sharply distinct borders, clear or finely granular cytoplasm and low N:C ratio. In case II, residual 10% of the neoplastic cells had lipid-like vacuolated cytoplasm. In both tumors, the cytoplasm resulted strongly positive with PAS. Treatment with diastase abolished PAS reactivity. Case I showed PAS+ and dPAS- staining also in lymph node metastasis. In case II, 10% of neoplastic cells were positive to Sudan III. No stain with AB was obtained. Case I showed positivity for CK19 and CK5/6, negativity for ER, PR and c-erbB2, resulting a basal-like phenotype in primary tumor and lymph node metastasis. They were both positive for EGFR, E-cad, c-KIT, and weakly for calponin. Case II was a basal-like phenotype, presenting CK 19, E-cad, c-KIT, weak CK14 and strong vimentin positivity. The proliferative ki67 index was 26.75% in case I and 8.2% in case II.

Based on the morphology, typical features of human MG GRCC are the “fried eggs appearance”, clear cytoplasm and small dark punctate nuclei (2). On the best of our knowledge, this is the first report regarding GRCC in canine MG. The diagnosis was confirmed by PAS+ of intracytoplasmic glycogen granules and lack of stain with dPAS. Case II was considered a GRCC with lipid rich differentiation. Both cases had a basal-like phenotype and the expression of EGFR and c-KIT was suggestive of an association of cell proliferation with signal transduction of surface molecules (3). GRCC can be considered a new rare histological subtype of canine mammary tumors, with clear cytoplasm, PAS+ and dPAS-, expressing the triple negative phenotype, a tumor with clinical aggressive behavior that should be differentiated from lipid rich carcinoma.

1. Tavassoli, Deville. Lyon: IARC Press, 2003
2. Rosen, Philadelphia. LWW press, 2001
3. Kim et al., Yonsei Med J 53:1142-1146, 2012

ANATOMIA PATOLOGICA

Canine, Mammary carcinoma, Glycogen rich

GRANULOMATOUS DERMATITIS OF THE AURICULAR PINNA IN A HEIFER

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This study is aimed to describe the morpho-pathological and etiopathogenic aspects of a unique case of granulomatous dermatitis.

A 2 y.o. Italian Friesian heifer showed a papillomatous-like lesion at the edge of the right auricular pinna. The lesion sized 9 cm x 11.5 cm, was exophytic, globular in shape, firm and with the outer surface uneven, depigmented and ulcerated. Cytological examination revealed the presence of an inflammatory population mainly composed by neutrophils and macrophages. On the basis of the cytological observation, the mass was excised and subjected both to histological staining (H&E, PAS, Gram) and molecular biology examination (PCR).

The histological examination allowed the diagnosis of a pyogranulomatous inflammation characterized by a epithelioid and giant cells, neutrophilic and macrophagic infiltrate organized around focal areas of eosinophilic homogeneous material consistent with the Splendore-Hoeppli phenomenon. PAS staining highlighted numerous coccoid formations within the piogranulomatous reaction or freely standing in the tissue. Gram stain revealed Gram-positive bacterial colonies confirmed by PCR as *Corynebacterium mucifaciens*.

Granulomatous dermatites are caused by agents against whom the body is sensitized and react through an immunomediated response(1). Sometimes the above reactions are histologically characterized by the Splendor-Hoeppli phenomenon that may represent the deposition of antigen-antibody complexes (immunoglobulins and major basic proteins) and debris from the host inflammatory cells(2). The lesion described in this work seemed worth of description because of the etiologic agent. In fact, *Corynebacterium mucifaciens* is a newly-described species belonging to the the largest genus in the group of coryneform bacteria(3). Moreover, the seat of development was extremely atypical for the bovine species. *Corynebacterium mucifaciens* is better differentiated from closely-related species by molecular biology techniques, such as sequencing of the 16S rRNA gene and is isolated from human blood or other normally-sterile body fluids, often considered as part of the normal skin flora or contaminants. To date, literature data report the occurrence of disease due to *Corynebacterium mucifaciens* only in humans where it has been related to severe infections, lethal bacteremia (4), cavitory pneumonia (5), corneal ulcer (6), otitis and nasal polyps (7). To the author's knowledge, this is the first report of a lesion induced by *C. mucifaciens* in veterinary medicine.

1. MARCATO PS (2002) - Patologia sistematica veterinaria, Edagricole, Bologna.
2. Hussein MR (2008) - Mucocutaneous Splendore-Hoeppli phenomenon. *Cutan Pathol* 35:979-988.
3. Funke G, Lawson PA, Collins MD (1997) - *Corynebacterium mucifaciens* sp. nov., an Unusual Species from Human Clinical Material. *Int J Syst Bacteriol.* 47(4):952-7.
4. Cantarelli V, Z.Brodt T, Secchi C, Inamine E, De Souza Pereira F, Pilger DA (2006) - Fatal case of bacteremia caused by an atypical strain of *Corynebacterium mucifaciens*. *Braz J Infect Dis.* 10(6):416-8.
5. Djossou F, Bebian MC, Moynet D, Le Fleche-Mateos A, Malvy D (2010) - *Corynebacterium mucifaciens* in an immunocompetent patient with cavitory pneumonia; *BMC Infectious Diseases* 10:355.
6. Sanz-Rodríguez N, Almagro-Moltó M, Vozmediano-Serrano MT, Gómez-Garcés JL (2014) - First case of a corneal ulcer associated with *Corynebacterium mucifaciens*. *Enferm Infecc Microbiol Clin* [Epub ahead of print; available on line at <http://www.sciencedirect.com/science/article/pii/S0213005X14000081>]
7. Morinaka S, Kurokawa M, Nukina M, Nakamura H (2006) - Unusual *Corynebacterium mucifaciens* isolated from ear and nasal specimens. *Otolaryngol Head Neck Surg* 135(3):392-6.

Patologia Veterinaria

C. mucifaciens, Skin granulomas, Splendore-Hoeppli

GRANULOMATOUS MIOSITIS DUE TO CORYNEBACTERIUM PSEUDOTUBERCULOSIS IN A HORSE

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This study is aimed to describe the morpho-pathological and etiopathogenic aspects of a case of granulomatous myositis in a horse.

A 12 years old Quarter horse mare was evaluated because of the presence of different subcutaneous nodules and masses. The lesions were cytologically diagnosed as pyogranulomas and were resistant to the given pharmacological treatment. The persistence of the lesions along with the deterioration of the clinical status suggested to surgically remove three of them. Macroscopically they were fixed to the muscles, painless and firm in consistency. On cut section, they showed a purulent exudate. Tissue samples from the lesions were fixed in 10% buffered formalin and paraffin wax embedded. Histological sections were stained with H&E, PAS, Masson trichrome, Grocott and Gram. Molecular biology assay (PCR) was performed too.

Histological examination revealed, in all samples, a diffuse mixed inflammatory infiltrate characterised by polymorphonuclear granulocytes (neutrophils and mainly eosinophils), macrophages, lymphocytes, plasma cells, epithelioid and multinucleated giant cells invading the endomysium. Different sized round foci of caseous necrosis with calcification and areas of collagenolytic degeneration were present in all sections. PAS and Grocott stains didn't show fungi whereas Gram stain revealed the presence of blue pleomorphic Gram + microorganisms free or inside macrophages.

The described results indicated a severe diffuse granulomatous myositis. PCR analysis revealed the etiologic agent as *Corynebacterium pseudotuberculosis*.

Corynebacterium pseudotuberculosis infection occurs world wide as caseous lymphadenitis in small ruminants and granulomatous infection in horse and cattle.

