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FORENSIC INVESTIGATION ON A DOG FOUND DECAPITATED. AN UNEXPECTED RESULT

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Trauma caused by the impact of a train is typically severe and instantly fatal. Nevertheless, in the absence of witnesses, determining the manner of death can be difficult. In human forensic medicine the most common dilemma is to clarify whether the death was due to accident, suicide or murder [1]. In May 2015 a Czechoslovakian wolfdog was found dead on the tracks, with the head completely detached from the body. The carcass, in advanced state of decomposition, was sent to IZSLT for necropsy and toxicological analysis. Due to the position of the remains, local authorities suspected that the animal was decapitated with an edged weapon, and that only later were the remains placed on the tracks to simulate having been run over by a train. In January 2016, since no new elements had emerged that could clarify the origin of the injuries, the remains were re-examined at the Referral Center for Veterinary Forensic Medicine to determine the cause of death and decapitation. A forensic necropsy was undertaken, including complete skinning of the carcass and photographic documentation with metric reference. Necropsy showed extensive splancno and neurocranial fractures and fractures of left scapula and tibia. Skinning revealed hemorrhages at the fracture sites and only on the left side of the skull. The neck was separated from the trunk at C7-T1; skin showed irregular margins. Protruding 10 cm from the distal surface of the neck were the stumps of the trachea and esophagus; the latter was longer than the tracheal stump. Stereomicroscopic examination showed thick, curled edges on the distal edge of the esophagus. The trachea was torn away due to tearing of muscle tissue between two cartilaginous rings at the level of the bronchial carina. Black oily material was observed on the left side of the body. Toxicological analysis of liver samples (HPLC, GC-MS) was negative.

Based on the suffusion of blood, visible only after skinning, it can be stated that the traumatic lesions were produced intravitam. It was excluded that the dog was already dead when hit by the train. It was excluded that the head was removed by a sharp

instrument since stab wounds were absent on skin and underlying tissues. Furthermore, trachea and esophagus were both excised by traction, having given way at the point of least resistance. In both cases the lacerated ends were located within the chest, thus in a position that cannot be reached by a cutting instrument. In cases of accidents involving people lying on the rails, impact of a train with speed >100 km/h may result in partial dismemberment; in the case of impact with a crouching person, the most severe lesions are produced on the skull [2]. The trauma described here is compatible with the collision of a high-speed train that hit the dog from back to front and on the left side. Cause of death was due to skull fractures that probably rendered the animal unconscious before being immediately followed by decapitation. The findings obtained from forensic necropsy allowed us to establish that decapitation occurred intravitam due to lethal blunt trauma associated with violent traction on the head.

[1] Nikolic et al 2013
[2] Driever et al 2002

A CASE OF A HUNTING DOG ALLEGEDLY KILLED AND CONSUMED BY WOLVES: VETERINARY FORENSICS FINDS THE REAL CULPRIT

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In the winter 2015-16 several cases of predation and consumption on domestic dogs were reported in Central Italy from local press and social media. The dogs were all used for wild boar hunting. The owners reported that dogs, unleashed in the woods to track the boars,were found within hours almost completely consumed. These cases caused uproar in hunters and dog owners, and the wolf was singled out as the responsible for the killings.

The remains of one dog allegedly killed and eaten by wolves were submitted to 'Centro di Referenza Nazionale per la Medicina Forense Veterinaria' for necroscopic examination, in order to ascertain the identity of the predator. The examined dog was an adult female Maremma hound. The carcass was devoid of the skin and of superficial and deep muscles. The skin was only preserved at the head and distal extremity of left rear limb. Lesions consistent with carnivore bite marks were not identified. The margins of the skin showed a clean edge and were not interested by hemorrhages. The left femur was exposed, with signs of deep furrowing on the distal bone surface. Abdominal and thoracic viscera were not affected by consumption. The appearance of the skin edges, unaffected by hemorrhages, was consistent with the defleshing having occurred postmortem. Lesions consistent with intravitam or postmortem carnivore bite marks were not identified. On the exposed bone surfaces furrowing had occurred, while no punctures or pits were identified that are usually present in cases of carnivore scavenging [1]. Therefore from necropsy findings the signs of consumption on the carcass were not consistent with the action of a carnivore. The carcass appeared to be consumed 'by layers', probably by an animal whose teeth do not allow wide and deep bites. Salivary swab samples were collected from the most apparent lesions produced by scavenging, such as furrows on the bones or torn muscles. The swabs were submitted to DNA extraction, and two panels of 15 and 20 nuclear loci (Short Tandem Repeats, STRs)

specific to *Sus scrofa* and *Canis lupus*, respectively, were applied [2, 3]. No wolf alleles were amplified from any sample, while 13 out of 15 STRs of *Sus scrofa* were successfully amplified. Genetic analysis at STR loci revealed that at least 2 wild boars fed on the dog. The use of forensic pathology and genetics allowed us to identify wild boar as the responsible for this case of consumption on a hunting dog. Other similar cases reported by the press have been likely attributed erroneously to the wolf, despite the absence of scientific proof and to the detriment of the actions undertaken for the conservation of the species. The wolf population has expanded in recent years, causing a resurgence of conflicts with human activities [4] and retaliation killings (with traps, poison or firearms). Poaching is still one of the main threats to the conservation of the wolf [5], thus it is essential not only to carry out appropriate measures for conflicts management, but also to correctly identify the predator in cases of attacks to domestic animals [6].

[1] Young et al 2015
[2] Lorenzini 2005
[3] Lorenzini et al 2014
[4] Lovari et al 2007
[5] Dondina et al 2014
[6] Fico et al 2005

THE EFFECT OF TEMPERATURE AND POST-MORTEM INTERVAL ON THE TRANSPARENCY OF THE EYE LENS IN DOGS

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The postmortem processes and the factors that affect them are very important in the estimation of the postmortem interval (PMI). The time of death and survival period may be used to determine criminal charges in animal cruelty cases. Several postmortem changes can provide valuable information in death investigations and most of these are affected by environmental conditions (1). Many post-mortem modifications have been studied in human as well as in animals, but, to our knowledge, no one considered the modifications of eye lens. The lens is a transparent, avascular organ (2) composed by a high concentration of protein (approximately 300 mg/ml) (3). The maintenance of transparency depend on the function of epithelial cells (2), on the interaction among lens proteins (protein-protein interaction) (3) and on intercellular communication (gap junction) that allows intercellular passage of molecules (up to 1 kDa) such as antioxidants (2). The opacification of the crystalline lens is also possible in the postmortem period because of the low temperatures of the body during the Algor Mortis (4, 5, 6). The aim of the study was to evaluate the modifications of the lens transparency of dead dogs over the time and the opacification of the lenses at low temperatures. We studied the lenses of twenty-five adult dogs at different time of death and at different storage temperature to assess variations of the optical density of the lens using a DOTTIE II transmission densitometer. We created a light source stabilized to calibrate the densitometer to 0.00. We observed and registered the opacification of the lens and the value of the transmitted light in the frozen dogs lens and over time postmortem. In frozen dogs at -18°C for 7 days, macroscopically, we observed opacification of the lenses and increase of optical density. Instead, the removed lenses, stored at room temperature (24°-26°C) showed increase of optical density starting after 8 days postmortem. These results will be useful in forensic veterinary medicine to better evaluate the period of death and temperature of storage of the cadavers. The understanding of the phenomena

underlying the postmortem opacification will also be useful to better understand the pathogenesis of in-vivo ocular diseases such as the cataract in humans and animals.

