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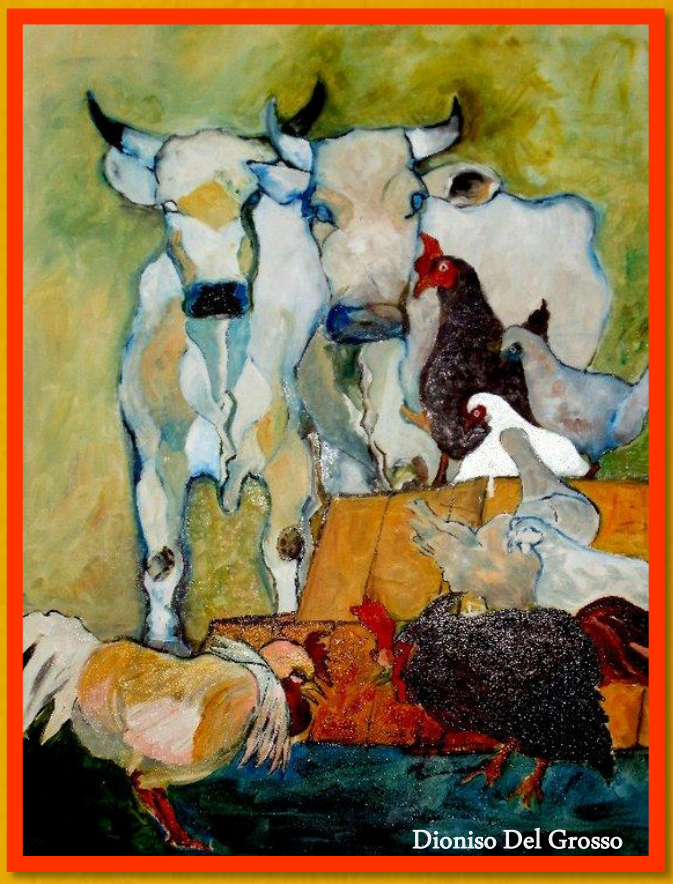

20-22 Giugno 2018

XVIII Convegno SICV

XVI Convegno SIRA

XV Convegno AIPVET

X Convegno ARNA

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Sede:
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Torino

Con il patrocinio di

CITTA' DI TORINO

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Università degli Studi di Torino

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Molecular Biotechnology Center (MBC)

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Workshop 1-ECM

Mercoledì 20 giugno 2018
(Aula ARISTOTELE)

Approccio clinico-patologico alle malattie renali del cane

Con la collaborazione di:
Associazione Italiana Patologi Veterinari (AIPVet)
Società Italiana di Clinica Medica Veterinaria (SICLIM-VET)
Federazione degli Ordini dei Medici Veterinari del Nord-Ovest

Con il patrocinio di
International Renal Interest Society (IRIS)

8.45	**Patto d'Aula e Introduzione**
9.00	**La biopsia renale nel cane: utilità clinica** **CLINICAL UTILITY OF RENAL BIOPSY IN DOGS** ***C. Brovida*** *ANUBI - Ospedale per animali da compagnia, Moncalieri (TO)*
9.35	**Il ruolo del patologo nella diagnosi delle malattie glomerulari** **THE ROLE OF THE PATHOLOGIST IN THE DIAGNOSIS OF CANINE GLOMERULAR DISEASES** ***L. Aresu*** *Dipartimento di Scienze Veterinarie, Università degli Studi di Torino*
10.10	**Marker ematici ed urinari di patologia renale** **BLOOD AND URINARY MARKERS IN RENAL DISEASES** ***S. Paltrinieri*** *Dipartimento di Medicina Veterinaria, Università degli Studi di Milano*
10.45	**Terapia standard ed immunosoppressiva in corso di glomerulopatie** **STANDARD AND IMMUNOSUPPRESSIVE THERAPY IN CANINE GLOMERULOPATHIES** ***F. Dondi*** *Dipartimento di Scienze Mediche Veterinarie, Università degli Studi di Bologna*
11.20	**Emodialisi veterinaria:stato dell'arte e prospettive** ***I. Lippi*** *Dipartimento di Scienze Veterinarie, Università di Pisa*
11.55	**Discussione e Test Finale**

CLINICAL UTILITY OF RENAL BIOPSY IN DOGS

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The renal biopsy is the diagnostic method that helps the assessment of chronic and acute renal damage. It defines the type of renal damage and allows discriminating between acute and chronic diseases that have undergone a sudden worsening. Also it's the gold standard to diagnose lesions of familiar, congenital or hereditary origin. The protocol defined by the World Small Animal Veterinary Association Renal Pathology Study Group (WSAVA RPSG) provides criteria through which renal biopsies should be evaluated such as light microscopy, immunofluorescence and electron microscopy. There are two continental referral centers that evaluate renal biopsies according to these criteria. One is the College of Veterinary Medicine of the University of Ohio, for the USA, and the second one is the EVRPS (European Veterinary Renal Pathology Service) for Europe. The coordinators are Prof. Luca Aresu (UNITO) and Dr. Silvia Benali (Lab. LaVallonea).
The renal biopsy is recommended in case of persistent and progressive proteinuria to evaluate the type of renal damage with information deriving mainly from electron microscopy. Other possibilities are to distinguish between chronic and acute renal injury, in order to motivate therapies such as hemodialysis and finally to correctly diagnose familial, congenital or hereditary renal diseases. The edge between reversible and non-reversible renal damage is always difficult to identify. Slow chronic renal pathologies can suddenly become acute as a result of infections or other concomitant diseases or immune stimuli. In these dogs, polyuria, polydipsia, worsening of clinical conditions associated with increased haematological parameters and consequent uremic symptomatology appear within few days. Renal biopsy precisely defines this diagnostic categories.
For the execution of the renal biopsy there are at least two techniques, which depend on the suspected disease, on the clinic and the diagnostic evaluation. Renal biopsy can be performed surgically to obtain an abundant portion of cortical tissue or by laparoscopic technique. In case of risky conditions or to avoid deep anesthesia, the most used method is the ultrasound-guided biopsy. A disposable Tru-Cut needle of 16, 18, 20 G is generally used depending on the size of the patient. Biopsies can be performed on both the left and right kidney; the left one is more mobile and allows an easier positioning. The right kidney is fixed and can be reached in dorsal position through the last right intercostal space. In some cases this characteristic helps the procedure. At least 5 glomeruli should be included in the biopsy for light microscopy evaluation. Two samples are taken and using a stereoscopic microscope glomeruli are counted. The two biopsies are separated and filled into Michel solution for immunofluorescence and glutaraldehyde for electron microscopy. The remaining part is fixed in formalin at 10%. The dog is subsequently monitored, kept in fluid therapy during the awakening and monitored to exclude bleeding. Renal biopsy results a very important diagnostic tool for the nephrologists. It allows the correct evaluation of glomerulopathies and the distinction between acute and chronic damage in case of ambiguity deriving from clinical evaluation parameters. However, some experience is needed to correctly perform biopsy at the renal cortical level and collaboration with an expert pathologist is essential to provide the correct diagnostic evaluations deriving from optical microscopy, immunofluorescence and electron microscopy.

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THE ROLE OF THE PATHOLOGIST IN THE DIAGNOSIS OF CANINE GLOMERULAR DISEASES