The bacterium can survive for extended periods in the environment and in the soil. Disease transmission is thought to occur thorough a contaminated environment by direct contact between animals and insects such as house flies (*Musca domestica*), stable flies (*Stomoxys calcitrans*), and other arthropods serving as mechanical vectors (1). Clinically, the infection in horses most commonly causes external abscesses or "pigeon fever" (90% of cases), however, internal abscesses (8%) or ulcerative lymphangitis (1%) may also occur (2).

In our case, none of the known pathogenetic pathways reported in the literature seems to be completely comparable being some aspects common to the so called pigeon fever and others to the chronic form of ulcerative lymphangitis. The presented case is, therefore, very rare and interesting for the pathological findings and, overall, from the epidemiological point of view.

1. Spier SJ, Leutenegger CM, Carroll SP, Loye JE, Pusterla JB, Carpenter TE, et al. (2004) - Use of a real-time polymerase chain reaction based fluorogenic 5' nuclease assay to evaluate insect vectors of *Corynebacterium pseudotuberculosis* infections in horses. *Am J Vet* 65:829–34.

2. Szonyi B, Swinford A, Clavijo A, Ivanek R (2013) - Re-emergence of Pigeon Fever (*Corynebacterium pseudotuberculosis*). Infection in Texas Horses: Epidemiologic Investigation of Laboratory-Diagnosed Cases. *Journal of Equine Veterinary Science* XX:1–7.

Patologia Veterinaria

horse, muscle granulomas, pigeon fever

HISTOLOGICAL ASPECTS OF AN UNUSUAL COLONIC DUPLICATION CYST IN A CONSTIPATED CAT

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In animals as in humans the main anomalies of canalization of enterocolic tract are represented by the persistence of Meckel's diverticulum, intestinal duplication cyst, vitelline or omphalic duct cyst or finally, diverticula losing a direct connection with the colonic lumen^{1,3}. In these condition histology may be characterized by the indistinguishable mucosal type between normal and accessories tract, or by the presence of metaplastic epithelial types. We describe a case of 5-year-old domestic short-haired spayed female cat presenting abdominal pain and suspended defecation, in which abdominal ultrasonography revealed the presence of an oval formation connected to the colon, whose wall was similar to the colonic one. Ultrasonographically the content appeared inhomogeneous. Surgery and related histopathology allowed a most likely diagnosis of a colonic duplication cyst.

For histology analysis, samples were routinely processed and stained with hematoxylin-eosin and Alcian-PAS using different types of mucins, then characterized also immunohistochemically.

Mucosal histology and histochemistry revealed particular heterotopic tissues represented by some spotted areas of gastric metaplasia, antral/pyloric in type, and a very particular area of mucosa that resemble respiratory mucosa in type. No heterotopic pancreatic tissue, Brunner glands or biliary epithelium, frequently reported in other cases¹, were observed in this cat. Two nonreactive lymphoid aggregates were observed in the lamina propria of the cyst, in absence of elements evidencing inflammation and/or subsequent bacterial superinfection of the cystic mucosa. After surgery the cat returned to defecate normally.

Duplication cyst is an uncommon congenital abnormality of the alimentary tract. Most often the patients are asymptomatic and colonic duplication cysts remain undiagnosed for years. In this case report we present a fourth description of intestinal duplication cyst case in a cat^{1, 2, 3, 4} with a descending colon location and a mucosal characterization by histology/immunohistochemistry. The importance of description of this rare congenital malformation is finalized to the inclusion in the differential diagnosis of cystic masses of the gastrointestinal tract.

1) Kook PH, Hagen R, Willi B, Ruetten M, Venzin C. Rectal duplication cyst in a cat. *J Feline Med Surg* 2010;12:978-981.

2) Kramer A, Kyles AE, Labelle P. Surgical Correction of Colonic Duplication in a Cat. *J Am Anim Hosp Assoc* 2007;43:128-131.

3) Parry-Smith P, Czerwinska M, Krudewig C. Duodenal duplication cyst in a young cat. *Vet Rec* 2008;162:826-827.

4) Radlinsky MAG, Biller DS, Nietfeld J, Enwiller T. Subclinical intestinal duplication in a cat. *J Feline Med Surg* 2005;7:223-226.

General and Special Pathology

disontogenic cyst , histochemistry, immunohistochemistry

HSP32 AND HSP90 IMMUNOEXPRESSION, IN RELATION TO GRADING AND OTHER HISTOPATHOLOGICAL FEATURES, IN CANINE CUTANEOUS MAST CELL TUMOURS

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Literature data indicate Hsp32 and Hsp90 as interesting molecular targets in canine neoplastic mast cells. However, their immunoeexpression patterns in canine mast cell tumours (MCT) have not been investigated so far. Thus, the aim of the present study was to evaluate the immunohistochemical expression of Hsp32 and Hsp90 in 22 samples of canine cutaneous MCT, in relation to histological grade and other pathological variables, such as absence/presence of epidermal ulceration or tumour necrosis, growth pattern and mitotic index

Grading was established on the basis of the systems proposed by Patnaik et al. (1984) and Kiupel et al. (2011) as follows: 13 grade II (10 low-grade and 3 high-grade) MCT and 9 grade III (all high-grade) MCT. A semi-quantitative method was used to analyse immunoreactivity. Fisher's exact test and Cramer's V were used to evaluate the associations between the examined parameters.

Hsp32 showed a variably intense and distributed cytoplasmic immunostaining, not associated with histological grade. However, the low Hsp32 immunoeexpression (<50% of positive cells) detected in the majority of grade III/high-grade MCT samples suggests the need for further investigating the efficacy of the pharmacologic Hsp32-inhibitors on aggressive canine MCT. Reduced Hsp32 immunoreactivity was associated with presence of tumour necrosis ($p=0.035$; Cramer's $V=0.542$), a finding in agreement with the pro-survival functions of Hsp32 in neoplastic canine MC.

Hsp90 cytoplasmic immunosignal was variably associated with nuclear and/or membranous staining. Proportion of Hsp90 immunoeexpression was not associated with histopathological features and grade.

Noteworthy was the detection of a membranous expression of Hsp90, in light of its molecular chaperone functions towards the receptor tyrosine kinase Kit. Further studies are currently underway in order to investigate the possible relationship between Hsp90 and Kit immunoeexpressions in canine MCT.

- Hadzijasufovic et al. (2008) Targeting of heat-shock protein 32/heme oxygenase-1 in canine mastocytoma cells is associated with reduced growth and induction of apoptosis. *Exp Hematol* 36:1461-70.
- Kiupel et al. (2011) Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behaviour. *Vet Pathol* 48:147-55.
- Lin et al. (2008) The novel HSP90 inhibitor STA-9090 exhibits activity against Kit-dependent and - independent malignant mast cell tumors. *Exp Hematol* 36:1266-77.
- Patnaik et al. (1984) Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. *Vet Pathol* 21:469-74.

disciplinare

HSPs, Mast cell tumor, Dog

IDENTIFICATION OF REGULATORY T CELLS IN CANINE MAMMARY GLAND TUMORS

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In the present study we evaluated foxp3 expression in benign and malignant canine mammary gland tumors by immunohistochemistry and we investigated its prognostic significance. Foxp3 is a member of the forkhead /widge-helix family of transcription regulators involved in regulating immune system development and function (2). This gene plays a crucial role in the generation of CD4+/CD25+ regulatory T cells. Tregs seem to enable tumors to elude host antitumoral immune response (1, 3).

Animals: a total of 33 samples of canine mammary gland tumors were selected from the archives of Veterinary Pathology of the Department of Veterinary Medical Sciences over a period between 2006 and 2013. 15 neoplasias were diagnosed as benign and 18 as malignant, based on the WHO (1999) classification. Among the benign tumors 10 were complex adenomas, 3 papillary adenomas and 1 mixed benign tumor. Among the malignant tumors 5 were solid carcinomas, 3 complex carcinomas, 9 tubulo-papillary carcinomas and 1 in situ carcinoma.