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ANGIOINVASIVE SUBCUTANEOUS LYMPHOMA IN THE CAT

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Angioinvasive lymphoma (AL), also called lymphomatoid granulomatosis, is a rare angiocentric/angiodestructive lymphohistiocytic proliferative disease that primarily involves the lung. Histologically, AL is characterized by a mixed mononuclear infiltrate containing large and small lymphoid cells, plasma cells and histiocytes with vascular infiltration. Although the pathogenesis of human AL is still unclear, cytotoxic T-cells activation likely play an important role in the development of the disease (2). The clonal B cells proliferation can be associated with Epstein Barr virus (EBV) infection (1). Few cases are reported in dogs (4) and cats (5) affecting mainly the lung. Only one study reported the skin and subcutis as the primary sites of AL in a cat (3). The present report describes the histopathological and immunohistochemical findings of recurrent subcutaneous nodules in an adult cat. A 12 years-old male domestic shorthair FIV FeLV negative cat was presented with single subcutaneous nodule in the right shoulder. The cat did not receive any injection for at least three years. A fine needle aspiration was consistent with a malignant round cell neoplasia. The cat did not show pulmonary radiographic involvement, lymph nodes enlargement and visceral ultrasound detectable lesions. The mass was subsequently removed and, on histology, a diffuse infiltration of atypical polymorphic lymphoid cells, admixed with moderate number of epithelioid macrophages was observed in the subcutis. Angiocentric cuffs of neoplastic lymphocytes, with mural invasion and confluent foci of coagulative necrosis were prominent. By immunohistochemistry, diffuse B cells (CD20+, CD79+) and multifocal angiocentric T cells (CD3+) were observed. Multifocal myeloid/histiocyte antigen positivity was also detected. The morphological and immunophenotypic features were consistent with a T cell-rich B cell angioinvasive lymphoma. One month later a fine needle aspiration of a new nodule in the neck showed the same features. As described in human medicine, the T-cells lymphoid angioinvasion is considered a peculiar feature to distinguish AL among other mixed lymphoid processes. Considering the relationship between AL and Herpesvirus in humans, a viral antigenic stimulation should be investigated also in

domestic animals. To the authors' knowledge, this is the first report of a primary cutaneous AL in the cat, without evidence of visceral involvement.

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VEGFA AND VEGFRS EXPRESSION IN CANINE APPENDICULAR OSTEOSARCOMA

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Osteosarcoma is the most common primary malignant tumour of bones in dogs, occurring most frequently in the axial skeleton (75%), it is locally aggressive with a high angiogenetic and metastatic potential. VEGFRs (VEGFR1, VEGFR2 and VEGFR3) are the most important receptors regulating tumoral angiogenesis following the interaction with VEGF factor.

The aim of this study was to investigate VEGFR-1, VEGFR-2 and VEGFR-3 and VEGFA expression in canine OSA tissues and cell lines to evaluate its prognostic value in relation to clinical outcome of the canine patients.

Thirty-one dogs diagnosed with canine appendicular were enrolled in the study. All the animals underwent a complete clinical staging and were treated with surgery and then they were followed until the recurrence of the neoplasm or death. All the samples were histologically evaluated and immunohistochemically tested for VEGFA, VEGFR-1, VEGFR-2 and VEGFR-3. Histological and immunohistochemical results were evaluated in relation to clinic-pathological data. Total RNA was extracted from 8 canine osteosarcoma cell lines and expression of VEGFR1, VEGFR2 and VEGFR3 canine gene were evaluated by qPCR.

VEGF was present in all analyzed cases and particularly was widely expressed in 33% of cases, moderately expressed in 46% of cases and poorly expressed in 20% of OSA analyzed. Regarding the expression of the receptors we found that the 64.52% of canine OSA were positive for VEGFR-1, 70.97% were positive for VEGFR-2, while 74.19% cases per positive for VEGFR-3. The positivity for VEGFR-1 was statistically associated with the positivity for VEGF ($P<0.05$) and VEGFR-3 ($P<0.05$). Statistical analyses comparing the immunohistochemical results with all clinical-pathological data revealed no statistical associations. Molecular data showed that only D22 cell lines over-expressed all three VEGFRs while VEGFR2 was expressed only by Wall and D22 cell lines and VEGFR3 by D22, Pedro, Lord and Wall cell lines if compared to osteoblastic cell line.

This is the first study investigating VEGFRs and VEGFA expression in canine OSA and cell lines demonstrating that those receptors are widely expressed in this tumor. No statistical association has been found between VEGFRs expression and clinical and histopathological features while a significant association between VEGFR1 and VEGFA has been found. In human osteosarcoma the autocrine loop VEGFR-1/VEGFA is correlated to the malignant progression while VEGFR2 and VEGFR3 seem not to be involved.

These preliminary data suggest that also in canine OSA this autocrine loop can be relevant in the progression of canine osteosarcoma as demonstrated in canine mammary tumors and that be considered a suitable target for innovative targeted therapies.

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EXPRESSION OF CD44, CD10, P63, VIMENTIN, TAZ/YAP, E-CADHERIN, AND BETA-CATENIN IN HUMAN, CANINE, AND FELINE MAMMARY TUMORS

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Mammary cancer is one of the most common type of cancer in women and female dog and cats. In cats it is an aggressive lethal (80-90%) tumor whereas in dogs it is a very heterogeneous tumor both in term of morphological subtypes and co-existing subpopulations. The aim of the study was to investigate different markers in a subset of human, canine, and feline mammary tumors with regards to stem cells and undifferentiated cells phenotype. Particularly, we investigated the expression of CD44, CD10, p63, Vimentin, TAZ/YAP, E-Cadherin and beta-Catenin in both single and dual immunohistochemical staining. Twenty-one canine mammary carcinoma, 15 feline mammary carcinoma, and 5 triple negative human breast cancers were included. Positivity was calculated in a semi-quantitative manner and also precise location of expression was monitored in the normal mammary gland counterpart of each sample. CD44, CD10 and p63 were expressed in the basal compartment of simple canine carcinomas despite not perfectly overlapping in term of percentages of expression. Solid pleomorphic canine carcinomas were predominately negative to CD44, whereas triple negative HBC were diffusely positive indicating only in the latter the predominance of undifferentiated precursors. CD44 was also very evident in the luminal canine compartment suggesting that this molecule is probably more widely expressed in the canine gland and tumors. CD10 was not observed in feline carcinomas and p63 was limited to residual basal/myoepithelial cells delimitation. Curiously, CD44 was variably expressed in feline tumors, also in discordance with a few published data. Vimentin and TAZ/YAP were often co-expressed in canine solid carcinomas showing also a negative correlation with the expression of membranous E-cadherin and beta-Catenin. Similarly, even if with less percentages triple-negative cancers were co-expressing Vimentin and TAZ/YAP. Feline carcinomas were diffusely a highly expressing and co-expressing

Vimentin and TAZ/YAP indicating an aggressive proliferative phenotype. E-cadherin and beta-Catenin were also diffusely decreased on the cellular membrane in aggressive grade III feline tumors. In conclusion, triple negative breast cancer, feline carcinomas, and canine solid pleomorphic carcinomas suggested an epithelial-to-mesenchymal transition phenotype with activation of YAP/TAZ pathway. CD44 was not observed as precisely marking undifferentiated cells since it was more diffusely distributed then expected also in the normal gland. This study would help elucidating the role and the distribution of these markers in mammary carcinomas to better understand the composition of cell subtypes in different tumors of different species.