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Glomerular diseases play a preponderant role in canine kidney diseases. Primarily, the glomerular damage alters the renal filter compromising the functionality of the nephron and secondary, the renal blood flow alterations lead to a weakening of the flow in peritubular cells causing the loss of the entire nephron (1). The renal biopsy represents a diagnostic test that is moderately used in clinical nephrology, but in the last years this procedure has become mandatory to discriminate immune mediated *vs* non-immunomediated glomerular diseases and essential for a correct therapeutic protocol (2). The experience in recent years and a number of scientific works have described how to obtain a correct diagnosis using renal biopsy. Optical microscopy (OM), immunofluorescence (IF), and electron microscopy (EM) are required and the complete report of a renal biopsy should include morphological data of the three techniques (3,4). Resuming the different stages, from the obtaining of the biopsy to the final diagnosis, the first goal after the biopsy sampling includes the assessment of the adequacy of the renal specimen. Through the aid of a stereomicroscope, the count of the number of glomeruli in the sample is necessary to determine in real time the quality of the sample. Considering the number of glomeruli, the next step is related to the division of the core for the three diagnostic examinations. With regard to OM, a number equivalent to eight glomeruli is considered the minimal amount to be representative of the renal lesions in toto. The fragment block must be fixed in formalin (1:10) for at least twenty-four hours. In the laboratory, cutting and staining histochemical analysis of the renal parenchyma are standardized for the purpose of examining all the compartments (glomerular, tubular and vascular). Serial sections are performed at 3 microns for the analysis of the structures in all their plans. For the histological examination, five histochemical stainings are run: 1) periodic Acid-Schiff (PAS) 2) Hematoxylin-Eosin 3) acid Fuchsin, and orange G (AFOG) 4) Masson's Trichrome 5) Silver Metanamina Acid Periodic (PASM). When a suspicious of amyloidosis is present, it is necessary to perform Congo Red staining for the identification of fibrils of amyloid within glomeruli, vascular and interstitial compartment (5). For IF, the specimen needs to be preserved fresh. For this porpoise two options are available: 1) freezing the sample rapidly 2) maintenance tissue homeostasis conditions, up to a maximum of 48 hours, through Michel's solution. This type of solution maintains osmolarity and pH conditions of the renal tissue by stabilizing the proteins. Folllwing, in the lab, the frozen sample is cut through cryostat and 5 µm thickness sections are incubated with antibodies directed against IgA, IgG, IgM, and complement C3. The positivity of the reaction is evaluated considering the pattern (granular or linear), localization of the deposits (mesangium and/or glomerular basement membrane), distribution (focal, diffuse, segmental and global) and intensity (3 degrees). For EM, the sample is generally small in size (a maximum of three glomeruli) and needs to be immediately fixed in glutaraldehyde at 2.5% or fixative Karnosky. The laboratory procedures require that the samples are post-fixed in osmium tetroxide, dehydrated and embedded in resin. Very thin sections (1 micron) are then stained with toluidine blue 1% for identification of glomeruli. After, the ultra-thin sections are colored first with acetate of uranyl at 4% and then in citrate to be observed in the transmission electron microscope.

The three techniques are now considered mandatory to provide a definitive diagnosis. The histological examination provides good indication for the identification of glomerular lesions. Under optical microscope, disseminated lesions, involving all glomeruli, or focal, involving only

some glomeruli, are easily identified. The examination of the glomeruli allows to define the presence of lesions within a single glomerulus. The distribution may be global, involving the entire glomerulus, or segmental, involving a portion of the glomerulus, mesangium primarily. Also, lesions can be classified according to the site of the lesion: 1) endothelial cells and glomerular basement membrane, such as deposition of immune complexes at subendothelial, intramembranous or subepithelial level. The histological appearance of the thickening can be diffuse or irregular (with spike formations). The deposition of immune complexes is shown by AFOG and Masson Trichrome, or by electron microscopy. The thickening of the basement membrane is the principal feature of membranous glomerulonephritis (6, 7). 2) Presence of neutrophilic inflammatory infiltrate within the Bowman space: this type of injury is pathognomonic in post-infectious glomerulonephritis, where numerous neutrophilic granulocytes and monocytes are observed (8). 3) Mesangial cells and mesangial matrix: mesangial cellularity and matrix increase are seen in the course of mesangioproliferative glomerulonephritis and membranoproliferative glomerulonephritis. Membranoproliferative glomerulonephritis is also associated with a thickened irregular glomerular basement membrane (9). 4) Podocytes: the fusion of the foot processes that alter the glomerular filtration rate is considered an early lesion and detectable only at the ultrastructural examination. It's generally associated to minimal change disease in dog (10). The loss of podocytes leads to the exposure of the glomerular basement membrane with consequent adhesion to the epithelium of the parietal epithelial cells, creating adhesions (sinechiae). The latter occurs in focal segmental glomerulosclerosis (FSGS), in which the areas of adhesion are associated to segmental sclerosis. This lesion has been described in the dog during idiopathic form of chronic kidney disease, after nephrectomy and in association with hyperlipemia and obesity (11).

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BLOOD AND URINARY MARKERS IN RENAL DISEASES

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The functional blood markers generally evaluate glomerular filtration (glomerular filtration rate or GFR) or tubular filtration considering analytes concentration in the blood. The principal direct marker of GFR is the clearance test, but due to the laboriousness this is scarcely available in clinics. Alternatively, indirect markers whose concentration depends mainly on the GFR are used. Recently, cystatin C and the symmetric dimethylarginine (SDMA) have been proposed but creatinine and urea concentration remain the gold standard to estimate GFR, especially when variations over time are suspected. Creatinine is the parameter that is widely considered when staging renal diseases through the international renal interest society (IRIS) score. Additional information are also obtained analyzing analytes whose blood concentration increase (eg: phosphorus, amylase) when GFR is reduced. Examples are electrolytes that help to formulate hypotheses on the pathogenesis of renal disease (ex: in acute or post-renal renal syndromes, potassium) and albumin concentration whose decrease leads to protein losing nephropathy suspect. In addition, new markers are currently studied to evaluate the possible association between inflammation and renal disease (C-reactive protein and endothelin), the presence of hypertension (homocysteine and endothelin) or endocrine disorders (aldosterone, renin).

Important diagnostic and prognostic information of renal disease are also obtained by complete urinalysis. Some of the innovative markers mentioned above are also proposed as urinary markers (endothelin, aldosterone, etc.), but standard urinalysis is based on more traditional tests such as the refractometric analysis for urinary specific gravity test (USG), dipstick and sediment analysis. The sediment analysis is generally useful to highlight erythrocytes or leukocytes, crystals, cylinders, bacteria or other cells. On the supernatant obtained after sediment preparation it is fundamental to evaluate USG which classifies the urine as: severe (USG> 1035 in the dog and USG> 1040 in the cat) or moderate hypersthenuric (USG=1012-1035 in the dog and USG=1040 in the cat), isosthenuric (USG 1008-1012, compatible with renal failure in dehydrated or azotemic animals); hyposthenituric (USG <1008), a condition most often associated with renal disease. The dipstick can provide an estimate of pH, glycosuria, ketonuria, bilirubinuria and hemoglobinuria, as well as proteinuria. Usually normal urine does not contain proteins because low molecular weight proteins (MWP) that exceed the glomerular filter are reabsorbed by the tubules. Proteinuria occurs when an excess of small blood proteins such as antibodies or hemoglobin are lost, even in the absence of renal damage (prerenal proteinuria), in case of transient pressure changes (physiological renal proteinuria), in case of glomerular lesions (renal proteinuria), in case of tubular damage (tubular proteinuria characterized by low-MWP, indicating an inability of the tubule to reabsorb the small proteins normally filtered by the glomerulus), in case of both glomerular and tubular damage (mixed proteinuria), and in case of urinary or genital alterations (post-renal proteinuria). Since renal proteinuria is a negative prognostic factor, it is always recommended to investigate the origin, such as possible hereditary nephropathies or vector-borne diseases. The first approach is to perform urine dipstick on urines collected by spontaneous urination. If the sediment is inactive, the positivity indicates the presence of renal proteinuria. The result of the dipstick must be evaluated in accordance to USG. However, a strong positivity always indicates proteinuria, conversely the negativity always excludes the proteinuria, while a weak positivity is generally not indicative of proteinuria. If the result of the dipstick is strongly positive, or weakly positive in dogs with low USG, the amount of proteinuria

must be confirmed by quantitative analysis by measuring the protein:urinary creatinine ratio (UPC) which, although potentially affected by analytical variability, is used to classify renal disease following IRIS guidelines. Once proteinuria is confirmed, its origin can be investigated by renal biopsy or electrophoretic methods such as SDS-PAGE or SDS-AGE that differentiate proteins according to molecular weight and therefore classify proteinuria as glomerular (high MWP), tubular (low MWP) or mixed (both). When tubular proteinuria is present, some molecules are excreted by tubular epithelial cells and released in the urine. Such molecules include N-acetyl-β-glucosaminidase (NAG) or γ-glutamyl transferase (GGT), which are measured on fresh urine to exclude conservation artifacts. The retinol binding protein (RBP), the Kidney Injury Molecule -1 (Kim-1), and Neutrophil gelatinase-associated lipocalin (NGAL) are considered important diagnostic and prognostic factors for acute renal failure.