All the samples were immunohistochemically tested with an anti Foxp3 primary antibody (purified Anti-Mouse/Rat Foxp3 clone FJK - 16s of the firm eBioscience), at a 1:200 dilution in PBS overnight 4°C.

Each slide was evaluated microscopically on 5, 20X fields and all the positive cells were counted in peritumoral and intratumoral infiltrates. Benign tumors showed fewer infiltrates than malignant neoplasias. Foxp3 expression was observed in 9 benign tumors out of 15. In 6 complex adenomas the number of tregs observed was between 1 and 12, only one showed more than 13 tregs, 2 papillary adenomas showed less than 12 tregs.

Foxp3 expression was observed in 14 malignant tumors out of 18. In solid carcinomas foxp3 was highly expressed (more than 40 tregs) while in complex carcinomas, expression was less (between 1 and 12) and in 3 tubulo-papillary carcinoma between 1 and 12 cells were positive and in 4 between 13 and 40. In the in situ carcinoma foxp3 was very highly expressed (more than 40)

In the present study the number of tregs was counted and compared with the histological grade of canine mammary tumors and we observed that foxp3 (tregs transcription factor) was mainly expressed in intratumoral and peritumoral infiltrating areas of neoplasias with poor prognosis.

These results suggest that the increase number of tregs lymphocytes in mammary neoplasias may play a role in tumor progression as they suppress the immune response against it, and allow the tumor progression.

1)Oh S.Y., 2012,Vet and Compar. Oncology DOI:10.1111/j1476-5829.201.00327x,

2)Kim J.H., 2012, The Vet, Journal 193 22-227

3)Horiuchi Y., 2009, Microbiol. and Immunol.53:468-474

IMMUNOREGULATION

T LYMPHOCYTES, MAMMARY GLAND TUMORS, FOXP3

MOLECULAR DETECTION OF OVIS ARIES PAPILLOMAVIRUS TYPE 3 IN FORMALIN FIXED, PARAFFIN EMBEDDED (FFPE) SHEEP SQUAMOUS CELL CARCINOMA SAMPLES.

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Recently (Alberti et al., 2010) the prototype of a novel papillomavirus genus (OaPV3) was detected in normal skin and squamous cell carcinoma (SCC) lesions of Sardinian sheep. OaPV3 belongs to the Dyokappa genus and its genome significantly differs from the 2 species of ovine papillomaviruses previously reported in Australia that instead group with the artiodactyl Deltapapillomavirus species, and have been isolated from benign cutaneous lesions. Here we investigate the relevance of OaPV3 in SCC by screening a panel of FFPE sheep SCC samples

Based on the sequence of OaPV3 L1 and E6 two DNA probes and a set of 4 primers were designed and respectively used to develop an In situ Hybridisation test (ISH) and two RT-PCR assays. These assays were applied to a collection of 41 FFPE sheep SCCs samples obtained from cutaneous tumours (5 nasal, 6 ear, 10 periocular, 3 dorsal, and 17 mammary tumours).

Diagnosis of SCC was confirmed by histopathological examination of the 41 samples. Molecular tests, summarised in the table, revealed that 26 out of 41 (63%) samples were positive to at least one test. Different tests showed different sensitivities. Also, tumours localised in different parts of the sheep body seemed to show variable degrees of positivity to OaPV3.

Results demonstrate a high prevalence (63%) of OaPV3 in sheep SCCs. This level of prevalence is particularly important and comparable to the prevalence of HPVs in human SCC (50 to 69%, Meyer et al., 2000), and suggests that OaPV3 represent an important risk factor for the development of sheep SCC. The level of positivity of nasal and periocular lesions was greater respect to other tumour locations. This can be explained by the greater level of solar exposition and/or to traumas of these area respect to other locations. As expected, RT-PCR has a greater sensitivity than ISH. However, only combining these two tests the total number of positives can be detected, and both the presence/expression and localisation in the tumour can be investigated. Concluding, we cannot rule out the presence of unknown viral types in negative tissues. Further investigation is needed to investigate the presence of viral variants associated to different tumour locations and the presence of uncovered papillomaviruses in negative samples.

1. Alberti et al., 2010. *Virology* 25:352-9.
2. Meyer et al., 2000. *Dermatology* 201:204-11

Anatomia Patologica e Malattie Infettive
OaPV3, squamous carcinoma, RT-PCR and ISH

Tumour location	N° samples	N° of positive	positive to all tests	ISH positive	RT-PCR positive	ISHE6 positive	ISHL1 positive	RT-PCR positive E6	RT-PCR positive L1
Nose	5	5	2	4	5	4	4	4	3
Ear	6	6	1	4	5	4	3	4	4
Eye	10	4	0	4	4	3	2	2	2
Flank	3	2	0	2	2	1	2	1	1
Udder	18	9	1	5	8	3	2	4	6
Tot.	42	26	4	19	24	15	13	15	16

MYOGENIC REGULATORY FACTORS EXPRESSION IN MURINE C2C12 CELLS TREATED WITH 17BETA ESTRADIOL: PRELIMINARY DATA

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Myogenesis is controlled by a family of transcription factors known as Myogenic Regulatory Factors (MRFs) including Myogenic Differentiation 1 (MyoD1), Myogenic Factor 5 (Myf 5), Myogenin (Myog) and Myogenic Regulatory Factor 4 (MRF4), which are differently expressed throughout the myogenic process. MyoD1 and Myf5 are mainly involved in the satellite cells commitment into proliferating myoblasts, whereas Myog and MRF4 are expressed during middle and late differentiation stages. MRF4 is also involved in fibers maturation (Dedieu 2002, Ferri 2009). These factors are regulated by extrinsic signals such as extracellular matrix substances, growth factors and hormones (Rhoads, 2009).

It is known that estrogens control myogenesis and skeletal muscle fibers changes. However the hormones mechanism of action are not yet completely understood (Boland 2008, De Jager 2011). In this preliminary study we evaluated the 17 β estradiol (E2) influence on MRFs expression in C2C12 cells.

C2C12 cells were seeded and, at about 80% confluence, cells were switched into a 10% horse serum medium to induce differentiation.

Cells were treated 5 times with 10⁻⁵ M or 10⁻⁸ M E2 every 48 hours and were harvested every 48 hours to evaluate expression of MyoD, Myf5, Myog, MRF4 by Real-time PCR and Western Blot. The evaluation of Myosin heavy chain I (MHCI) gene expression was carried out to confirm myotubes formation. Data were properly analyzed using GraphPad InStat (vers. 3.05).

No difference in myoblasts MRFs gene expression was revealed, while a difference in MRF4 gene expression was detected between treated myotubes and controls culture. In particular, 10⁻⁸ M E2 administration induced a greater MRF4 expression in myotubes than in untreated culture and 10⁻⁵ M E2 administration. Western Blot analysis confirmed these data.

The study confirmed that MRFs expression varie throughout the myogenic process, as previously described by Dedieu, 2002 and Ferri, 2009. In particular MRF4 expression, which is physiologically up-regulated during the differentiation phase, is further increased on myotubes by E2 administration.

Results confirm the over expression of MRF4, already described in adult bovine skeletal muscle cultures treated with E2 (Divari, 2013), and could help to understand the estrogen pathway regulation in muscle hypertrophy

Boland, R., et al. 2008. *Steroids* 73, 859–863.

Dedieu, S., et al. 2002. *Int J Dev Biol*, 46, 235-241

De Jager, N. et al. 2011. *Physiol. Genomics* 43, 467–478.

Divari S., et al., 2013. *Food Chem.* 141, 4358-4366

Ferri, P., et al. 2009. *J. Cell. Biochem.* 108, 1302–1317

Rhoads, R. P., et al. 2009. *Domest. Anim. Endocrinol.* 36, 111–126.