EXPERIMENTAL INDUCED HYPOFERTILITY IN SARDA BREED RAMS BY BLUETONGUE VIRUS SEROTYPE 1 INFECTION.

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Bluetongue virus (BTV) infection can be clinically unapparent or provoke a fatal clinical manifestation, which includes fever, prostration, stiffness, dispnea, nasal discharge, and anorexia. Among the several symptoms, male infertility has also been described, either in natural illness or after using attenuate BTV strains for vaccination. In these cases BTV infection clinically resulted with alteration of the morphology, decreasing of the number and reduced viability of the spermatozoa or complete azospermia. However, the pathogenesis of such effects has never been investigated. Herein, we experimentally studied the pathogenesis of BTV serotype 1 infertility in Sarda breed rams by using a field isolate. Nine 2-year-old rams were inoculated intradermally and subcutaneously with 3 and 10 ml, respectively, of total BTV serotype 1 infected blood originated from a clinically affected Sarda breed ram. After, 3 rams were serially euthanized at 5, 7 and 15 days post-inoculum (p.i.), respectively. At necropsy a wide sampling of tissues was collected for viral RNA quantification by Real-Time PCR, histopathology as well as for viral VP7 and NS2 proteins immunohistochemical detection (IHC).

No clinical signs were observed in the animals sacrificed at 5 days p.i.. While, in the 3 rams sacrificed at 7 and 15 days p.i., the infection was clinically apparent and merely characterized by the involvement of the genital tract, with severe hyperthermia and edema of the scrotum at 7 days p.i.. BTV RNA was detected in the animals sacrificed at 5, 7 and 15 days p.i., in blood, lymphoid tissues and testicles. Histologically, no lesions were found in the testicles of the rams at 5 and 7 days p.i., while edema and inter-tubular vasculitis with consequent severe degeneration of the tubular germinative epithelium were found in the testicles of the rams sacrificed at 15 days p.i.. By IHC, BTV

was found in the endothelial cells of the testicular, epididymal and scrotal skin capillaries only at 5 and 7 days p.i.. Hypofertility has been reported in rams vaccinated with BTV serotype 2 live modified vaccine and naturally affected by BTV serotype 8. Interestingly, BTV serotype 1 and BTV serotype 8 field strains have not been considered responsible for any lesion in the reproductive tract of rams after experimental infection. In this study, we observed that rams experimentally infected with 2013 Sardinian BTV serotype 1 field strain displayed severe degeneration of spermatic epithelial cells. The IHC results coupled with the histopathological findings clearly indicate that in the testicle the degeneration of the germinative epithelium during BTV infection is ascribed to the endothelial damage of the intertubular capillaries.

E5 ONCOPROTEIN OF BOVINE DELTAPAPILLOMAVIRUSES IS EXPRESSED IN CONGENITAL CARCINOSARCOMATOSIS OF LAMBS

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Papillomaviruses are oncogenic, double stranded DNA viruses responsible for epithelial and mesenchymal tumors in humans as well as in domestic and wild animals.

Bovine Deltapapillomaviruses (δPVs) are known to be the only Papillomavirus causing a cross-species infection. In sheep, the association of Papillomavirus infection with tumors and other disorders has been poorly investigated; indeed, Papillomavirus-associated lesions were described only in adult animals.

To our knowledge, congenital carcinosarcomatosis (CCS) in lambs has not previously been reported. The present study deals with the pathological findings observed in Sarda breed lambs affected by severe CCS at gengiva, palate and muzzle skin level.

Proliferative lesions were macroscopically observed just few days after birth in lambs, which died at about one month of age as they were not able to feed.

Tissues from two lambs were collected for molecular, histopatological and ultrastructural investigations.

Histologically, a mixture of epithelial (keratinocytes) and mesenchymal cells was seen. Numerous mitoses, many of which atypical, were observed in both cell types. In all samples the oncoprotein E5 was detected immunohistochemically. Ultrastructurally, neoplastic cells showed abnormal nucleoli-containing nuclei, the morphology of which was characterized by the presence of deep meandering invaginations giving them a bizarre and lobulated appearance. Atypical nuclei such as micronuclei were also seen. Intranuclear electron-dense particles, 40 nm in diameter, consistent with virus particles were shown.

Herein we documented that E5 oncoprotein of bovine δPV is expressed in sheep and appears to be involved in a malignant lesion of lambs. Our study shows, for the first time, a possible interspecies infection between sheep and cattle. Bovine δPVs are known to be responsible for vertical transmission in cattle as it has been shown that they can infect trophoblasts in vivo. It is conceivable to think that they could be responsible for transplacental infection also in sheep offspring.

Neverthless, the role, if any, of bovine δPVs in carcinogenetic and reproductive disorders of sheep warrants further studies in order to improve our knowledge about molecular pathways leading to neoplastic and no-neoplastic events.

1. IARC, 2007
2. Roperto et al., 2010a; 2010b; 2011; 2012; 2013; 2014
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POXVIRUS INFECTION ("TATTOO SKIN DISEASE") IN TWO STRIPED DOLPHINS (*STENELLA COERULEOALBA*) STRANDED ALONG THE ITALIAN COASTLINE

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Tattoo skin disease (TSD) is a Poxvirus-induced cetacean disease characterized by typical skin lesions. Few pathological descriptions and a limited number of TSD reports are available worldwide. We describe herein the histological, biomolecular and virological identification of TSD in two striped dolphins (*Stenella coeruleoalba*) stranded along the Latium and Tuscany coasts of Italy in 2015 and 2016, respectively. A full necropsy was performed on the two male, juvenile and well-preserved dolphins under study, followed by detailed histopathological and transmission electron microscope (TEM) investigations. DNA extraction from skin lesion samples and PCR amplification of Poxvirus DNA polymerase were also carried out. The first striped dolphin showed wide, coalescing, lightly gray skin lesions with dark edges (tattoos) on the head, while the second one had a single, 2 cm-wide, round, yellowish lesion with a slightly dark edge, affecting the mandibular region. Numerous Poxvirus-like particles were observed in both animals' skin samples by means of TEM. In their skin, a multifocal, severe, hydropic degeneration of the keratinocytes of stratum spinosum was also apparent, with numerous round, 5-10 μm in diameter, eosinophilic, glassy structures (intracytoplasmic eosinophilic inclusion bodies), compatible with type-B poxviral inclusions ("Guarnieri bodies") being additionally found. The overlying stratum corneum was mildly hyperplastic (1.5 times over normal), with heavily hyperpigmented keratinocytes, occasionally hosting Guarnieri bodies. Viral DNA polymerase PCR allowed to confirm the presence of Poxvirus in the skin from both dolphins. To the best of our knowledge, this should be the first report of TSD in cetaceans stranded along the Italian coastline. It has been suggested that anthropogenic factors may play a major role in the emergence of skin diseases, with special emphasis on immunodeficiency originating from exposure to high levels of immunotoxic pollutants, which can be directly linked to TSD occurrence. Further studies are underway, in order to assess the tissue loads of immunotoxic contaminants in these

two dolphins, as well as in order to investigate the potential relationships between the level of exposure to the aforementioned pollutants, on one side, and Poxvirus infection's development, on the other.