STANDARD AND IMMUNOSUPPRESSIVE THERAPY IN CANINE GLOMERULOPATHIES

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In 2013, the International Renal Interest Society (IRIS) has proposed guidelines for the treatment of glomerular diseases in dog. Overall, glomerular diseases are considered more frequent in dogs than in cats, probably also due to a greater difficulty in obtaining a histopathological diagnosis in the feline species. The guidelines included a standard therapeutic approach, common to all glomerular pathologies, and specific therapies aimed at to treat specific disease. It is also important to consider that although we are discussing standard treatments, the therapeutic approach to glomerular diseases must always be carefully considered by the nephrologists and individualized to the dog's condition. The novelty of this type of approach does not lie so much in new therapeutic benefit, but in a more careful of drugs by the clinician and in a more rational and stepwise approach. The patient's renal disease should be evaluated first and therapeutic objectives must be achieved in order to have a real benefit when setting the treatment.

The severity of a glomerulopathy is usually expressed, especially in the initial stages, by the proteinuria level and evaluated as urinary protein/creatinine ratio (UPC - Urinary Protein to Creatinine ratio). Whenever we are faced with persistent renal proteinuria with UPC> 0.5 (or 0.4 in the cat), a pharmacological approach must be considered.

Nutrition plays a fundamental role in the management of renal diseases in veterinary medicine. Reduced protein intake and optimal ω-3 fatty acid supplementation can reduce the severity of proteinuria and slow the progression of chronic kidney disease (CKD).

The most common anti-proteinuric treatment consists in the use of RAAS (Renin-Angiotensin-Aldosterone System) drugs. Thromboembolism, following a generalized thrombophilia, is a serious complication in the course of protein loosing glomerular diseases, recognized both in humans and in dogs. The most commonly used tromboprofilactic drugs in small animals are heparin and acetylsalicylic acid (ASA). In the course of glomerular diesease another important issue to be considered is arterial hypertension. Established that the patient is hypertensive, it is necessary to set up an antihypertensive therapy to prevent the damage to target organs such as kidneys, eyes, myocardium, and brain. The objectives of antihypertensive therapy are to minimize the future risk of organ damage and to support a significant reduction in proteinuria. Alterations of body fluid homeostasis are common in small animals with glomerular pathology and include excess, deficit and misdistribution. However, little is known about the pathogenesis of these imbalances, which for these reasons are extremely complex and often frustrating to treat. The use of diuretics in dogs with edema should be limited to those cases in which there is severe respiratory distress or abdominal pain. Patients with nephrotic syndrome should not undergo fluid therapy unless strictly necessary (acute vomiting or diarrhea, worsening of atrial, and preoperative procedures).

Current recommendations include the administration of immunosuppressive / anti-inflammatory therapy to dogs with severe, persistent and progressive glomerular disease, when renal biopsy demonstrates an active immune-mediated pathogenesis and after excluding underlying or concomitant infectious diseases (which ideally they should be treated before starting immunosuppressive treatment). Immunosuppressive therapy, in the event of a complete or partial

response and if well tolerated, should be continued for at least 12-16 weeks. After that it may be considered to progressively reduce the dosage of drugs at the lowest effective dose. Another particular case is represented by those patients who present a serological positivity for infectious agents recognized as a cause of glomerular pathology (eg: *Borrelia burgdorferi*, *Leishmania* spp.). Serological positivity for a given etiologic agent does not confirm a cause-effect relationship with glomerulopathy. However, the safest treatment approach in positive subjects, who have been exposed to the infectious agent and who have a glomerular disease (UPC> 0.5) is to set up a "standard therapy" along with treatment against the suspected infectious disease. The use of potentially nephrotoxic drugs should be avoided.

AIPVET

PROGNOSTIC SIGNIFICANCE AND POSSIBLE THERAPEUTIC IMPLICATIONS OF TUMOR-INFILTRATING LYMPHOCYTES IN DE NOVO CANINE DIFFUSE LARGE B-CELL LYMPHOMA

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In human medicine, there is a growing body of evidence that tumor cells are able to escape T-cell mediated anti-tumor immunity [1]. Therefore, immunotherapy represents the new frontier of cancer treatment [2] and clinical trials with customized cancer vaccines are ongoing [3]. Dogs with diffuse large B-cell lymphoma (DLBCL) benefit from the inclusion of immunotherapy in the treatment regimen [4]. However, the immunity status of dogs affected by DLBCL has been scarcely characterized. The aim of this study was to describe the composition of the intra-tumoral non-neoplastic lymphoid population in lymph node aspirates of dogs with DLBCL, and to assess the possible prognostic role of different immune patterns. Twenty-three cases with histopathological diagnosis of DLBCL were retrospectively extracted from the flow cytometric (FC) database of the Department of Veterinary Medicine (University of Milan). All cases were obtained from a single oncological referral center (Centro Oncologico Veterinario), underwent a standardized complete staging workup and received chemo-immunotherapy. The percentage of CD21+, CD5+, CD4+ and CD8+ cells out of small non-neoplastic lymphocytes was extracted from FC data. Hierarchical cluster analysis separated two groups: group 1 (12 dogs) had a lower percentage of small cells and most of them were CD21+; group 2 (11 dogs) had significantly higher percentage of small cells and most of them were CD5+, either CD4+ or CD8+. CD5/CD21 ratio accurately discriminated between the two groups, with a cutoff value of 1.0 having 100% sensitivity and specificity. Breed, sex, age, anemia, thrombocytopenia, LDH levels, disease stage and achievement of complete remission (CR) were equally represented among groups, whereas all symptomatic dogs (n=5) were in group 1. The achievement of CR was the only variable significantly influencing lymphoma-specific survival (LSS). Still, dogs in group 1 had a shorter LSS compared to dogs in group 2 (median 148 days and 623 days, respectively) (p=0.187). Based on our results, about a half of dogs with DLBCL showed a poor number of T-cell infiltrating the tumor. This was associated with uneffective response to immunotherapy, as median LSS was similar to that obtained in dogs treated with chemotherapy and placebo in a previous clinical trial [4]. Although preliminar, our data suggest that a higher component of intraneoplastic T-cells predict a good response to immunotherapy. Further studies are needed to assess the activation status of T-cells in these dogs and the strategies used by the neoplastic cells to escape immune surveillance. A better characterization of these population in DLBCL before treatment will help to stratify dogs and drive therapy, eventually.

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Hermetia illucens MEAL INCLUSION IN DIETS FOR PIGLETS: MODULATION OF INTESTINAL MICROBIOTA, MORPHOLOGY AND HISTOLOGICAL FEATURES

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Insects are considered as a novel protein source for animal feed, because of their nutritive properties and rearing characteristics (1). In livestock species, gut health can be considered a synonymous of animal welfare and is of vital importance to animal performance (2). Insect meal has been reported to improve growth performance in pigs, but limited information about post mortem findings are currently available. The present study aims to investigate the gut health modifications and histopathological findings in piglets fed with *Hermetia illucens* (HI) meal. A total of 48 piglets were randomly allotted to 3 dietary treatments (control, 5% and 10% HI meal inclusion with 4 replicates/diet each). A total of 12 animals per treatment (3 piglets/replicate) were slaughtered at 61 days of age and submitted to anatomopathological investigations. Cecal content was collected, subjected to DNA extraction and used by 16S rRNA amplicon based sequencing. Samples of gut (duodenum, jejunum and ileum), mesenteric lymph nodes, liver, spleen, lung, stomach and kidney were collected, fixed in Carnoy's and 10% buffered formalin solutions and paraffin embedded in order to obtain 5µm sections stained with Haematoxylin & Eosin. Gut morphology was evaluated through morphometric measurements of villus height, crypt depth and villus height to crypt depth ratio on Carnoy-fixed gut segments. Histopathological alterations were scored on formalin-fixed gut segments and organs using a semiquantitative scoring system as follows: absent (score 0), mild (score 1), moderate (score 2) and severe (score 3). Data were analyzed by IBM SPSS Statistics V20.0.0 and R softwares (P value and false discovery rate [FDR]<0.05). The study was performed according to animal welfare regulations (93/119/EC).
β-diversity calculation showed a clear separation of the cecal microbiota composition depending on diet. In particular, *Prevotella*, *Roseburia* (butyrate-producing genera), *Blautia*, *Ruminococcus*, unclassified members of *Ruminococcaceae* family (short chain fatty acids-producing bacteria), *Collinsella* and *Methanosphaera* (inhabitants of swine intestine) were characteristic of HI piglets (pairwise Kruskal-Wallis test, FDR<0.05). Gut morphology was not affected by dietary HI meal inclusion (General Linear Model, P>0.05), with duodenum and jejunum showing the highest morphometric indices in all the dietary treatments (General Linear Model, P<0.05). Gut and stomach showed mucosal/submucosal lymphoplasmacytic inflammation with or without Gut-Associated Lymphoid Tissue (GALT) activation, also accompanied by reactive follicular hyperplasia and/or depletion in mesenteric lymph nodes. Lymphoplasmacytic inflammation and vacuolar degeneration were also identified in liver and kidney, while lung and spleen showed no significant alterations. Dietary HI meal inclusion did not influence either development or severity of the observed histopathological alterations (Kruskal-Wallis test, P>0.05). In conclusion, dietary HI meal inclusion positively modulates gut microbiota of piglets by preserving the physiological bacterial communities and increasing the potential beneficial bacteria, with no negative effects on gut morphology and health status.