Wyzykowski, J. C. et al. 2002. *Mol. Cell. Biol.*, 22, 6199-6208

Pathology

myogenic factors, estrogen, mouse cell culture

NECROSCOPIC FINDINGS ASSOCIATED WITH EARLY REPLACEMENT IN BREEDING DOES

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In commercial rabbit farms, mortality and culling are of great relevance from the financial viewpoint.¹ Lesions in breeding rabbits are recorded mainly on animals at the end of their reproductive life and the replacement is usually performed based on reproductive parameters without further diagnostic investigations. The aim of this study was to assess the main necroscopic lesions in does replaced from reproduction and not slaughtered.

Sixty-seven dead does were collected from a commercial rabbit farm (Centro genetico Martini s.p.a., S. Maria Codifiume, FE) housing 5000 does, and submitted for postmortem examination. During necropsy, samples for histological and microbiological examinations were also collected. Information about age, number of deliveries and number of mating were recorded. To evaluate the effect of age, does were arbitrarily divided into two categories based on the median of number of deliveries (if available).

The age of does ranged from 115 to 587 days (median 286), the number of deliveries ranged from 0 to 13 (median 2). On postmortem examination, respiratory and genital tracts were the most frequently affected (n = 40; 59.7% each), the digestive system was involved in 30 does (44.77%) and other concomitant lesions, such as traumatic fractures, abdominal effusion, splenomegaly or cardiac injuries were seen in 36 cases (53.73%). Presence of pododermatitis was also observed in 42 animals (62.69%). Genital lesions included 16 uterine torsion (40%), 12 endometritis (30%), 12 uterine retention (30%) and others, such as dystocia or rupture (n= 6; 15%). Uterine torsion was significantly more frequent in does with more than 2 deliveries (P< 0.05). No other significant differences were observed in the 2 sets of animals grouped according to the number of deliveries. Bacteriological examinations performed on randomly selected pleural effusions were positive for *Pasteurella* spp. (n = 2); *Staphylococcus* spp. were isolated from endometritis (n = 2), macerated fetus (n = 1) and pododermatitis (n = 1).

Mortality in does was highest during the first two deliveries, similar results of highest mortality risks in the first three deliveries are reported also in literature.² Respiratory and genital lesions were the most relevant, in contrast with other authors,² who reported a higher prevalence of respiratory and digestive pathologies. Moreover, in our study, uterine torsion resulted the most important lesion associated with increased number of deliveries, although the related pathogenesis is still unclear.

1 Gómez EA (2006): Producción industrial de gazapos: algun puntos críticos. Boletín de cunicultura 145:12-23.

2 Rossel JM & de la Fuente LF (2009): Culling and mortality in breeding rabbits. Preventive Veterinary Medicine, 88:120-127.

Pathology

Necropsy, Rabbit reproduction, Rabbit Pathology

PARASITIC DISEASES IN MEDITERRANEAN JOHN DORY (ZEUS FABER)

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The aim of the present paper was to identify, describe and compare tissue changes due to parasites occasionally found in different subjects belonging to a wild teleost species, Zeus faber.

A total of 28 John dory (*Z. faber*) were examined. 4 fish had been collected during an experimental trawl survey carried out along the southern Tyrrhenian coasts in May 2012. 24 fish were collected from the fish market of Porto Empedocle and were coming from southern Mediterranean sea (Strait of Sicily). Scientific investigations were performed following routine histopathology methods. For parasitology investigation, parasite specimens were fixed in 70% ethanol, clarified by lactophenol and finally observed under the stereoscope. The molecular investigation was performed for anisakid larvae identifications by RFLP-PCR, amplifying ITS1, ITS2 and the 5.8S subunit.

Two fish, out of a total of four coming from Tyrrhenian sea, showed macroscopic parasites on the skin. Particularly, on the external surface of one fish a marine leech (*Hirudinea*) belonging to the genus *Calliobdella* was found. In the second specimen an adult taenia, 24 cm long, was found. The parasite was identified at genus level as *Alloptychobothrium* sp. At macroscopical evaluation of internal organs, within coelomic cavity of the same fish few anisakid larvae encysted in the stomach serosa were seen.

Only a single fish, out of a total of 24 subjects coming from the Strait of Sicily, at macroscopical external examination, showed grossly evident tissue changes. This fish showed a nodular bulge, 1 cm in size, in the left jaw. Histologically, such newborn tissue, was characterized by a granulomatous reaction containing pieces of parasitic bodies surrounded by an external chitinous cuticle; these parasites were identified as copepods, likely belonging to the genus *Chondrachanthus*. At the level of the caudal and the left pectoral fin, several whitish nodules (more than 50), about 1-2 mm sized, were seen as seriated or grouped growths; histologically, such nodules resemble granulomas found in the jaw. Within the coelomic cavity of all the fish a large number of anisakid larvae, identified by PCR as *Anisakis pegreffii*, were detected. Only two fish were negative for parasites. Moreover, in two fish, adult cestoda, belonging to the same genus described above, were detected within the intestinal lumen. Finally, slight fatty liver degeneration was demonstrated in all the examined fish.

By the results here obtained and according to the literature on teleost parasitoses, it can be assumed that parasites are fully integrated in all the different aquatic ecosystem and can be found in both wild and farmed teleost fish, although generally in wild conditions parasites have a low impact and only rarely can cause mortality. Although copepods and leeches are a common finding in the John dory, no data are available on intestinal cestoda. Finally, according to Angelucci et al. (2011), the presence of anisakid larvae in a so high percentage of specimens is in contrast with data recently reported on *Z. faber* fished in Mediterranean sea (Costa et al., 2010).

1) Angelucci G., Meloni M., Merella P., Sardu F., Madeddu S., Marrosu R., Petza F., Salati F. Prevalence of *Anisakis* spp. and *Hysterothylacium* spp. larvae in teleosts and cephalopods sampled from waters off Sardinia. *J Food Prot.* 2011 Oct;74(10):1769-75. doi: 10.4315/0362-028X.JFP-10-482.

2) Costa A., Sciortino S., Reale S., Alio V., Cusimano M., Caracappa S. 2008. Indagine sulla presenza di Nematodi Anisakidae in specie ittiche marine. *Atti X Congresso Nazionale S.I.Di.L.V.* Available at: http://www.sidilv.org/index.php?option~com_docman&task~cat_view&gid~4&Itemid~6. Accessed 10 September 2010.

fish pathology

ZEUS FABER, MEDITERRANEAN SEA, PARASITE

PATHOLOGICAL AND IMMUNOHISTOCHEMICAL EVALUATIONS OF EQUINE INFECTIOUS ANEMIA INFECTION IN MULES

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Following the implementation of a National surveillance program for equine infectious anaemia (EIA), a high seroprevalence was detected among Italian mules population. EIA infection in mules has been studied limitedly. To increase the knowledge about the pathogenesis EIA virus (EIAV) in mules and to better understand their role in the epidemiology of the infection, a study was conducted.

Naturally EIA-infected mules were immunosuppressed. At the end of the observation period, the mules were euthanized, and their organs were examined for gross and microscopic lesions. Furthermore, immunohistochemistry (IHC) was conducted to investigate the distribution of the virus.

At necropsy, no relevant gross lesions were observed. Microscopic examination of the different animals revealed mild multifocal haemorrhages in several tissues, a moderate to severe hemosiderosis in spleen and lymph nodes and mild to moderate lymphoid infiltrates, mainly in kidney, and lesser in liver and in the adrenal glands. Membranous glomerulonephritis was detectable in about 40% of the kidney. In the lungs, a mild interstitial pneumonia characterized by slight hypercellularity and thickening of the alveolar septa was evident. Interestingly, mild lymphocytic myocarditis with fibres degeneration, together with a multifocal, mild to moderate, lymphocytic meningoencephalitis, with perivascular cuffings thicker in the brain stem, were observed.