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MULTIVISCERAL TETRATHYRIDIOSIS WITH GENITAL INVOLVEMENT IN AN EUROPEAN CAT

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Tetrathyridium bailleti is the second larval stage of *Mesocestoides lineatus*. Human, dog, cat and other carnivores are definitive hosts. *Tetrathyridium* can pierce bowel wall of dog or cat to reach body cavity producing peritoneal larval cestodiasis also known as tetrathyridiosis (1). Aim of this work is to describe the first report of multivisceral tetrathyridiosis with genital involvement in an european cat, highlighting the salient injuries and discriminative framework of infection as well as the important role played by molecular investigation to diagnose the parasite species involved. To the author's knowledge no report describes a parasitic oophoritis and metritis caused by *T. bailleti* and takies into account the rarity as well as the distinctive characteristics of this disease in cat rather than in dog (2, 3). The domestic cat lived in a garden with other conspecifics and it has not been vaccinated or treated against infectious agents and parasites. Physical examination and x-ray evaluation showed moderate abdominal swelling, cough, dyspnea and several pulmonary nodules that at first oriented clinicians towards a diagnosis of cancer. Necropsy carried after the owner request, showed the parasitic etiology of disease. Tissue samples collected during necropsy were routinely processed for histopathological examination. Adult tapeworms and larvae were stored in 70% alcohol, then placed in Petri dishes and observed with stereomicroscope (Zeiss Discovery V12), while flatworms belonging to the larval stage were processed for the observation with the electron microscope Cambridge Stereoscan 240 (SEM). Adult (n=1) and larval (n=1) tapeworms stored in 90% ethanol were sent to confirm morphological identification by means of PCR amplification and sequencing. Necropsy and histopathology showed

multivisceral parasitosis, with free and encysted worms in both body cavities, on serosal surface of the abdominal wall. Several multifocal granulomas were detected in spleen, lungs, uterus and ovary. The framework of pulmonary edema, granulomatous inflammation and emphysema led the cat to death. Morphological and molecular investigation confirm the diagnosis of Tetrathyridiosis. The features of oophoritis and metritis due to tetrathyridia could be interesting for clinicians, since despite the lack of reproductive history, on the basis of the observed lesions, it is possible to hypothesize reproductive function disorders, like oestrus disorders or persistent anoestrus, infertility or primary uterine inertia due to the injuries observed.

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EXPLANTS FROM BOVINE AIRWAYS: A SUITABLE TOOL TO INVESTIGATE THE EARLY PATHOGENESIS OF CONTAGIOUS BOVINE PLEUROPNEUMONIA?

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Contagious bovine pleuropneumonia (CBPP) is a relevant disease caused by *Mycoplasma mycoides* subsp. *mycoides* (Small Colony type, MmmSC). CBPP has been eradicated from most of Europe, while it is endemic in many Sub-Saharan Countries. The pathogenesis of CBPP is poorly understood and mainly investigated in cattle, infected by endotracheal intubation or by contact with infected/diseased animals (Scacchia et al., 2011).

The present study aims at evaluating in vitro models, which could be suitable to investigate the early stages of MmmSC infection and CBPP pathogenesis. The respiratory tracts (trachea, bronchi and lungs) from apparently healthy and regularly slaughtered cattle (n=7) were collected and studied. All samples were tested for *Mycoplasma* spp. by polymerase chain reaction (van Kuppeveld et al., 1994) and proved to be negative.

Two MmmSC strains, both alive and inactivated, were used: "57/13" (Italy, 1991) and "Caprivi" (Namibia, 2003). MmmSC inactivation was obtained by heat-treating (10' at 100°C) or by 10% formalin (50%, v/v). According to van Riel et al. (2007), the adhesion of MmmSC to the host cells was first evaluated on formalin-fixed tissue samples, routinely processed for histopathology. Tissue sections were then submitted to a "double-step" immunohistochemistry (IHC), by sequential incubations with MmmSC and with a murine anti-MmmSC primary antibody.

In addition, MmmSC "colonization" of the respiratory tract was investigated in living explants, which were incubated with both MmmSC strains (1 h at 37°C) and then formalin-fixed, processed for histopathology and submitted to IHC using a murine primary antibody anti-MmmSC.

To better detail the localization of MmmSC, laser scanning confocal microscopy (LSCM) tests were carried out using primary antibodies anti-MmmSC, cytokeratins, von

Willebrand factor and lysozyme. Negative and positive controls were included in each IHC and CLSM run.

MmmSC showed a marked tropism for the lower respiratory tract, while it spared the tracheal and bronchial tissues. IHC and CLSM detected MmmSC attached on and/or inside the epithelial cells of bronchioles and alveoli, the macrophages and the endothelial cells lining the blood and lymphatic vessels, these results closely resembling the IHC pattern observed in naturally infected cattle (Bashiruddin et al., 1999). Inactivated MmmSC strains were able to "colonize" different cell types residing within the cattle airways, thus suggesting that MmmSC could also "passively" adhere to and penetrate inside the host cells.

Our data suggest the suitability of in vitro models - complementary if not alternative to the use of experimental animals which sound useful to investigate the early stages of infection. Further studies are currently ongoing to evaluate the kinetics of MmmSC infection, as well as the host reactions, by means of medium-to-long term organotypic cultures.

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POLYAMINES ACTIVITY IN CANINE INFLAMMATORY COLORECTAL POLYPS BEFORE AND AFTER A PROBIOTIC BACTERIA TREATMENT

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Recent researches demonstrate a correlation between polyamine intake or intestinal exposure, to risk of colorectal neoplasia (Vargas et al., 2012). Furthermore, the role of polyamines, spermidine (SPD) and spermine (SPM), and their precursor putrescine (PUT), regulated in their cellular levels by ornithine decarboxylase (ODC), in cell growth and proliferation is very well recognized. Increased polyamine levels are observed also in patients with IBD with their corresponding inflammatory index revealed that increased concentrations of polyamines found in the most severe inflamed mucosal areas. Some probiotics seem to have anti-inflammatory and tumor inhibitory properties, but few studies have investigated their actions on mucosal polyamine levels. Recently, a demonstration that dysbiosis is associated with canine inflammatory colorectal polyps (ICRPs) development, and that this may represents a potential therapeutic target, was published (Igarashi et al, 2016). In this study, the effects of probiotic mixture on colonic polyamine biosynthesis in dogs with colonic polyposis (CP) were investigated. Histological sections of dogs with a long-time diagnosis of colonic polyposis (n=5) were analyzed. These dogs had received between 112 and 225 billion (112 to 225 x 109) of lyophilized bacteria daily for 60 days, and samples were obtained at baseline (T0) and 30 days after the end of treatment (T1; i.e. 90 days after T0). Histology scores, the expression of PUT, SPM, ODC and DAO positive cells, and the clinical activity index (CIBDAI) were compared at T0 and T1 using paired t-tests or Wilcoxon matched pairs tests, where appropriate. Additionally, levels of cellular proliferation (Ki-67 expression), and apoptosis (Caspase 3 protein expression) in the polyp were also evaluated. After probiotic treatment, significant decreases were observed for CIBDAI ($p=0.006$) and histology scores ($p<0.001$). In

contrast, SPM, PUT, and ODC expression increased ($p<0.01$) after probiotic treatment. Specifically, a significant decrease in colonic polyamine levels, ODC activity and Ki-67 was noted at T1 to T0. In contrast, a significant increase in caspase-3 positive cells and DAO expression ($p=0.005$) was also observed. Polyamines levels suggest a potential anti-proliferative effect of probiotics in hyperplastic mucosa, but also an anti-inflammatory effect associated with a reduction of mucosal infiltration. These effects could be related to increase in some bacteria genera such *Faecalibacterium* after probiotic treatment. Interestingly *Faecalibacterium* catalyzes the irreversible transfer of a propylamine group from the amino donor S-adenosylmethioninamine (decarboxy-AdoMet) to putrescine (1,4-diaminobutane) to yield spermidine, increasing PUT and SPD levels (van Vliet MJ, 2010). In conclusion, this study provides data about the ability of a cocktail of probiotics, administered for 8 weeks, to regulate polyamine levels, by enhancing polyamine biosynthesis and degradation in canine inflamed polypoid colonic mucosa, and to reduce cell proliferation in hyperplastic/neoplastic areas.