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FIRST DETECTION OF *Helicobacter canis* AND RELATED GASTRIC PATHOLOGY IN CHEETAHS (*Acinonyx jubatus*)

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Gastritis or, in general, gastro-intestinal diseases, causes significant morbidity and mortality in cheetahs (*Acinonyx jubatus*), especially in captive animals. The condition is characterized by vomit, diarrhea, anorexia, weight loss, until the death of the animal (1). In free-range cheetahs, clinical signs are weaker or even absent. Although currently a multifactorial condition is related to the pathogenesis of cheetah gastritis, four main factors interact in gastritis determinism: the lack of cheetahs genetic polymorphism; the captivity; the diet; and the presence of Gastric *Helicobacter*-like organisms (GHLOs) infection, in particular *Helicobacter acinonychis* and *Helicobacter heilmannii* (2). It is undoubted that the *Helicobacter* infection is always present in all samples of cheetahs gastric mucosa with gastritis of varying degrees and severity. Fecal samples from 18 cheetahs, with different severities of gastritis, were selected for this study. Nine wild cheetahs were located in Cheeath Conservation Fund (CCF), in Namibia, they had not evident clinical signs, with rare episodes of vomit, diarrhea and weight loss. Nine captive cheetahs, housed in different Italian Zoo Parks, had various degrees of gastritis clinically characterized by a going light syndrome, until the death of the animal. To detect *Helicobacter* species we used a highly sensitive and specific qualitative PCR assay. In addition, a subset of PCR products (= 9) was sequenced to confirm their identity: 60% of cases has been identified as *Helicobacter heilmannii* whereas 40% of cases has been identified as *Helicobacter canis*. From *Helicobacter canis* infected cases, two cheetahs showing severe clinical signs and subjected to the endoscopy, evidenced a multifocal and atrophic severe gastritis, with large numbers of inflammatory cells in both the superficial and deep regions of the lamina propria, as well as abundant intra-epithelial lymphocytes (IELs). Inflammatory cells consisted predominately of lymphocytes and plasma cells with variable numbers of large globule leukocytes. Disruption of normal glandular structure, loss of parietal cells, necrosis, and intraglandular neutrophils were also present, with a constant evidence of a heavy GHLOs colonization of the glands or free in the superficial mucus covering the mucosa. In both cases, neutrophils were a minor component of the inflammatory cell infiltrate. Atrophic gastritis characterized by large lymphoid aggregates at the base of the lamina propria, mucosal atrophy, and variable lamina proprial fibrosis were also seen in bioptic samples especially belonging to the antral region of the stomach. In our knowledge this could be the first report of *H. canis* detection from cheetahs with severe gastritis; previously this specie was isolated from feces of diarrheic or healthy dogs, cats, humans and sheep (3).

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Encephalitozoon cuniculi IN DOMESTIC RABBIT: BRAIN PATHOLOGY AND BIOMOLECULAR DATA IN CLINICALLY AFFECTED ANIMALS

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Encephalitozoonosis is a chronic parasitic disease that affects many mammal species, primarily including rabbit [1]. It represents a potential zoonosis, especially for immunocompromised individuals [2]. *E. cuniculi* is an obligatory intracellular parasite, belonging to the *Microsporidia* Phylum, which mainly localizes in brain and kidneys. The symptoms are neurological, ocular and/or renal [1]. Aim of this study was to evaluate the encephalic lesions due to *E. cuniculi* in rabbits affected by torticollis through confirmation with a biomolecular test [3]. Histological examination evaluated the lesions distribution, correlating both their localization and severity. Furthermore, the differential diagnoses observed were taken into consideration. Fourty one meat rabbits with torticollis, aged from 1 month to 2 years, were selected from two breeding farms in the province of Cuneo, Piedmont. After euthanasia, each subject was subjected to a necropsy, paying attention to any presence of medium-internal otitis, which is one of the main differential diagnoses [4]. The brain was collected and split in two portions: one part was 10% formalin fixed, paraffin embedded, sectioned and stained with haematoxylin and eosin to evaluate the morphological lesions, while the other one was frozen to perform biomolecular investigation by PCR. On rabbits with histopathological lesions attributable to *E. cuniculi*, whose presence was confirmed by PCR, both the prevalence and distribution of the lesions were evaluated, for each anatomical area (cerebral cortex, thalamus, hippocampus, pons and cerebellum). Statistical analysis was also performed to correlate the lesions severity with their localization. Data were analyzed by Shapiro-Wilk normality test, Cochran Q test, Kruskal-Wallis and Dunn's Multiple Comparison test ($P<0.05$) by means of GraphPad Prism® software. The study confirmed that the observed torticollis can have different etiologies. Typical histological lesions of *E. cuniculi* characterized by non-suppurative perivascular infiltrations, granulomas and meningitis were observed in 17/41 individuals. 14 cases were also confirmed by PCR that showed a lower sensitivity compared to histopathology due to the focal nature of *E. cuniculi* brain lesions. Histologically lesions with lower severity were more present in the rostral areas, such as cerebral cortex, thalamus and hippocampus. Individuals, without lesions attributable to parasitic disease, showed: mild non-suppurative encephalitis (2 cases); suppurative encephalitis (3 cases), secondary to suppurative otitis or trigeminal nerve infection. Degenerative lesions (white matter vacuolations) potentially related to metabolic, toxic or nutritional conditions, were found in 11 cases; whereas, no encephalic lesions were observed in the other 8 rabbits. Further studies, on a larger number of neurologically affected rabbits, is ongoing to better classify the differential diagnosis of torticollis in this species.

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PRELIMINARY HISTOPATHOLOGICAL AND IMMUNOPHENOTIPIC CHARACTERIZATION OF TISSUES FROM SARDINIAN CATTLE INFECTED BY *Echinococcus granulosus* S.S.

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The mechanisms of immune evasion, host-parasite interplay and immune pathogenesis of *Echinococcus granulosus* (EG) in cattle are poorly characterized, and the scientific literature lacks information on the local inflammatory response. Cystic echinococcosis (CE) caused by EG is the most widespread zoonotic disease in both developed and developing countries. In Italy, CE is considered endemic in Sardinia where the prevalence rates are of 75% in sheep and 41.5% in cattle [1]. The aim of this study was to identify the immune reaction surrounding cysts in livers and lungs from naturally infected bovines slaughtered in Sardinia between 2015-2017. In this study, a total of 70 hydatids, 55 from lungs and 15 from livers, were collected from 21 cattle. Each cyst was measured during macroscopic examination, processed by routine histology and stained with both haematoxylin/eosin and Masson's trichrome. Fertility was assessed by microscopic examination of protoscoleces' presence and vitality in cystic liquid. Germinal layer (GL) was used for molecular characterization by polymerase chain reaction (PCR), carried out by amplifying fragments within 2 mitochondrial genes, NADH dehydrogenase 1 (ND1) and cytochrome C oxidase subunit 1 (cox1). Cysts were classified according to the degree of inflammatory infiltrate into four categories: absent, mild, moderate and severe. The evaluation of the immune response was carried out by indirect immunohistochemistry (IHC) using the following antibodies: CD3, Cd79α, MAC387 and FoxP3, to identify T and B lymphocytes, macrophages and Treg cells, respectively. Stained tissue sections were analyzed at 200X magnification. CD3 and CD79 positive cells were scored in 5 random fields of the adventitial layer of the cyst. Two fertile pulmonary cysts did not show any inflammation, and the remaining 68 cysts were classified as infertile: of these, 6 cysts (2 lungs, 4 liver) showed mild inflammation, 44 (40 lungs, 4 livers) moderate and 18 (11 lungs, 7 livers) severe inflammatory reaction. PCR results demonstrated that all isolates belonged to *E. granulosus* sensu stricto (former G1 or sheep strain). IHC showed a majority of T lymphocytes vs B lymphocytes in the 68 samples analyzed. Presence of MAC387 and FoxP3 positive cells were negligible. Furthermore, in the infertile cysts there was a cellular layer, adjacent to the capsule wall, probably derived from macrophages. Our results are in agreement with observations in sheep [2] with a lower prevalence of fertile cysts in cattle. To better understand the pathogenesis of the disease, our future goal will be to carry out proteomic analysis to investigate the molecular cross-talk between host and parasite and to identify novel markers with potential applications in clinical diagnostics.