IHC, performed with a monoclonal antibody anti-p26 protein, detected EIAV infected cells in different tissues, both in sites as well as in the absence of lesions; this is particularly true in the adrenal glands, where a high number of positive reacting macrophages were detected in the medulla, and in the heart, with a lower positivity in the interstitial space. In contrast to what described in horses, the amount of positive cells in spleen seem to be lower. A positive signal was revealed mainly in the periportal areas of the liver, the alveolar septa of lungs and interstitium of the kidney cortex, and generally limited to cells morphologically resembling macrophages.

Microscopic lesions and EIAV localization in mules, highly resembles the infection described in horses. However, differently from what is reported for certain strains of EIAV in horses, no virus was detected in the endothelia. The present description is a preliminary contribution for the study of pathological alterations and EIAV localization in non-horse species.

T. C. McGuire et al. Immunofluorescent localization of equine infectious anemia virus in tissue. *Am J Pathol.* Feb 1971; 62(2): 283–294. Spyrou V et al. Equine infectious anemia in mules: virus isolation and pathogenicity studies. *Vet Microbiol.* 2003 Aug 29;95(1-2):49-59. J. Lindsay Oaks et al. Equine Infectious Anemia Virus Is Found in Tissue Macrophages during Subclinical Infection. *J Virol.* Sep 1998; 72(9): 7263–7269.

Sanità animale, virologia, anatomoistopatologia
EIA virus, Mules, Immunohistochemistry

PRIMARY “AMYLOID TUMOR” OF THE MAMMARY GLAND IN A DOG

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The present report describes an unusual mammary lesion in a dog, with gross and histological features similar to the lesion known as “amyloid tumor of the breast” in the woman.

A fourteen year-old female spayed Dalmatian dog was presented to the practitioner with a large, bilobated lump involving left and right IV mammary gland. The mass was surgically excised and submitted for histopathological examination.

Grossly, two large subcutaneous nodular, firm masses, merging on the median line, expanded left and right IV mammary glands. Histologically, masses were encapsulated and mainly composed of large, coalescing lakes of pale eosinophilic, homogenous hyaline amorphous material, that stained positive with Congo Red (amyloid). Numerous plasma cells with moderate anisocytosis and anisokariosis and rare mitoses were multifocally recognizable. Multinucleated giant cells (MNGCs) with nuclear features similar to plasma cells were occasionally present. Moreover, MNGCs with scant eosinophilic cytoplasm and up to 25 dense nuclei were visible closely associated with amyloid deposits. Moderately atrophic mammary parenchyma was present at periphery. Immunohistochemistry (ABC method) was performed to characterize MNGCs and amyloid, applying anti-Lysozyme; Lambda-light-immunoglobulin-chains and HLA antibodies. Plasma cells and MNGCs were Lysozyme and HLA negative. Lambda-chains stained plasma cells, the majority of MNGCs and amyloid (AL amyloid).

Bilateral mammary extramedullary plasmacytoma with abundant AL amyloid deposition was diagnosed. Clinical workup did not reveal systemic amyloidosis or multiple myeloma. Sixteen months later the dog was humanely killed because of cardiac failure. No signs of multiple myeloma or recurrence of the tumor were reported.

Primary amyloidosis of the breast not associated with mammary carcinoma (amyloidoma), is a rare entity that has been documented in women since 1973, affecting mostly elderly patients, with bilateral involvement and related neither to systemic amyloidosis nor to multiple myeloma. Despite similar gross and histological appearance, some cases have been described as primary amyloidosis, while others as plasmacytoma with massive amyloid deposition

In the canine species, mammary gland amyloid deposition was described in association with mammary carcinoma whereas mammary primary amyloidosis or plasmacytoma with massive amyloid deposition have never been reported so far. To the best of author’s knowledge this is the first report in the dog of a bilateral mammary extramedullary plasmacytoma with features consistent with primary amyloidoma of the breast in the woman.

1. Balbino BF, Hernandez FJ. Amyloid tumor of the breast. Arch pathol 1973; 95, 102-105
2. Taniyama H, Kitamura A, Kagawa Y, Hirayama K, Yoshino T, Kamiya S. Localized amyloidosis in canine mammary tumors. Vet Pathol. 2000 Jan; 37 (1): 104-107

Veterinary and comparative pathology
amyloid, dog, mammary gland

SKELETAL MUSCLE EXPRESSION OF MYOSTATIN, IGF-1 AND GATA-2 IN CATTLE TREATED WITH GROWTH PROMOTERS

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Hormones are key regulators of mammalian muscle metabolism both in health and disease. Myostatin (MSTN), a member of transforming growth factor-beta family is a negative regulator of skeletal muscle mass. Several authors reported a direct relationship between myostatin expression and hormones treatment (Santos et al., 2012). Moreover, insulin-like growth- factor 1 (IGF-1) as well as GATA-2 have been broadly implicated in skeletal muscle growth, hypertrophy and regeneration (Musarò et al., 1999). This study aimed to determine the effects of different growth promoters on myostatin, IGF-1 and GATA-2 expression in skeletal muscle of veal calves and beef cattle.

Trial 1: 24 Charolaise beef cattle, randomly divided into 3 groups received: group A (n=6) 5 doses of 17beta-estradiol (20 mg/week/animal, IM), group B (n=6) DEX per os (0.7 mg/day/animal) for 40 days, group C (n=6) prednisolone per os (15 mg/day/animal) for 30 days; and group K1 (n=6) was untreated. Trial 2: 24 Friesian beef cattle, divided in 4 groups were treated as follows: group D (n=8) Revalor-200 subcutaneous implant for 89 days; group E (n=8) Revalor-200 subcutaneous implant for 89 days and dexamethasone per os (0.7 mg/day/ animal) for 40 days; group F (n=8) Finaplix-H subcutaneous implant for 89 days; group K2 (n=8) was the control.

Trial 3: 18 Friesian veal calves, randomly allotted in 3 groups received: group G (n=6) 35 mg/week of 17beta-estradiol, for 6 times; group H (n=6) 175 mg/week of testosterone, for 6 times; and group K3 (n=6) was untreated.

Trial 4: 30 Friesian veal calves, divided in 4 groups received: group L (n=8) was 17beta-estradiol (5 mg/week/animal) for 6 times and brotizolam (0.25 mg/day/animal) for 31 days; group M (n=8) 17beta-estradiol (5 mg/week/animal) for 6 times and dexamethasone (0.4 mg/day/animal) for 31 days; group N (n=8) nandrosol (150 mg/biweekly/animal) for 4 times and ractopamine (80 mg/day/animal) for 31 days; and group K4 (n=6) was untreated.

Samples of the sternocleidomastoideo muscle were collected from each animal and subjected to quantitative PCR for MSTN, IGF-1 and GATA-2 genes.

No statistical difference was observed in skeletal muscle for MSTN and GATA-2 genes examined in all experimental groups compared to controls.

The IGF-1 expression was significantly up-regulated in the group A ($P<0.01$) as well as in the group F beef cattle ($P<0.01$). Also in veal calves IGF-1 gene expression was significantly up-regulated. In particular, group L and group N gene expression were respectively 2.18-fold increase ($P<0.05$) and 2.48-fold increase ($P<0.01$) compared to controls.

The growth-promoting effects of sex steroids administration in livestock are well known.

IGF-1 is a potent anabolic factor that can activate myocyte proliferation and differentiation, leading to muscle hypertrophy. This study demonstrate the up-regulation of IGF-1 in skeletal muscle of cattle treated with estrogenic and androgenic molecules, as reported in humans and rats (Gentile et al., 2010; Pöllänen et al., 2010). However, in our study the increase of IGF-1 did not induce the up-regulation of mRNA GATA-2, as previously reported by Musarò and colleagues (1999). Further studies are needed to understand the role of sex steroids in the regulation of skeletal muscle hypertrophy.