LYSOSOMAL STORAGE DISEASE (LSD): MORPHOLOGICAL ASPECTS OF GANGLIOSIDOSIS (GM) IN A FAMILY OF BOARS

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Lysosomal storage diseases (LSD) are heterogeneous group of progressive, lethal, multisystemic diseases. Generally, they have autosomal recessive inheritance and are gene-dose dependent. Inherited LSD show deficient enzymatic function of specific enzymes due to a genetic defect. These pathologies can be classified by the underlying molecular defect or according to the accumulated product. Gangliosides are membrane constituents of neurons; genetic defects in catabolism of these glycosphingolipids cause a neuronal LSD defined Gangliosidosis (GM). The aim of this work is to describe morphological features of GM in all boars of the same brood. 3 littermate boards, from a free ranging farm, presented neurological signs at 9 month of age. Due to the worsening conditions, they were euthanized at 1 year of age and submitted for necropsy. Tissue samples were submitted for viral and bacteriological analysis, routine histology (formalin-fixed and paraffin-embedded) and for TEM (glutaraldehyde-fixed). Also tissue samples were frozen. Tissue were negative for bacteria, CSF and Aujeszky virus. Paraffin sections (brain, spinal cord, peripheral ganglia and retinal ganglion cells) showed enlarged foamy neurons, with finely diffusely vacuolated cytoplasm. Nucleus moved from to the center to the plasma membrane and Nissl substance was effaced. Diffusely hepatocytes were characterized by the same cytoplasmic vacuolization that affects neurons, as well as Kupffer cells. TEM analysis revealed numerous swollen and degenerated neurons. Their cytoplasm was enlarged by the presence of numerous perinuclear membrane bound vesicles and lysosomes were severely filled by membranous material arranged in concentric lamellae and whorls (membranous cytoplasmic bodies). Some neurons appeared completely filled by the pathological lysosomes, that peripheralized or efface the nucleus. These aspects of lysosomes are referred to LSD, particularly ultrastructure

aspects of the content can be ascribed to GM. GM in swine has been described only in 1978 in purebred Yorkshire swine (*Sus scrofa domestica*) and has never been described in boars (*Sus scrofa*). Biochemical investigations are in progress.

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NEUROFIBROMATOSIS TYPE 1 GENE AFFECTS THE TUMORIGENESIS EVENTS IN *CARASSIUS AURATUS*

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Neurofibromatosis type 1 (NF1) is a common dominantly inherited genetic disorder that results from various mutations in the neurofibromin 1 (NF1) gene. The mutational events generates abnormalities in neural-crest-derived tissues that include hyperpigmented skin lesions, glioblastoma, schwannoma and malignant peripheral nerve sheath tumors. The nf1 gene encodes the large protein neurofibromin, which contains a GTPase-activating protein-related domain (GRD) capable of inactivating the RAS proto-oncogene (1). Furthermore, loss of nf1 in a p53-deficient background results in highly penetrant malignant formation (2). Zebrafish is currently the most suitable animal model for studying NF1. Homozygous loss of both alleles in combination generates larval phenotypes that resemble aspects of human diseases and results in larval lethality in 10 days (3). In our studies goldfish (*Carassius auratus*) was used and breeded at the CISS facilities, of the University of Messina (Italy). Little is actually known on the genome of *C. auratus* that up to date has not been entirely sequenced. We selected wild types as a control and diseased fish. Fish showed obvious damages as non-pigmented areas with raised or lost scales and dermal thickening. DNA was extracted from both the tumour site as well as the normal tissue. Since the nf1 gene has never been described in goldfish, we employed heterologous primers based on zebrafish nf1 gene in order to obtain an amplicon. Zebrafish nf1 gene has been properly described, whereas no literature has been shown related to goldfish homologue DNA. Firstly we identified the NF1 gene in goldfish and compare it with the zebrafish homologues, correlating among mutations and characteristics of neurofibromatosis. Later, the sequences with mutations within the goldfish gene were compared with the normal genotypes in order to identify nucleotide changes not linked to specific species-related markers through goldfish and zebrafish. The comparison of protein sequences of the goldfish gene on GeneBank revealed amino

acid changes following nucleotide substitutions in the positions 2279, 2289, 2293 and 2294. This work suggests that mutations of nf1 gene may be involved in the loss of function of neurofibromin protein. Because the gene plays a negative regulation on cell proliferation by inactivating Ras proto-oncogene it could be hypothesized the same molecular mechanism in goldfish. Gene expression test targeted to Ras in *C. auratus* with mutated nf1, could confirm the oncogenetic mechanism. Actually, we conclude that the founded mutations for nf1 genes could give the same models demonstrated for zebrafish.

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NON-HUMAN PRIMATE MODEL OF TYPE 2 DIABETES MELLITUS: CEREBRAL EXPRESSION OF GLUCOSE TRANSPORTERS GLUT1 AND GLUT3 AND ALTERATIONS ASSOCIATED WITH INSULIN RESISTANCE AND COGNITIVE IMPAIRMENT

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There is a large body of evidence documenting the cognitive impairment associated with type two diabetes mellitus (T2DM). These impairments range from mild amnestic effects to almost Alzheimer's Disease-like dementia, but the underlying mechanisms are unknown. Hence, the Insulin Resistance Hypothesis, which theorizes that there is a significant role for insulin in normal cognitive functioning and that T2DM-related insulin dysregulation may contribute to cognitive impairment. For the present study, 15 aged female Rhesus macaques were investigated to evaluate aspects of the insulin resistance hypothesis using a three-pronged approach: confirm body composition changes with physiological testing; gauge cognitive capability using a Novel Object Recognition (NOR) task; and measure expression levels of two glucose transporters, GLUT1 and GLUT3, in the brain regions associated with NOR-related learning and memory.

Body composition and physiological changes consistent with insulin resistance, characterized by hyperinsulinemia and euglycemia were confirmed. In particular, a strong correlation has been observed between abdominal fat, body weight and insulin resistance. More specifically, omental fat weight correlated positively with Intravenous Glucose Tolerance Test (IVGTT) mean insulin levels, indicating that animals with more omental fat have higher insulin levels.

Many physiological parameters correlated positively with NOR task performance such that degree of disease progression predicted poorer cognition in primates with altered glucose metabolism, in particular pronounced insulin resistance. We found both positive and negative correlations among physiological parameters and the expression levels of the glucose transporters. Clear associations are between GLUT1 and GLUT3 immunohistochemical expression levels in brain regions of interest (hippocampus and

entorhinal cortex) and increased body fat and insulin resistance. However, NOR performance did not correlate significantly with the expression of glucose transporters. Overall, our results provide some support for the insulin resistance hypothesis of T2DM-mediated cognitive deficits, although deficits do not appear to be mediated through glucose transporter-regulated mechanisms.

NEUROAXONAL LEUKODYSTROPHY IN THREE CHIHUAHUAS

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Neuroaxonal dystrophy (NAD) is a neurodegenerative disorder characterized by severe degeneration of neuronal cells and their processes. The predominant neuropathological feature is the presence of a large number of spheroids in the CNS.