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ENDOCARDIOSIS OF VALVULAR COMPLEX IN AGING STURGEONS (*Acipenser* SPP.): FIRST REPORT

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Valvular endocardiosis is a degenerative process characterized by matrix proliferation and degeneration affecting cardiac valves. It is a common age-related lesion in animal and human species [1,2,3]. A limited documentation of fish valvular lesions is available [4] and to date no data have been reported on *Acipenser* species. The adult sturgeon heart is formed by sinus venosus, atrium, ventricle and outflow tract (conus arteriosus and bulbus arteriosus). Valve systems appear at the sinoatrial and atrioventricular junctions and in the conus arteriosus [5,6]. The present study represents the first description of morphological features of valvular endocardiosis in *Acipenser* spp. A total of 54 specimens of *Acipenser* spp. were collected in an italian fish farm and divided into three groups according to age and body weight: group 1 (G-1), 2-5 years, 0.5-2 kg, male, *A. baerii* and *A. transmontanus* (13 animals), ; group 2 (G-2), 6-10 years, 6-9 kg, *A. baerii* (5 male), *A. transmontanus* (6 female); group 3 (G-3), 12-16 years, 27-60 kg, female, *A. gueldenstatedtii* and *A. transmontanus*, 30 animals. Hearts were fixed in 10% buffered formalin solution and submitted to macroscopical evaluation. Samples of valve systems were processed for histological evaluation, embedded in paraffin and stained with Haematoxylin & Eosin, Weigert Van Gieson, Toluidine Blue and Alcian Blue stains. Valves lesions were scored on a 0 to 3 scale on the basis of the severity. Data were analyzed by Shapiro-Wilk normality test and Mann-Whitney U test ($P<0.05$) by means of GraphPad Prism® software. Valvular endocardiosis affected all the animals of the group 3. Particularly severe lesions characterized by verrucous proliferation with distortion of the valves and increase of myxomatoid Alcian positive matrix affected the valves at the bulboventricular junction. Fish of the group 1 and 2 generally showed no endocardiosis or low grade lesions; in particular atrioventricular valves were always less affected. All valves of group 3 showed a statistically significant increase of severity. The presence of most severe lesions in aged animals permits to consider endocardiosis an age-related lesion as observed in other species. A systematic study on a larger number of young fish is ongoing to confirm these preliminary results.

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MAST CELLS IN CANINE NORMAL, HYPERPLASTIC AND NEOPLASTIC PROSTATE TISSUES

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Normal microenvironment plays an important role in maintaining tissue homeostasis and counteracting tumorigenesis. Recent works focused on the role of inflammatory cells in modifying the microenvironment by producing different signals, that can promote or initiate tumor growth [1]. Mast cells are involved in angiogenesis, tissue remodeling and immunomodulation in human cancer, by synthesizing and releasing potent angiogenic cytokines, such as VEGF, FGF-2, NGF, Tryptase and Chymase, although their exact role is still controversial. In fact, mast cells can exert pro- or anti-tumor effects depending on tumor type and microenvironment [2]. Most of the studies showed the presence of mast cells mainly at the peripheral part of the tumors in humans. It has also been shown that reactive stroma initiates during early human prostate cancer development and is associated with prostate cancer progression. However, no information is available for canine prostate tissues. The aim of this study was to evaluate mast cell presence, distribution, as well as Tryptase and c-Kit expression in 6 normal, 15 hyperplastic and 8 carcinomatous canine prostate tissues. All samples were stained with Hematoxylin-Eosin and Toluidine Blue for mast cell evaluation. Immunohistochemistry for Tryptase, c-Kit (CD 117) and Von Willebrand Factor was also performed. Quantification of mast cell density (MCD) was made by the hot-spot method, by selecting three intraglandular/intratumoral and periglandular/peritumoral fields in areas with highest MCD. Individual mast cells were counted at 200X magnification, with each microscope field corresponding to an area of 0.785 mm^2. Statistical analysis was performed using GraphPad. MCD was significantly increased in periglandular/peritumoral areas (Normal 6.11 +/- 1.55; Hyperplasia 4.84 +/- 0.8; Carcinoma 9.37 +/- 1.89) when compared with intraglandular/intratumoral areas (Normal 2.22 +/- 0.98; Hyperplasia 2.82 +/- 0.6; Carcinoma 1.91 +/- 0.8) in all groups (P=0.03). However, increased number of mast cells was observed in intraglandular/intratumoral zones in association with inflammatory infiltration in hyperplastic samples. Mast cells were mainly detected in small cell clusters around blood vessels, particularly in the peritumoral stroma. A positive correlation between Tryptase and c-Kit expression (ρ=0.64 P=0.01) was observed at the periglandular/peritumoral zone in hyperplastic samples, whereas a strong correlation for c-Kit expression between the intraglandular/intratumoral zone and the periglandular/peritumoral area was observed in neoplastic samples. Our data confirm the importance of c-Kit receptor in the regulation of mast cell survival. In addition, predominant location of mast cells in the periglandular/peritumoral zone in both normal/hyperplastic and neoplastic canine prostate was similar to humans. This peripheral location was particularly evident in prostate carcinomas, strongly suggesting that neoplastic cells can produce substances attracting mast cells to the tumor microenvironment, where they can exert a proangiogenic activity [1]. On the basis of these results, mast cells can be suggested to play an important role in neoangiogenesis and tumor growth even in canine prostate cancer.

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INTEGRATED ANALYSIS OF GENE EXPRESSION PROFILING AND COPY NUMBER VARIATIONS IN CANINE B-CELL INDOLENT LYMPHOMAS

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Canine indolent B-cell lymphomas (CIBCL) are a heterogeneous group of malignancies arising from neoplastic transformation of mature B-lymphocytes. The definition of “indolent” is related to the low mitotic rate and slow clinical progression, therefore CIBCL are often clinically presented in advanced stages. However, late-stage CIBCL tend to clinically behave as aggressive lymphomas and outcome is generally poor [1]. Two WHO histotypes of CIBCL are more frequent: marginal zone lymphoma (MZL) and follicular lymphoma (FL). Beyond morphological and clinical classifications of tumors, the development of novel technologies such as NGS has led to an enhanced understanding of molecular heterogeneity within cancer development and progression. Little is known regarding molecular basis of cancer in dogs, and only few studies have been conducted on CIBCL [2,3]. In our work, we have combined gene expression profiling using RNA-seq and copy number variation (CNV) analysis by aCGH to provide insights on genetic signatures of CIBCL and possibly define new treatment options. RNA-seq data of 13 lymph nodes (CTRL) obtained by healthy dogs and 12 CIBCL, morphologically classified as FL (n=7) and MZL (n=5), were analyzed. Briefly, after quality reads check and mapping, differential expression (DE) analysis and functional studies were performed with EDAseq-EdgeR and GSEA, respectively. For aCGH, the 12 CIBCL were matched with the corresponding normal tissue and analysed as previously described [4]. DE analysis identified 509 upregulated genes and 2,056 downregulated genes in CIBCL compared to CTRL. The most upregulated transcripts in tumors were SDC1, FOS and BLNK and the most enriched signatures were related to MYC-interacting genes and E2F transcription factors targets. By STRING database, B-cell receptor signalling pathway resulted significantly upregulated. When investigating the two histotypes independently, gene ontology terms involved in ribonucleoprotein complex biogenesis and organization were enriched in MZLs, whereas FL showed functional enrichment of genes implicated in regulation and interaction of T cells and macrophages, similar to human FL. CNV analysis revealed a high heterogeneity among samples and a low number of aberrations when compared to canine DLBCL. The most frequent gain (25%) was along the length of chr13 where MYC is located. Focal losses with high penetrance (>60% of cases) and corresponding to clonal rearrangement of B-cell receptor loci on chr8, chr17 and chr26 were also identified. In FLs, CD8A and CD8B showed a significant correlation between losses and down-expression confirming a possible modulation of the immune response in this histotype. In conclusion, this study provided the first integrated analysis exploring the molecular profiles of CIBCL and revealed distinctive molecular patterns between FL and MZL. Functional pathways in FL seemed to reflect human FL in its interactions between immunologic and neoplastic cells. Given the critical role of MYC highlighted in our analysis, the use of agents able to modulate MYC functions, such as BET degraders, might represent a novel therapeutic opportunity in CIBCL treatment. Further studies with larger sample sizes are required to define detailed molecular signatures within CIBCL.