Gentile et al., (2010). *J. Mol. Endocrinol.* 44, 55–73.
Musarò et al., (1999). *Nature.* 400, 581-585
Pöllänen et al., (2010) *Growth Horm. IGF Res.* 20, 372–379.
Santos et al., (2012). *Neuromuscular Disorders.* 22, 864.

pathology

skeletal muscle, growth promoters, cattle

SPONGY POLIOENCEPHALOPATHY IN TWO BELGIAN MALINOIS PUPPIES

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Spongy degeneration is seen in prion diseases and in a variety of progressive, invariably lethal neurodegenerative disorders. In various dog breeds it can involve the white or the grey matter. Amongst those involving the gray matter, cases are described in Bull Mastiff, Rottweiler, Saluki, Cocker Spaniel and Australian Cattle dog. A hereditary spongy degeneration has been reported in Malinois puppies, which suffer from congenital tremors with ataxia. In this report two cases of spongy degeneration in Malinois puppies are described, emphasizing the morphological and immunohistochemical features of the CNS lesions.

Two unkindred Belgian Malinois puppies were presented for mild depression, tremors of the head, cerebellar ataxia, and progressive ascending paraparesis. Complete blood count and serum biochemistry were normal. Serology for canine distemper virus, Toxoplasma spp. and Neospora spp. was negative. Cerebrospinal fluid showed no abnormalities. Because of the progressive ataxia and development of proprioceptive deficits on all limbs, and poor prognosis, the owners elected for humane euthanasia.

Brain, spinal cord, and samples of major organ were fixed in 4% buffered formaldehyde. Transverse sections of brain and spinal cord were embedded in paraffin, sectioned at 5 µm, and stained with haematoxylin and eosin, Luxol fast blue. Immunohistochemistry for 2',3'-cyclic nucleotide-3'-phosphohydrolase (CNPase), Olig2, glial fibrillary acidic protein (GFAP), phosphorylated neurofilaments, heat shock protein (HSP)70 and ubiquitin were performed on selected sections.

No lesions were detected at gross examination.

Histological lesions were restricted to the brain and were characterized by the presence of bilateral and symmetric large interneuronal vacuoles in the area of cerebellar nuclei, granular cell layer and foliate white matter of the cerebellum. Phosphorylated neurofilaments revealed numerous axonal spheroids; no ubiquitin-positive intracellular deposits were detected and a normal myelin pattern was evident with Luxol Fast Blue and CNPase immunohistochemistry. Oligodendrocytes showed a normal nuclear reaction for Olig2.

Lesions detected in these puppies are similar to previously described cases (1,2); the predominant lesions were found in cerebellar nuclei and flocculonodular cerebellar cortex, whereas spongy degeneration was not detected in the cerebral cortex. These are the first cases of spongy polioencephalopathy of Malinois dogs described in Italy, and the first time that immunohistochemical features are reported.

1.Cachin et al., 1991, 2.Kleiter et al., 2011

neuropathology

neuropathology, malinois, immunohistochemistry

STEM CELL MARKERS IN BENIGN AND MALIGNANT CANINE PROSTATE TISSUES: AN IMMUNOHISTOCHEMICAL INVESTIGATION

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Canine prostatic carcinoma (PCa) is considered a relevant model for studying advanced, hormone refractory PCa in men. It has been proposed that cancer contains a minor population of cells that can self-renew while simultaneously giving rise to tumour cells (cancer stem cells). Survivin is an acknowledged cancer therapy-resistance factor overexpressed in several tumour types, proposed as a valid cancer biomarker for human prostatic cancer for an early screening for malignancy. Sox9 is a stem cell marker expressed in several adult tissues, required for prostate development. Accumulating evidence indicates that it contributes to the development of human PCa. No studies have been published concerning the immunolocalization of survivin and Sox9 in canine prostatic hyperplasia (BPH) and neoplasia. AIM: to evaluate the patterns and levels of expression of survivin and Sox9 in canine BPH and PCa, in order to correlate their expression with malignant histological features.

immunohistochemistry with specific antibodies in a set of canine BPH and PCa.

Survivin nuclear and rare cytoplasmic immunostaining were present among the basal/reserve cell layer of normal and hyperplastic prostatic lobules. An increase of survivin expression was observed in PCas compared with BPHs. 6/11 PCas showed rare positive nuclei, mainly among the "basaloid" neoplastic cells in the areas with a tubular-papillary arrangement. In the areas with a solid pattern the cytoplasmic immunostaining was more diffuse. Sox9 expression was absent in normal prostatic glands and in all BPHs. 6/9 cases of PCa were highly positive.

Based on the role of survivin as a stem cell marker and the main role in proliferation of nuclear survivin, the positive cells among basal cell layer in normal and HBP cases could represent transit amplifying cells maintaining some stem cell proprieties. The increased survivin expression in Pcas would indicate the molecule as a valid prognostic marker. The absence of expression of Sox9 in the normal gland and all the BPHs suggests that Sox9 is not a stem cell marker of canine adult prostatic stem cells. The high expression observed in PCas clearly suggests an important role of the molecule in canine prostatic carcinogenesis and malignant progression. Further studies should be done in order to confirm this hypothesis.

Argyle DJ. Prostate cancer in dogs and men: a unique opportunity to study the disease. *Vet J.* 2009; 180: 137-138.

Liao CP, et al. Cancer stem cells and microenvironment in prostate cancer progression. *Horm Cancer.* 2010;1(6):297-305.

Li XL, et al. On-chip selective capture of cancer cells and ultrasensitive fluorescence detection of survivin mRNA in a single living cell. *Lab Chip.* 2013 Oct 7;13(19):3868-75.

Huang Z, et al. Sox9 is required for prostate development and prostate cancer initiation. *Oncotarget.* 2012;3(6):651-63.

Disciplinare

dog, stem cell, prostate cancer

STUDY OF THE HIPPO-TRANSDUCER TAZ IN CANINE AND FELINE MAMMARY TUMORS: PRELIMINARY DATA

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Among others, HIPPO signaling was recently indicated as a key regulator in both physiological and neoplastic cell growth, differentiation, and survival. One of the major output of HIPPO pathway is the inhibition of the coactivator transducer TAZ which controls cell growth, proliferation and survival but also cell differentiation, stem cell renewal, reprogramming, and patterning. TAZ activity correlates with high grade, metastasis, and Cancer Stem Cells (CSCs) content in human breast cancer. Particularly, TAZ is required to sustain self-renewal and tumor-initiation capacities in breast CSCs, moreover its biological activity is associated with epithelial-mesenchymal transition which disrupts TAZ inhibition, increasing its activity. The aim of our study was to evaluate TAZ expression in canine (CMCs) and feline mammary carcinomas (FMCs) particularly investigating correlation to grading and histopathological morphology.

We investigated TAZ expression in 60 CMCs and FMCs and surrounding non-neoplastic mammary gland by immunohistochemistry. The selected mammary tumors equally belonged to the following histopathological groups: simple tubular carcinomas grade I, simple carcinomas grade III, ductal-associated tumors grade I (cats), complex carcinoma grade I (dog), simple-carcinoma-and-myoepithelioma (SCMM) grade I (dog). Three different scoring systems were applied and compared.

TAZ was found to be diffusely overexpressed in grade III simple CMCs and FMCs. In addition, basal cells were TAZ positive both in neoplastic and hyperplastic canine glandular tissue. Interestingly, a moderate positivity was detected also in basal undifferentiated cells of ductal-associated FMCs. Canine SCMMs showed higher TAZ expression compared to complex CMCs.