The aim of this study is to describe the neuropathological findings in 3 cases of NAD in Chihuahua puppies. Two 2-month-old male Chihuahuas from the same litter and a presumably not related 2-month-old female Chihuahua were presented with neurological signs including severe depression, tetraparesis, ataxia, absence of menace response and bilateral strabismus. Post-mortem examination revealed lesions restricted to the CNS in all cases. Grossly there was moderate to severe dilation of lateral ventricles accompanied by atrophy of the cerebral cortex and flattening of the cerebral convolution, as well as cavitation of the subcortical white matter, thinning of the corpus callosum and rupture of the septum pellucidum. Coronal samples of fixed brains were routinely processed for histology and sections were stained with H&E, Luxol Fast Blue, CNPase, neurofilaments and GFAP. Histopathological examination revealed marked and widespread axonal swelling with formation of round to irregularly shaped spheroids, accompanied by gliosis and severe myelin loss. The lesions primarily affected the white matter in the cerebrum and cerebellum, and both white and gray matter in the medulla oblongata, pons and spinal cord. Spheroids were numerous and large in the white matter of the cerebrum, cerebellar medulla, and several nuclei of the brain stem including lateral cuneatus, spinal tract of trigeminal nerve, olivary, solitary tract, lateral lemniscus, cochlear, trapezoid body, and lateral and medial geniculate. The presence of spheroids was moderate in the pontine nuclei, transverse and longitudinal pontine fibers, caudal colliculi and periaqueductal grey matter. A moderate number of spheroids was found in the cerebellar nuclei and in the nucleus of vagus. Spheroid of smaller caliber were found in the cerebral and cerebellar cortices. Scattered spheroids were evident in the reticular substance of the medulla oblongata and pons. Segmental loss of Purkinje cells was observed in all cases, accompanied by cytoplasmic vacuolation in one case.

The lesions observed in our cases were consistent with a form of NAD. In canine NAD, dystrophic axons are mainly found in the grey matter of the sensory brainstem nuclei, accompanied by cerebellar atrophy. In Chihuahuas and Papillons spheroids are also described in the white matter and in the cerebral and cerebellar cortices. Our cases differed from previous reports showing severe cerebral atrophy and high involvement of cerebral and cerebellar white matter with spheroids accumulation, while cerebellar atrophy was limited to mild loss of Purkinje cells. Findings in our dogs resembled Hereditary Diffuse Leukoencephalopathy with Spheroids described in adult humans.

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RISK FACTORS OF GASTROINTESTINAL PARASITES LUNGWORMS TICKS AND LICE IN DONKEYS IN THE ASINARA NATIONAL PARK (SARDINIA - ITALY)

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From June to November 2015 a total of 113 Asinara donkeys (41 albino, 72 coloured) were sampled (91 fecal samples from 36 albino and 55 grey donkeys). All donkeys were surveyed for ticks and lice. Sedimentation, Baermann and modified McMaster methods were performed for endoparasites. The EPG/OPG were calculated. Larval cultures were performed and L3 were recovered by a Baermann technique. Ectoparasites were morphologically identified. The infection's ticks level were recorded defining 3 categories: no infestation, low (1-10 ticks) and high infestation (>10 ticks). Three land cover types were defined to estimate the risk: sparse vegetation; mediterranean shrubland; grassland. Statistical analysis were performed through GLM with a ordinal logistic regression (SPSS 20.0, Chicago, IL). Ninety out of ninety-one donkeys were infected by intestinal strongyles (98.9%), *Strongyloides* (6.6%), *Parascaris equorum* (15.4%), *Oxyuris equi* (2.2%) and *Eimeria leukarti* (2.2%). No eggs of cestodes and trematodes were found. *Dictyocaulus arnfieldi* L1 were found in 46.1% of samples. Fecal pools were positive for *Cyathostominae* (61%), large strongyles (30%) and *Trichostrongylus axei* (9%) L3. Strongyles showed the highest egg excretion (mean abundance=1176.4 EPG; min-max=0-4575 EPG). Significant risk factors associated to strongyle infection (EPG) were: season; geographical distribution of herds and the land cover types. Egg shedding was 10.887 times higher in autumn than in summer and 2.865 times higher in donkeys from the North than those in the rest of the island. Donkeys from spare vegetation areas shed more eggs than other animals (OR=2.507). Albino and young donkeys were more at risk for *P. equorum* than coloured and old donkeys (OR=4.289 and OR=0.978 respectively). *D. arnfieldi* larvae shedding was higher in autumn than in summer (OR=5.577). *Haemaphysalis punctata* (46.2%), *Hyalomma marginatum* (10.7%) and *Rhipicephalus bursa* (43.1%) were found. A total of 58.4% (66/113) of donkeys were infested by ticks

(28.3% albino; 30.1% coloured). The prevalence was 78% (32/41) and 47% (34/72) respectively in albino and coloured donkeys. Albino donkeys group had the highest percentage with high infestation (39% vs 15%; OR=2.865; P=0.021). The highest percentage of donkeys with no ticks (57.77%) were from land with "sparse vegetation" and had a low number of ticks (OR=0.185; P=0,001) than donkeys from other areas. *Haematopinus asini* were found on nine donkeys (8%), 8 albino and 1 coloured (OR=17.212, 95% CI 2.067-143,321, P= 0.009). Significant risks to tick infestation were associated to the colour of coat and the types of land cover. Albino donkeys show a 3.120 times higher risk than coloured donkeys to be infected by ticks. Donkeys from areas with sparse vegetation cover showed a lower risk to be infected by ticks (OR=0.227).

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HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDIES OF PARASITE ASSOCIATED GRANULOMA DEVELOPMENT IN VISCERAL ORGANS OF GREY MULLETS (*OSTEICHTHYES*: *MUGILIDAE*) FROM SARDINIAN LAGOONS

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Grey mullets (*Osteichytes: Mugilidae*) are cosmopolitan fish that inhabit estuaries and lagoons and are particularly important in the fisheries and economy in particular Sardinian areas. Parasitic diseases have been found to be the main health problem in these populations (1). Fish immune response against these parasites is mainly represented by chronic granuloma development (2). The aim of this work was to describe the structure of parasitic granulomas and their temporal progression in visceral organs of grey mullets by histological and immunohistochemical techniques.

A total of 239 grey mullets were collected from four different Sardinian lagoons (western Mediterranean Sea) in two seasonal samplings. Fish were euthanized (Tricaine Methanesulfonate) and a complete necropsy was performed. Samples of visceral organs, where macroscopic granulomas were detected, were processed for histological examination, stained with Hematoxylin-eosin (HE), Masson's Trichrome (MT) and investigated by immunohistochemical techniques using anti-cytokeratin AE1/AE3 and anti-Vimentin antibodies. Quantitative assessment of epithelioid cells, fibroblasts and collagen component of granulomas was performed with a semiquantitative grading score system, whereas rodlet cells (RCs) and eosinophilic granular cells (EGCs) were quantified with an image analysis software (Rasband, W.S., ImageJ). Microscopical features of the lesions were analysed using Stata 11.2 software (StataCorp LP).

Histopathological examination revealed two groups of different granuloma categories according to the aetiological agent (digenean trematodes or *Myxosporea*). Granulomas associated to metacercariae of digenean parasites revealed a higher number of EGCs

(ρ=0.5197, P<0.05), whereas granulomas due to spore of *Myxobolus* sp. were significantly associated with a higher number of RCs (ρ=0.4296 , P<0.05).