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OCULAR SURFACE CYTOLOGY: COMPARISON BETWEEN IMPRESSION CYTOLOGY AND CYTOBRUSH IN DOGS WITH OCULAR DISEASES

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Diseases of the ocular surface are very common in dogs. The etiologies include bacterial, viral, fungal, allergic, degenerative, and neoplastic causes [1]. Exfoliative cytology of the ocular surface's epithelium, obtained by cytobrush, is a routinely applied complementary diagnostic tool for the diagnosis of these diseases [2]. Impression cytology (IC) is a newly, alternative, non invasive method to sample the superficial layers of ocular surface's epithelium [3]. The aims of this study are 1) to evaluate sensitivity (Se), specificity (Sp), and diagnostic accuracy of IC and cytobrush compared to the clinical diagnoses; 2) to assess the intraobserver agreement between IC and cytobrush; and 3) to assess the agreement between two observers with different experience in cytopathology. Forty-six IC samples and forty-six cytobrush samples were evaluated by light microscopy. Twenty-six samples from pathological eyes (including 4 neoplastic cases, 13 inflammatory cases, 8 degenerative cases, and 1 congenital abnormality) and twenty samples from healthy eyes were collected. All the cytological samples were stained with May-Grünwald-Giemsa stain and evaluated by two observers with different cytological expertise: one board-certified clinical-pathologist and one post-doc researcher with 5 years of cytological experience. Se, Sp, and diagnostic accuracy were higher using IC compared to the cytobrush for both the observers (IC: Se 80%, Sp 71%, diagnostic accuracy 77% for the less experienced observer; Se 72%, Sp 100%, diagnostic accuracy 83% for the experienced observer. Cytobrush: Se 75%, Sp 53%, diagnostic accuracy 65% for the less experienced observer; Se 46%, Sp 79%, diagnostic accuracy 60% for the experienced observer). The intraobserver agreement was substantial (K=0.64) for the less experienced observer and moderate (K=0.46) for the experienced observer. The interobserver agreement was moderate (K=0.58) for IC and fair (K=0.34) for cytobrush. IC has proven to be better than cytobrush in term of Se, Sp, and diagnostic accuracy. However, IC samples cannot be evaluated at high magnification, so IC is an excellent screening tool to discriminate between inflammatory and neoplastic processes, while cytobrush is more appropriate to evaluate the cytoplasmic and nuclear malignancy features in case of neoplasia. Based on the higher interobserver agreement using IC compared to the cytobrush, and the substantial intraobserver agreement obtained by the less experienced observer, IC samples can be evaluated also by unskilled observer.

This study was approved by the Animal Welfare committee of the University of Padua (authorization number 70/2015)

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A SURVEY ON FELINE MORBILLIVIRUS IN DOMESTIC CATS IN PIEDMONT: VIROLOGICAL, MOLECULAR AND ANATOMO-PATHOLOGICAL INVESTIGATIONS

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Feline Morbillivirus (FeMV) was discovered in Hong Kong in 2012 [1] and later reported in other countries, as the first member of the *Paramyxoviridae* pathogenic in domestic cats; in Italy, it was identified in the urine sample of a stray cat suffering from CKD [2]. Currently, the scientific community is providing detailed investigations involving cats with and without urinary tract diseases from different areas to determine the prevalence of FeMV infection, its pathogenetic role and the genetic diversity of viruses. Aim of this study was to investigate the presence of FeMV in urine and kidney samples from cats of Piedmont region. Urine samples individually collected from cats referred to two veterinary clinicians (n=118) and one pool of urine belonging to a colony were investigated. Animals were mainly European breed (96/118, 81%), aged from 1 to 20 years old, of both sexes (36 females, 82 males). On the basis of clinical evaluation (blood and urine analyses), cats were classified into 4 groups: affected by kidney disease (acute: 5/118, 4%; chronic - CKD: 29/118, 24%), pathology of the lower urinary tract (24/118, 20%) and other not related to urinary tract pathologies (60/118, 50%). Kidneys (n=40) from different cats submitted to necropsy were also investigated. Animals were mainly European breed (32/40, 80%), aged from 4 months to 17 years old, of both sexes (23 females, 17 males). Molecular investigations were performed by one-step real time RT-PCR according to the protocol kindly provided by Lorusso et al. (IZSAM) targeting a conserved 76 bp-region of FeMV. Positive samples were submitted to a nested PCR targeting 400 bp of the L-gene [3], and amplicons were submitted to sequencing and phylogenetic analysis. Formalin fixed and paraffin embedded sections of kidney were examined by means of standard methods. Six urine samples (European breed, 4 males - 2 females, one 14 years old and the remaining adult 4-6 years old) and the pool tested positive for FeMV; only two positive animals were affected by CKD. Kidney lesions were classified as: interstitial chronic nephritis (20/40, 50%), other lesions (tumors, granulomas, abscesses) (14/40, 35%), no significative lesions (6/40,15%). Four kidney resulted positive by real time RT-PCR assay (3 European/1 Norwegian, 2 males - 2 females, 7 to 11 years old). Histologically multifocal to diffuse chronic interstitial infiltrates were present in three kidneys and a metastatic lymphoma in the fourth. The nested PCR used for virus typing was successful only for two samples, likely because of low viral load. The two sequences were from an infected cat and a urine pool of its cattery. Phylogenetic analysis showed that the samples clustered within a clade of German and Turkish strains, not related to the first Italian strain, Piuma/2015. These two isolates were 97.9% similar each other, suggesting that a second strain might be circulating in the same cattery. Despite the large sample set of cats examined, the presence of the FeMV seems to be low and not closely related to CKD or inflammatory kidney lesions.

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PERCUTANEOUS BIOPSY OF THE EQUINE SUSPENSORY LIGAMENT: AN EX-VIVO STUDY AND VALIDATION OF THE HISTOLOGIC METHOD

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Tendon and ligament injuries are a common cause of lameness and athletic wastage in sport horses [1]. Suspensory ligament (SL) injuries are a significant concern for horse owners and veterinarians, due to the high probability of recurrence [2]. Tendon percutaneous biopsy techniques have been investigated in horses to evaluate an alternative method for monitoring the healing process through the histological analysis [1]; however, biopsy techniques of the equine SL have not been previously described in literature. The first aim of this study was to elaborate an ex-vivo percutaneous biopsy technique of the SL to collect samples suitable for a histomorphological exam. Artifacts may occur during the histological processing of collagenous structures, limiting the use of this technique [3]. Therefore, different histological methods were compared in order to define the optimal processing for a routine microscopic evaluation of such tissues. Four healthy equine forelimbs obtained at the slaughterhouse were placed on a metallic support reproducing a physiological weight-bearing position of the distal limb. Biopsies of the body and the lateral branch of the SL were performed using a manual biopsy system (HandCut, MDL) with a 14-gauge needle, adopting a transverse (90°) and a longitudinal (30° to 45°) approach each site. Collected specimens were fixed in 10% formalin (16) and in Bouin's solution (16), paraffin embedded and H&E stained for a morphological evaluation; twenty selected specimens were stained also with Picrosirius Red to observe collagen fibers arrangement under polarized light microscopy. The Bouin's fixed samples were further divided in two portions for resin (Technovit 7100®) and paraffin embedding. Histological quality was evaluated using a semiquantitative score scale, ranging from 0 to 2, based on contrast, shrinkage and splitting of the tissue. The Bouin's fixation, followed by paraffin embedding, significantly improved morphology and histologic quality of the specimens in comparison to the standard formalin fixation. In such samples contrast was excellent, tissue shrinkage was absent (56%) or moderate (37,5%) and splitting was absent (37.5%) or moderate (50%) resulting in significantly better samples compared to the other histological methods (Fisher exact Test, $p<0.001$ and $p<0.05$ respectively). The morphological quality of the resin embedded specimens was equivalent to the paraffin ones, although resin embedding was realized only in few samples (31%), due to its low feasibility for routine histological analysis. The majority (81%) of biopsy samples were suitable to evaluate collagen fibers parallelism under polarized light microscopy. This methodological work will be useful for future researches in the field of sport medicine, reproducing this biopsy technique also in vivo to evaluate the healing process and to guide full rehabilitation of the affected animal.