These preliminary results indicate that also in CMCs and FMCs TAZ may be an important pathway transducer for aggressiveness and might regulate basal cells proliferation and fate. Further samples collection and analyses are required to give insights into its role and regulation in mammary cancers and eventually its additional prognostic information.

Liu S, Wicha MS. Targeting breast cancer stem cells. *J Clin Oncol*, 2010 1;28(25):4006-12.

Cordenonsi M, et al. The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. *Cell*, 2011 11;147(4):759-72.

Ferletta M, et al. Canine mammary tumors contain cancer stem-like cells and form spheroids with an embryonic stem cell signature. *Int J Dev Biol* 2011;55(7-9):791-9.

Blacking TM, et al. CD44 is associated with proliferation, rather than a specific cancer stem cell population, in cultured canine cancer cells. *Vet Immunol Immunopathol*, 2011 15;141(1-2):46-57.

Pang LY, et al. Feline mammary carcinoma stem cells are tumorigenic, radioresistant, chemoresistant and defective in activation of the ATM/p53 DNA damage pathway. *Vet J*, 2013;196(3):414-23.

Anatomia patologica veterinaria - Tumori mammari

Hippo, TAZ, Mammary tumor

THE ASSESSMENT OF FEED EFFECTS ON LIVER AND INTESTINE OF REARED FLATFISH: WHAT IS THE BEST STANDARD ANATOMOPATHOLOGIC PROTOCOL?

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This presentation highlights the importance of using standardized methods by the fish pathologists to assess the effects of feed on reared flatfish. The liver and intestine are the most important organs for the digestion and absorption of nutrients from feed, and monitoring their histological structure is of fundamental importance in assessing the effects of nutrient mixtures. A critical analysis of the histological methods on feed effects will be presented, aiming to suggest a standardized protocol that should be adopted, on the basis of the advantages and disadvantages that have been encountered in the course of our recent research trials.

Six feeding trials have been carried out on juvenile common sole (*Solea solea*), Senegalese sole (*Solea senegalensis*) and turbot (*Psetta maxima*) (fed diets with increasing dietary protein levels; 2, 3 increasing dietary plant protein in replacement of fishmeal; 4 increasing lipid levels 5 and increasing mussel meal 6, respectively).

Routine histology (haematoxylin-eosin stain on formalin-fixed and paraffin-embedded sections) was carried out to check degenerative and/or inflammatory changes. In the liver, as well as the gradation of the lipid content, further histochemical techniques have been applied in order to characterize the type of intracellular accumulation (OilRedO, Toluidine blue, PAS) and transmission electron microscopy (TEM), while in intestine in situ techniques to assess the cellular turnover and trophism of epithelial mucosa (anti-PCNA immunohistochemistry and TUNEL method for apoptosis) evaluated by image analysis and TEM were employed.

The routine histological evaluation is helpful only to demonstrate degenerative-inflammatory changes and its usefulness increases when combined with a grading system for the lipidic content of the liver that revealed the most frequent degeneration assessed. To confirm the lipidic content, Toluidine Blue on semithin sections revealed better than OilRedO which run on frozen sections that require a more difficult management of the samples.

IHC on intestinal sections and the evaluation of cellular kinetics and mucosal turnover are necessary tools to assess the influence of a diet on mucosal trophism.

For both tissues TEM is the elective method to further characterize the type of intracellular accumulation and the early signs of cell damage.

On the basis of our experience it is necessary to adopt a standardized approach to evaluate the effects of experimental diets on liver and intestine; in particular, investigations in liver require Toluidine Blue on semithin sections and TEM, while for intestine IHC together with digital image analysis and morphometry are suggested in addition to routine histology.

1Raskovic et al, 2011; 2Gatta et al., 2011; 3Bonaldo et al., 2011; 4Mandrioli et al., 2012; 5Bonvini et al., 2014 (Proceedings of ISFNF); 6Bonaldo et al., 2014 (Proceedings of ISFNF).

fish pathology

flatfish, liver and intestine, histopathology

Unusual mass stranding of *Caretta caretta* in the North Adriatic Sea: relevant gross and histological lesions.

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Caretta caretta is the most abundant species of marine turtles in the Mediterranean Sea. The greatest threats for the sea turtles in many parts of the world include, marine pollution, loss of nesting habitats, accidental capture by surface fishing with longline hooks or to lesser extent other types of fishing. Worldwide, the loggerhead sea turtle is currently classified as an "endangered" species (IUCN), and its situation is probably critical in the Mediterranean Sea. Between October and December 2013 an unusual mass stranding involving 300 dead *Caretta caretta* occurred on the coasts of the North Adriatic Sea. Depending on the tidal currents and on the wind directions, the sea turtles stranded in 4 main waves on the coasts of Emilia Romagna (ER), Friuli Venezia Giulia (FVG) and Marche (Ma). 54 necropsies were performed at the Department of Comparative Biomedicine and Food Science (BCA) of the University of Padua, 10 subjects from FVG and 44 from ER. Regarding the biometrical data the sex distribution was females 63%, males 28%, and 9% undetermined. On the basis of Curved Carapace Length (CCL) they were 11% juveniles, 72% subadults and 17% adults. The conservation status of the carcasses was very uniform with 46% of the subjects in moderate decomposition and 45% in advanced decomposition; only 7% of the carcasses were mildly decomposed and 2% mummified. Generally, all the subjects were in good status of nutrition (70%); only 24% had scarce fat deposits (6%, not detectable). About the main lesions, 63% of the subjects had haemorrhagic oedema between the pectoral ventral muscles, 60% haemorrhagic effusions within the body cavities (30% in both coelomic and pericardic cavities), and 56% severe and diffuse often haemorrhagic enteritis, lack of ingesta and moderate quantity of haemorrhagic catarrhal contents. Considering all the data together, the dead stranded *Caretta caretta* seem to belong to a singular group that died suddenly and later drifted to the Italian coasts. Parasitological, bacteriological and histological analyses were done; toxicological and biological analyses are in progress. Unfortunately, the scarce status of conservation of the turtle tissues prevent any interpretation of possible lesions (except very rare granulomas or parasite eggs), but strange small round formations appear in different organs of most of the histologically examined turtles: in 18 out of 21 turtles (86%), unidentified roundish eosinophilic cell-like structures are visible, mostly with dark brown crowns, often with very small basophilic structures inside, from 5 to 50 µm large. These corpuscles are mainly in the intestine (48%), spleen (48%) and thymus (29%) suggesting that from the gut they have a tropism for the lymphoid system. Electron microscopy analyses are in progress. Until now, the data does not support any valid theory for the death of so many turtles.

UTERINE LEIOMYOMA ASSOCIATED WITH CYSTIC ENDOMETRIAL HYPERPLASIA-PYOMETRA COMPLEX IN ASIAN ELEPHANT (ELEPHAS MAXIMUS).

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Leiomyoma is the most common uterine tumor in women, occurring also in many domestic animals³. Most captive female elephants are nulliparous and aged and many have endometrial diseases, factors that may hinder fertility^{1,4}. We here describe a case of uterine leiomyoma associated with cystic endometrial

hyperplasia—pyometra complex occurred in a 42-year-old female Asian Elephant (*Elephas maximus*), maintained at the Rome Zoological Garden.

The Elephant, nulliparous, occasionally expelled necrotic material from uro-genital opening for nine years before death. Histologically, this material was always identified as an exudate in the process of organization, resulting positive to *Escherichia coli*. Repeated cycles of antibiotic (moxifloxacin) were made. Two days before death, the animal showed systemic malaise, weight loss and ataxia.