Three developmental stages were identified during the evolution of the granulomas on the basis of common histopathologic features in both parasitic groups. Early stage granulomas were characterised by an intact parasite and minimal inflammatory response. In the intermediate stage granulomas, epithelioid cells (CK AE1/AE3 positive) were evident and represented the most characteristic cells. In late stage, fibroblasts (Vimentin positive) were noticed in large numbers in the outer portion (capsule) of granulomas. At this stage, collagen fiber development showed a significant correlation with the presence of EGCs (ρ=0.4707, P<0.05).

The immunitary response of *Mugilidae* to different classes of metazoan parasites seems to display a low specificity but, even if characterised by a common encapsulation mechanism, some differences were identified in the cell composition and associated inflammation development.

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MOLECULAR EXPRESSION OF GENES RELATED TO WNT/BETA-CATENIN AND HIPPO PATHWAYS IN CANINE AND FELINE MAMMARY TUMORS

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Mammary tumor is the most common cancer and the third most frequent cancer in dogs and cats, respectively. To date is well known that several molecular pathways, such as Wnt/beta-Catenin and Hippo, involved in cell proliferation, tumor progression and metastasis were altered in human breast cancer. On the contrary, there is only few information concerning alterations of these pathways in canine and feline mammary tumors. The aim of the present study was to investigate the expression of several genes directly or indirectly associated with these pathways, such as beta-Catenin, TAZ, YAP, CTGF, ANKRD1, and CYCLIND1 in canine mammary tumor and corresponding non-tumor tissues. Specifically, RNA from 17 canine mammary tumors and healthy tissues was extracted and subsequently, reverse transcribed into cDNA. A semi-quantitative PCR was performed in order to assess the expression level of beta-Catenin, TAZ, YAP, and CTGF. Expression level of these genes was quantified by densitometric analysis using Image J. The expression of beta-Catenin, TAZ, YAP, and CTGF were significantly ($P<0.05$) higher in canine mammary tumors than in non-neoplastic tissues. These results indicates for the first time neoplastic deregulation of the beta-Catenin and Hippo pathways in canine mammary tumors as in human breast cancer. Because feline mammary carcinomas are, in more than 80% of cases, malignant, aggressive, and associated with rapid development of metastasis we extended the study also in 6 feline mammary tumors and 6 healthy tissues. The obtained results were in agreement with those obtained in canine mammary tumors, with a greater difference in the expression level of beta-Catenin between tumor tissues and healthy tissues, probably due to the greater aggressiveness of feline mammary tumors. If this represents a scientifically valid study for dogs and cats, with direct implication in veterinary medicine, it also has implications in comparative oncology with a potential social and economic beneficial impact. Domestic animals represent an interesting model for human cancer.

DIAGNOSTIC RESULTS ON THE POPULATION OF ROCK-PARTRIDGE (*ALECTORIS GRAECA WITHAKERI*) DURING THREE YEARS LIFE PROJECT IN WESTERN SICILY.

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Sicily (Italy) hosts a "relict", endemic population of *Alectoris (graeca) whitakeri*, commonly known as Sicilian Rock partridge (Lucchini & Randi, 1998;). Today, *A. graeca* is included in the IUCN Red List rating of vulnerable species (IUCN, 2012). Recently, in order to improve the residual population, an EU funded Life Natura 2000 project (2011-2012; SICALECONS: "Azioni urgenti per la conservazione della coturnice di Sicilia") has been founded, involving IZS of Sicily for welfare and veterinary aspects. Fifteen Sicilian Rock Partridge (*A. (g) whitakeri*) carcasses were collected. All birds were identified by presumed age and sex. Necropsy was performed for each carcass for laboratory investigations. Additionally, fecal samples collected from wild birds were also screened to identify pathogens carriage as a risk for this specie. For every sample were performed an oropharyngeal, cloacal and oculo-conjunctival swabs. Tissues of suspected lesions were collected from bird carcass and fixed in 10% buffered formalin for standard histological investigations; for putative fungal infection some sections were also stained by PASM (Periodic Schiff-Methenamine). Samples from suspected lesions were also subcultured by standard procedures. The presence of internal parasites was investigated microscopically by direct mount examination through preparation of smears taken either from fecal samples and from 3 portion of the intestine (duodenum, jejunum and caecum). External and internal parasites were also collected and fixed in 70% (v/v) alcohol for future identification. Prevalence rates and intensities value of each parasite species found was calculated for every sample, then for each specimen. Necropsy involved no. 9 females and no. 6 males. Almost all birds showed ematiation and ruffled feathers, and in 4 cases *Goniodes colchici* was found. Mucosal swabs showed in one single case a pathogen strain

of *E. coli* related to granulocytic lesions in liver. Another case of death was due to nodular lung lesions caused by infiltration of *Aspergillus fumigatus* hyphae. The other oropharyngeal, cloacal and oculo-conjunctival swabs were negative for pathogenic bacteria. The evidence of internal parasites was the most relevant finding, showing different types of infestation by nematoda as *Ascaridia compar*, cestoda as *Railletina tetragona* and 4 species of coccidia as *Eimeria legionensis*, *E. caucasica, E. kofoidi* and *Isospora* spp.. Furthermore in 60% of these cases a concomitant pluri-parassitosis was found. Weak body condition have been associated only in two cases to chronic and lethal bacterial or fungal infections. This study represents the first veterinary report on this rare species and underlines the importance to monitor the health status of wild species in the Italian environment in order to preserve local biodiversity.

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EFFECTS OF 17B-ESTRADIOL AND OXYTOCIN RECEPTOR ANTAGONIST IN MURINE SKELETAL MUSCLE

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Although estrogens (E) are considered to participate in the development of the skeletal muscle phenotype, their role is still poorly understood (1). Recently, the oxytocin role (OXT) in muscle regeneration was demonstrated (2). An unexpected induction of oxytocin precursor gene (OXT) in bovine skeletal muscle of estrogen treated animals has been previously described (3-4). A potential cross-talk between skeletal muscle and adipose tissue through adipomyokines activity has been proposed (5).

Aim of the work was to evaluate the possible hypertrophic effect of 17β-estradiol (E) on murine skeletal muscle and OXT role in muscle and adipose growth. In order to evaluate the OXT role in skeletal muscle the competitive oxytocin receptor antagonist (Atosiban, OTA) was employed.

C57BL male mice were randomly divided into groups and orchidectomized except for 5 animals that were sham operated (placebo surgery). Animals were treated with subcutaneous 21-day release pellets as following: group S: sham+placebo pellet (n=5); group K: control with placebo (n=5); group A: 2mg E pellet (n=6); group B: 2mg E + 1.2mg OTA pellet (n=6); group C: 1.2mg OTA pellet (n=6). Procedures were authorized by D.M. 182/2010.

At euthanasia blood sample was collected to perform ELISA test for OXT on plasma. Vastus lateralis (VL) muscle and perirenal white adipose tissue (pWAT) were collected for morphometric analysis, for gene expression studies of OXT, OXTR, myogenic regulatory factors, myosin heavy chain 1 and 2 (MhC1 and 2), atrogin1, insulin-like growth factor 1 (IGF1), adipokines genes and peroxisome proliferator-activated receptor γ (PPAR γ) by quantitative PCR.