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A FORM FOR AN EXHAUSTIVE INVESTIGATION

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In the veterinary practice, a good knowledge of the scene is crucial for a correct interpretation of the necropsy findings especially to determine cause, manner and method of abuse or death. A good practice is the presence of a veterinary pathologist at the scene, in fact reviewing crime scene photographs and reports is beneficial but the information that can be collected from a direct crime scene examination is irreplaceable. Nowadays the best veterinary practice requires an accurate collection of the evidence associated with the scene and the animal victim. This material is then passed to other forensic scientists for evaluation, interpretation and, when requested, presentation in the court. In this way veterinary pathologist plays a fundamental role in the correct documentation and collection of evidence for analyses performed later by other forensic experts (eg. BPA, traces analyses, ballistics, DNA, etc.). Aim of the present work is to propose a protocol facilitating the field and postmortem activities of the veterinary pathologist when an injured or deceased animal is found, in order to guarantee the quality of the forensic process from the crime scene to the reconstruction of the case. Based on the protocol developed by human forensics scientists we conceived the present form. The form is divided in five sections (Pathology, Entomology, Osteology, Genetics and Toxicology). Each section is marked with an alphanumeric code at the top of each page. The letter indicates the discipline involved and the number the numerical progression of the pages.

The method of compilation is partly guided for optimizing the time needed to fill in the form and takes into account all the details of the case. Tables, graphs and images are inserted for the description of the sites, environmental conditions, status of the carcass (position, degree of preservation, injury, etc.) and presence of insects to aid the veterinary pathologist in collecting information and samples. A copy of the form can be provided to each specialist and used as a starting point by other experts later involved in the case analysis.

The result of the work is a form that can be easily used in the veterinary practice. Although the ideal situation would be that all experts are present at the scene, this is not feasible in real cases and the veterinary is frequently the first and/or the only expert at the scene. Therefore, this form provides an initial tool for a multidisciplinary activity in close synergy with other experts offering an initial complete documentation of the scene.

In conclusion, in order to evaluate how the form can be an effective, user-friendly tool, it is proposed to the attendants of the 72nd SISVET meeting and the authors are happy receiving comments, suggestions and criticisms.

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MORPHOLOGICAL STUDY OF NEOPLASTIC CELL INTRAVASATION IN MOUSE

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Tumor distant progression is related to malignant cell spreading enhanced by mediators of vascular permeability such as vascular endothelial growth factors. (VEGFs). The morphological transendothelial migratory “mode”, also related to Epithelial-Mesenchimal-Transition (EMT), of the invasive neoplastic cells into the tumor-associated lymphatic and blood vessels was investigated. The aim is to investigate the EMT morphological changes of neoplastic cell during intravasation (IV) “in vivo” model. Eighteen nude female mice and ten SCID/Nod female mice [1] were inoculated, in the mammary gland, with a neoplastic cell suspension expressing VEGF-D: $1x10^7$ cells of the VEGF-D EBNA 293 cell line (Ethics Committee UniPr, prot. 54/11 del 14.06.2011). At 24 and 35 days after inoculation mice were euthanized. Tissue samples of neoplasia were collected for histology, immunohistochemistry (IHC) and ultrastructure (TEM) investigations. Specimens for histology and IHC were formalin-fixed paraffin-embedded and 5µm serial sections were stained for histology (H&E) or immunostained. IHC was performed according to data sheet for Lyve-1, CD31 and F-actin. Specimens for TEM, 3-4 mm wide, collected at the periphery and centre of tumor, were fixed in a 1% osmic acid buffer solution (pH 7.3), embedded in Durcupan and ultrathin serial sections were stained using a negative uranyl acetate protocol. Gross pathology showed a sub-cutaneous tumor, 0.8-1.8 cm Ø, in the right lateral sub-umbilical region. Lyve-1^+ lymphatic vessels were absent in the core of the tumor while were detected in periphery as well as in peritumoral connective tissue. This vascular arrangement was similar to what described in other experimentally induced tumors [2,3]. $CD31^+$ and Lyve-1^- blood vessels were morphologically characterized by a thin endothelial wall without continuous basal membrane and wide fenestrated areas alternated with pore lacking areas. $CD31^+$ and Lyve-1^- blood vessels showed the neoplastic cell during trans-endothelial migration (TEmi). TEmi is connected after the detachment of the neoplastic cells from the tumor. The neoplastic cells modify their shape, from rounded to elongate. Modified neoplastic cells are arranged in parallel lines to the abluminal wall: a cytoplasmic protrusion follows the directional TEmi feature. The IV occurs via an intra-endothelial space (IEs) (1.8-2.7 µm Ø) between adjacent endothelial cells and does not compromise the inter-endothelial junctions. The ultrastructural pictures from ultrathin serial sections describe the dynamics of cytoplasmic protrusion. Neoplastic cell TEmi was characterized by different moments and IEs appeared determinant in the IV processes during metastasis. The EMT remodelling of the cytoskeletal actin supports cell motility. F-actin microfilaments and microtubule polymerization and depolymerisation generate movement in neoplastic cells. An innovative migratory mode of the EMT neoplastic cells is proposed and points out the active role of the vascular endothelium in tumor distant progression.

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MORPHOMETRIC ANALYSIS OF TISSUE CHANGES INDUCED BY RADIOFREQUENCY THERMAL ABLATION ON ISOLATED SWINE THYROIDS

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Radiofrequency thermoablation (RFA) is a minimally invasive technique that induces tissue coagulative necrosis by means of thermal energy, locally applied by needle electrodes. RFA has many applications in human oncology, especially for the treatment of small size masses, and potential applications also in veterinary medicine. Its use for treating thyroid nodules has been described [1] using non-perfused needles. One of the major drawbacks of RFA is the reduced size of the lesions that this technique can induce, due to thermal energy losses in presence of increased tissue vascularity (which alters tissue conductivity). Inducing small lesions in the thyroid tissue potentially increases the risk of incomplete nodule ablation, which in turn could damp lesion volume reduction at follow-up [2]. New perfused needles have been developed for RFA to increase lesion size, but they have never been tested in thyroid tissue. The aim of this study was to compare the size and geometry of the lesions induced by perfused and non-perfused RFA needles in isolated swine thyroids. RFA was performed on 44 freshly isolated swine thyroids using internally cooled needles (RF Medical Co. Ltd., Seoul, Korea), either perfused or not. When non-perfused needles were used, the time of delivery of thermal energy was fixed at 20 seconds. When perfused needles were used, 3 solutions were tested, namely saline 0.9%, hypertonic saline 7% and hypertonic saline 18%. In the latters, procedural endpoint was determined based either on fixed time (20 seconds, to allow comparison with non-perfused needle-induced lesions) or on tissue impedance values (variable time, the instrument stopped automatically energy delivery when tissue impedance reached its maximum and conductivity dropped). Then, thyroids were transversally and longitudinally cut, and pictures obtained for macroscopic lesion morphometry. After formalin fixation, pictures from thyroids were obtained to evaluate tissue shrinkage and then paraffin embedded. Microscopic lesions morphometry was performed on PAS stained sections. When the effect of a single variable was assessed among multiple groups, one-way ANOVA test (or Kruskal-Wallis test) was used. When the effect/interaction of two variables was evaluated among multiple groups, the two-way ANOVA test was applied, followed by the optimal post-tests. Paired Student's T-test was used to compare lesions before and after fixation.
Both macroscopic and microscopic analysis revealed that perfused needles produce significantly larger lesions ($p<0.01$) when hypertonic saline 7% is used rather than isotonic saline with variable but not fixed energy delivery time. Histologically, perfused needles induced significantly larger lesions ($p<0.01$) compared to non-perfused needles, both with isotonic saline and with hypertonic saline 7% at fixed energy delivery time. In conclusion, needle electrodes perfused with 7% hypertonic saline increase the size of the thermal lesions induced on ex vivo thyroid tissue.