At necropsy, the uterus was significantly increased in volume and abundant purulent material was present in the lumen; the wall was considerably thickened, with the presence of numerous cysts mixed with nodular lesions of variable size, whitish in color and with increased compactness. Masses of necrotic material could also be seen adhering to the wall or free in the lumen. Histologically, numerous nodular lesions that showed neoplastic proliferation of smooth muscle cells in diffusion-type arrangement were observed. The mitotic index was low and there was absence of nuclear atypia. In addition, diffuse hyperplasia of the endometrium with formation of numerous cysts containing liquid amorphous eosinophilic were seen. In some areas there were extensive purulent-necrotic foci. Histological sections stained with Masson's method emphasized the presence of smooth muscle cells and, immunohistochemically, neoplastic cells were constantly positive for vimentin and for α -smooth muscle actin, while they were negative for cytokeratin and S-100. In this case report, the features of the neoplastic cells and the positivity for Masson's method, vimentin and for α -smooth muscle actin clearly express the origin of the tumor from smooth muscle². In addition, *Candida* sp., *Aeromonas* sp. and *E. coli* were isolated from the uterus.

To our knowledge, this is the first case of uterine leiomyoma associated with cystic endometrial hyperplasia-pyometra complex described in an Asian Elephant.

1)Aupperle et al., Chronic endometritis in an Asian elephant (*Elephas maximus*). *J Zoo Wildl Med.* 2008;39(1):107-10. 2)Ilha et al., Uterine lesions in 32 female miniature pet pigs. *Vet Pathol.* 2010;47(6):1071-5. 3)Meuten DJ, *Tumors in Domestic Animals*, 4th Edition Iowa State Press, 2002 Ames, Iowa, USA, 4)Sapundzhiev et al., Fibroleiomyoma in elephant uterus. *J Vet Med A Physiol Pathol Clin Med.* 2007;54(9):499-500. 5)Schaudien et al., Omental leiomyoma in a male adult horse. *Vet Pathol.* 2007;44(5):722-6.

ONCOLOGIA ANIMALI ESOTICI

UTERINE LEIOMYOMA, CYSTIC ENDOMETRIAL H, ELEPHAS MAXIMUS

WINTER DISEASE IN ASSOCIATION WITH INTESTINAL NON-FORMING XENOMA MICROSPORIDIA IN GILTHEAD SEABREAM (SPARUS AURATA)

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Winter disease (WD) is a multifactorial disease found primarily in sea caged gilthead sea bream (*Sparus aurata*) along the Mediterranean coast.^{1,2} An emaciative syndrome has been recently observed in Spain and *Enterospira nucleophila*, a new microsporidian species of the family Enterocytozoonidae has been described; these intracellular, non-forming xenoma microsporidia, opportunistic in nature exploit a weakened host immune status, as it could happen in WD.⁶ Teleost intestine contains Mast Cells (MCs), whose functional properties are similar to those of mammalian mast cells;⁵ recruitment of MCs to sites of persistent inflammation is a general response in parasites-affected fish. An increased number of the MCs is also reported in WD-affected fish.^{3,4} In December 2013 a disease outbreak in sea caged gilthead seabream in Italy, affecting 0+ year fish occurred. The aim of this study was to evaluate the histopathological changes related to these two conditions, to characterize the MCs by immunohistochemistry and the microsporidia by PCR.

From twenty gilthead sea bream intestinal tracts were fixed in buffered formalin at the sample site and sent to DIMEVET. Routine histological sections were obtained; Luna stain was also performed. Immunohistochemistry with CD117 antibody (1:100, Dako) was also carried out. Intestinal tissue was also subjected to molecular analysis; a fragment of the 18S rDNA was amplified and then sequenced.

Hindgut showed a moderate dilatation of the lumen in association with whitish casts, similar to the milk-like mucous casts reported in WD outbreaks.^{1,2} A severe mucosal atrophy with total folds flattening was present; within lamina propria and submucosa a mild to moderate MCs hyperplasia and mild mucous cells hyperplasia were observed. Multifocally, within enterocytes and rodlet cells, the nucleus and/or cytoplasm contained microsporidian spores, more evident with Luna stain. The sequences obtained from intestines showed 99.9% identity with *E. nucleophila*. Within perivisceral exocrine pancreatic acini, focal necrosis and MCs infiltration have been observed, as reported by other authors during WD outbreaks.

MCs are interpreted as “standing force” in particular tissues consistently exposed to pathogens, in contrast to a “mobilization force” that has been an advantage in those being exposed to noxious agents only occasionally.⁷

The severe mucosal flattening could be interpreted as an effect of a chronic insult, not only related to the microsporidia infection; other concurrent predisposing factors as those reported in WD could be then considered into the development of an overt pathology.

¹Birincioglu et al, 2013; ²Colorni, 2011; ³Contessi et al,2006; ⁴Ibarz et al, 2010; ⁵Lauriano et al.,2012;⁶ Palenzuela et al.,2014; ⁷Reite and Evensen,2006.

fish pathology

winter disease, *sparus aurata*, microsporidia

DILATION OF THE VERTEBRAL CANAL IN A PARETIC BOA CONSTRICTOR (BOA CONSTRICTOR IMPERATOR) AFFECTED BY PROLIFERATIVE OSTEOARTHRITIS

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Proliferative vertebral lesions are a common finding in snakes and have been classified into three distinct categories: bacterial osteoarthritis, including active septic osteomyelitis or osteoarthritis with multiple inflammatory foci, and non-bacterial degenerative ankylosing osteoarthritis. Computed tomography (CT)-guided biopsy could be especially useful to rule out active bacterial infections.

AIM. To describe a case of proliferative osteoarthritis and dilation of the vertebral canal in a paretic boa constrictor (*Boa constrictor imperator*).

Radiography and CT-guided biopsy, haematoxylin-eosin staining and histopathological examination.

A 1-year-old, male boa constrictor presented with paresis of the trunk originating cranial to the cloaca. Radiographies were consistent with proliferative bone lesions involving several vertebrae. CT demonstrated the presence of lytic lesions, characterized by widening of the spinal canal. CT-guided biopsies of the lesions were performed. Histology was consistent with bacterial osteomyelitis and osteoarthritis. Gram-negative bacteria (*Salmonella* sp.) were isolated from cultures of the biopsies. A medical treatment was attempted for several weeks without clinical or radiographical improvements. The animal was euthanized and necropsy confirmed the findings observed upon CT. The main lesion comprised 3 vertebral bodies and the vertebral foramen appeared dilated and filled with friable gray/green to brown (purulent/necrotic) material, surrounded by abnormal vertebral bone. Another lesion, which was clinically undetected, consisted in accumulation of purulent material on the ventro-lateral aspect of the vertebra, involving the left rib. Histologically, the vertebral foramen was filled with degenerated heterophils and necrotic material, with bacterial colonies, completely replacing the spinal cord, surrounded by a rim of reactive macrophages and fewer multinucleated giant cells.

Dilation of the vertebral canal is an uncommon finding in snakes presenting proliferative osteoarthritis and possibly carries poor prognosis. Considering that proliferative vertebral lesions may be secondary to bacterial or other causes, and blood culture is not one hundred per cent sensitive, CT-guided bone biopsies may be useful to reach a definitive diagnosis.

Frye, F. L., & Carney, J. Osteitis deformans (Paget's disease) in a boa constrictor. *Veterinary medicine, small animal clinician* 1974; 69,186-188.

Isaza, R., et al. Proliferative osteoarthritis and osteoarthritis 208 in 15 snakes. *Journal of Zoo and Wildlife Medicine* 2000; 31, 20-27.

Kiel, J. L. Spinal osteoarthropathy in two southern copperheads. *The Journal of Zoo Animal Medicine* 1977; 8, 21-24.

Vignoli, M., et al. Computed tomography-guided fine-needle aspiration and tissue-core biopsy of bone lesions in small animals. *Veterinary Radiology & Ultrasound* 2004; 45, 125-130.

generale

snake, *Salmonella*, osteoarthritis