No differences were highlighted regarding body weight or plasma OXT concentration between groups. Morphometric analysis of VL muscle did not reveal any fiber size change. Gene expression data on VL muscle showed the up-regulation of OXTR (6.7±3.3 fold increase) in group A ($p<0.05$) compared with K and of MhC1 and fatty acid binding

protein 3 in group B (2.1±0.9 and 2.2±1.1 fold increase respectively, $p<0.05$). Gene expression data on pWAT described a significant decrease of adiponectin, leptin and PPAR γ expression in all treated groups compared with K ($p<0.0001$ in A and B, $p<0.001$ in group C). Contrary to the cattle, E does not induce OXT serum increase in mice, but still increases mRNA OXTR expression in muscle. The results confirm that E exerts effects on several adipokines and in particular represses adipogenic differentiation in pWAT (6, 7) and promotes fatty acids metabolism. Those effects are more intense in group B and are still present in group C supporting that OTA is an antagonist still able to activate some pathways of OXT metabolism (8).

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DIFFERENT APO A-IV TISSUE PROTEIN EXPRESSION PATTERNS IN APOA4 GENE INDUCIBLE AND NOT INDUCIBLE SPECIES AND ITS IMPLICATIONS IN LIPID METABOLISM

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Apolipoprotein (apo) A-IV is a protein known to participate in the regulation of various metabolic pathways, with special regards to lipid absorption, transport and metabolism. Only rodents can synthesizes apo A-IV by both liver and small intestine, whereas its synthesis is restricted to the enterocytes in the other species, human included (Wu, 1979). A study demonstrates apo A-IV role in mouse liver in enhancing triglyceride secretion and reducing hepatic lipid content by promoting very low density lipoprotein particle expansion (VerHague, 2013). In a previous study we demonstrate an inverse proportion between hepatic lipid content and apo A-IV liver expression in *Felis catus*, *Felis silvestris* and *Rattus norvegicus*, highlighting differences between cat and rat lipid metabolism (Piccinini, 2015).

In this study, the apo A-IV protein expression and tissue distribution patterns in domestic, wild cat and rat (n=5) were analyzed. Anamnestic data were collected to exclude any interfering pathology. Histological sections of intestine, liver and adipose tissue were analyzed by immunohistochemistry to assess apo A-IV tissue expression levels and protein cytolocalization.

Significant differences in apo A-IV intestinal levels between cat and rat samples were observed. Specifically, feline enterocytes showed low cytoplasmic apo A-IV protein expression, in contrast to the rat group. Rat liver revealed a marked cytoplasmic immunopositivity, with weak nuclear signal. In contrast, felids hepatocellular signal was weak in cytoplasm and marked in the nucleus membrane. Nuclear apo A-IV signal was also observed in adipocyte from all species samples.

Feline apo A-IV intestinal and cytoplasmic liver low levels are strictly related to the previously observed high hepatic lipid content. In addition, apo A-IV hepatocytes nuclear signals are related with the gluconeogenesis suppression mediated by nuclear receptors. In conclusion, these results confirm apo A-IV "key role" in lipid metabolism, suggesting new interesting hypothesis on the physiopathology of feline hepatic lipidosis and new possible therapeutic strategies in lipid disorders, also in other species, human included.

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MUTATIONS AND POLYMORPHISM IN ALBUMIN GENE OF BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*): FIRST IDENTIFICATION OF MUTATIONS RESPONSIBLE OF INHERITED BISALBUMINEMIA

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Hereditary bisalbuminemia is an asymptomatic and heterozygous condition, characterized by the presence of 2 different albumin fractions with different electrophoretic mobility that leads to typical electrophoretic pattern with double albumin peak (1). In human medicine, is usually revealed by chance, is not been clearly associated with a specific disease and the causative genetic alteration is a point mutation of human serum albumin gene inherited in an autosomal codominant pattern (2). Previously, bisalbuminemia was diagnosed by capillary zone electrophoresis in two Bottlenose dolphin's families (3), but mutations responsible of this condition and the inherited pattern were not identified. The aims of this work are: 1) to investigate polymorphism of Bottlenose dolphins' albumin gene and to identify possible mutations responsible of bisalbuminemia, 2) to identify the inherited pattern of this condition in two Bottlenose dolphin's families. Coding regions of albumin gene were screened for mutations in 15 Bottlenose dolphins kept under human care (9 from family 1, 4 of them bisalbuminemic; 6 from family 2, 3 of them bisalbiminemic) using PCR on DNA extracted from peripheral blood and tissue, and the sequence were compared to the reference sequence to identify DNA alterations. In order to identify mutations able to cause bisalbuminemia, for each non-synonymous variation identified, we studied the genotype-phenotype correlation within the two families. Eighteen albumin mutations (3 synonymous and 15 non-synonymous) were identified in the 2 families. The non-synonymous variations were identified in exon 4, 5, 6, 7, 8, 9, 10, 12 and 13. Based on the genotype-phenotype analysis we identified two heterozygous non-synonymous

variations that co-segregate with bisalbuminemic phenotype: p.Phe146Leu in exon 4 and p.Tyr163His in exon 5. The amino acid change in mutation in exon 5 seems to cause a variation into the secondary and /or tertiary structure of the albumin protein and it's already describe as causative mutation of bisalbuminemia in human beings (4). Our study confirmed that, as human albumin gene, Bottlenose dolphins' albumin possessed a significant degree of polymorphism and we identified 2 mutations potentially responsible of bisalbuminemia. Moreover, we confirm the autosomal codominant trait of this condition also in these animals.

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HEART VALVE PATHOLOGY IN WILD-CAUGHT SWORDFISH (*XIPHIAS GLADIUS*)

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The heart of marine teleosts can be affected by several infectious and parasitic diseases. Little is known about cardiac pathology and, in detail, investigations on heart valve diseases of Swordfish (*Xiphias gladius*) have never been reported.

Aim of this work is to evaluate gross and histopathological lesions of heart valves of wild-caught swordfish hearmarked for human consumption.

Thirty-sevenhearts of swordfish, aged between 3 and 13 years, caught in the Ligurian Sea (Italy), were referred to the Department of Veterinary Sciences of the University of Turin. Gender of the animals was determined by macroscopic observation of the gonads. The age was estimated by counting the bands of skeletal growth on the anal fin spines. Serial sections 1.0 mm thick of the condyle base were obtained, dried for 24 hrs, observed with a stereomicroscope, and the number of rings was counted to assign an estimated age. Heart samples were collected directly on boats and stored in 4% buffered formalin for gross and histopathologic investigations, stained with Haematoxylin-Eosin, Weigert-Van Gieson, Periodic acid-Schiff, Toluidine blue, and Alcian blue PAS.

Gross evaluation of the hearts showed in 34 out of 37 animals (91.9%) chronic pericarditis and in 15 (40.5%) and 8 (21.6%) cases a valvular thickening respectively of the atrio-ventricular and bulbo-ventricular valves. Histological examination of the atrio-ventricular leaflets revealed in 27/37 (73%) Lambl's excrescences; in 20/37 (54%) fibrosis grading 1/3 and 2/3 in 10 subjects; in 5/37 (13.5%) endocardiosis grading 1/3 and 2/3 respectively in 2 and 3 animals. Ichthyophoniasis (n=1; 2.7%), endocarditis (n=1; 2.7%) and lymphocytic infiltration (n=1; 2.7%) were also detected.

The bulbo-ventricular leaflets showed Lambl's excrescences in 14 out of 37 animals (37.8%); fibrosis in 10/37 animals (27%; n=2 grading 1/3; n=8 grading 2/3), and endocardiosis in one case (2.7%).