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NLRP3 INFLAMMASOME AND AUTOPHAGY CROSS-TALK IN BOVINE BRAINS

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"*Immunosenescence*" is one of the most recognized effects of aging that consist in the dysregulation of the immune system as a result of defects in both initiation and resolution of immune responses. Immunosenescence is accompanied by a low-grade and chronic pro-inflammatory environment in multiple tissues known as "*inflammaging*" and it has been linked to an increased incidence of several disorders, including neurodegenerative diseases [2]. NLRP3 (NOD-like receptor protein 3) inflammasome is a pattern-recognition receptor in the innate immune system that has been implicated in age-related chronic inflammation [3]. Several authors also suggest that autophagy contributes as negative regulator of NLRP3 inflammasome [3]. Here, we describe our findings concerning the expression of MHC II as a marker of microglia senescence and NLRP3 inflammasome in brains of aged bovine. We also evaluated the cross-talk between inflammasome, autophagy and ROS production. Samples of hippocampus were collected from 42 Podolica cattle. Animals were divided in three groups: group A (aged 15 to 24 years) (n=14), group B (aged 5 to 14 years) (n=14) and group C (aged up to 5 years) (n=14). Immunohistochemistry and double color immunofluorescence were performed on 4 µm thick sections to evaluate, respectively: 1) the expression of MHC II and NLRP3 and 2) the relationship between NLRP3, SOD1 and autophagy marker Beclin 1. Moreover, Western blot analysis was performed in order to determine the expression levels of NLRP3. Immunohistochemistry revealed a statistically significant ($p<0.0001$) increase of MHC II–labeled microglial cells and NLRP3 expression in group A and B compared to group C. Double color immunofluorescence indicated an association between NALP3 and SOD1 expression in adult and aged brains, whereas there was no co-expression of NLRP3 and Beclin 1. Our results show that MHC II and NLRP3 are up-regulated in the brain of aged cattle suggesting the presence of an age-related chronic inflammation. We also propose that the age-related decline of autophagic capacity leads to increased ROS production with subsequent overexpression ofSOD1resulting in oxidative stress-related injury, upregulation of NLRP3 inflammasome and neuroinflammation.

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SCORING PLEURISY IN SLAUGHTERED PIGS – THE OTHER SIDE OF THE COIN

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The European legislation regards the slaughterhouse as “an establishment used for slaughtering and dressing animals, the meat of which is intended for human consumption” [1]. In addition, the slaughterhouse is widely recognized as a useful check point for assessing the health status of livestock, as well as the effectiveness of strategies implemented to treat and/or prevent disease conditions [2].

The present work aims to assess an alternative method to score pleurisy in slaughtered heavy pigs, based on the inspection of the parietal pleura.

At the beginning of the study, 216 pigs were investigated, the presence/absence and the severity of pleurisy being evaluated in parallel by two different methods: a) inspection of the visceral pleura and scoring pleurisy according to the “Slaughterhouse Pleurisy Evaluation System” (SPES), which is currently regarded as the “gold standard” [2]; b) inspection of the chest wall (“Pleurisy Evaluation on Parietal Pleura”, PEPP), scoring lesions as follows: absence of pleurisy = 0 points; pleurisy of the 1st-to-3rd intercostal spaces = 1 point; pleurisy of the 4th-to-6th intercostal spaces = 2 points; pleurisy affecting the remaining caudal surface of the parietal pleura = 3 points. Statistical analysis demonstrated a very high and significant correlation between the two scoring methods (Pearson’s coefficient $r=0.91$; $p<0.01$). Likewise, the coefficient of determination was high and statistically significant ($R^2=0.833$; $p<0.0001$). Afterwards, the possibility of scoring pleurisy on digital images was assessed. To this aim, a veterinarian scored 260 pigs by the PEPP method and took pictures of all the animals under study. Two other veterinarians, unaware of the score given at the slaughterhouse, independently applied the PEPP method to the digital images. The correlation between different investigators proved to be very high, Pearson’s coefficient ranging between 0.85 and 0.94.

Overall, our data indicate that the PEPP method represents a suitable alternative to the SPES method for the scoring of pleurisy in slaughtered pigs. It appears to be a really fast and easy method, which is compatible with slaughter line operations. Similarly to other scoring systems, the PEPP method has both advantages and disadvantages. For example, the inspection of the parietal pleura in a later point along the slaughter line does not permit the scoring of pneumonia, pericarditis and parasitic hepatitis at the same time. On the other hand, scoring pleurisy on the parietal pleura seems to be less influenced by confounding factors (e.g. inspiration of blood), thus also being easily applicable to photographic images, independent of the inspector’s presence at the slaughterhouse. This would make it possible to obtain a large amount of data, in a more efficient and potentially “automated” way.

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NUTRIA (*Myocastor coypus*) HEALTH STATUS IN THE NATURAL PARK "LA MANDRIA". ANATOMOPATHOLOGICAL AND MICROBIOLOGICAL INVESTIGATIONS

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Nutria (*Myocastor coypus*) is a medium-sized rodent native of South America introduced in North America and Europe, where it has been managed to establish naturalized populations. In Italy the first specimens of Nutria were imported for commercial breeding (fur production). After World War II, faced with a crisis of this business, small entrepreneurs, to avoid the costs of abatement, intentionally released animals, causing their rapid distribution in the area and modifying the original zoogeographic profile, with a considerable impact on environmental components [1]. Aim of this work was to evaluate by means of necropsy, histopathological and microbiological investigations the sanitary status in nutrias included in an eradication programme in the Regional Park "La Mandria" (Northwestern Italy), with special interest for viral, bacterial and parasitic diseases.

Following the post-mortem examination of 44 carcasses of Nutria (25 males and 19 females), samples of organs were collected and frozen at -20°C and/or fixed in buffered formalin; laboratory investigations have been performed according to standard methods.

Histologically, the organs showing the highest number of lesions were the liver (activation of periportal lymphoid tissue: 44.4%), kidney (non-purulent lymphocytic interstitial nephritis: 87%) and lung, in which alterations were detected in all the analysed samples (parenchymal: 81.8% and perivascular: 72.7% lymphocytic inflammatory infiltrate). Bacteriological tests provided negative results in all the samples for Francisella spp., as previously reported [2]. Bacteriological examination performed on lung yielded in 25/44 cases (64.1%) the isolation of different bacteria. In detail: polymicrobism (15.4% of samples), *Enterococcus* (17.9%) and *Pseudomonas* (10.3%), whereas in the remaining 20.5% of samples bacteria of the genus *Achromobacter, Nocardia, Streptococcus, Brevibacillus, Ochrobactrum* and *Corynebacterium* were detected. Although, previous investigations reported seropositivities in nutrias for viral encephalomyocarditis (EMCV) [2, 3], no subject was tested positive in this study. No Nutria was tested positive for Hepatitis E Virus (HEV), as previously reported [4]. Two samples out of 35 (5.7%) resulted positive for *Toxoplasma gondii*, while no *Neospora caninum* infection was detected. Toxoplasmosis is a common infection in nutria [2, 3]. Besides being potential source of *T. gondii* for scavengers, they constitute relevant species to monitor the burden of oocysts in the wild environment. A continuous health monitoring of the populations of nutria is necessary in order to assess and prevent any health risk for wildlife and humans.

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POST-MORTEM INVESTIGATION TO ASSESS THE ROLE OF PLASTIC INGESTION DURING A PESTES DE PETIT RUMINANTS VIRUS (PPRV) OUTBREAK: A FIELD STUDY IN THE SAHARAWI REFUGEE CAMPS, ALGERIA, 2010

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Peste des petits ruminants (PPR), also known as 'sheep and goat plague', is an acute febrile viral disease of small ruminants and camelids caused by a highly contagious RNA virus, belonging to *Paramyxoviridae* family [1]. To date the disease is endemic in Africa except the Southern Countries, in the Arabian Peninsula, throughout most of the Near East and Middle East, and in Central and South-East Asia [1]. In May 2010, Veterinary authorities of the Saharawi Refugee Camps (Algeria) reported a mortality outbreak in the small ruminants population characterized by respiratory signs, diarrhea, fever and depression mainly young animals. Nasal swabs obtained from 9 alive animals confirmed PPR virus circulation [2]. Despite sampling during necropsies and analyses were strongly limited by field conditions, postmortem examinations carried out on 96 small ruminants (53 sheep, 43 goats) showed pathological changes consistent with this agent: 50% of the animals (n=48) showed lungs lesions with differences in type and severity of inflammatory involvement with interstitial pneumonia, epithelial cells damages and macrophage exudation (21%, n=30). Multinucleated giant cells were observed in 10 specimens (10.4%). These findings were frequently complicated by severe diffuse pleuritis (18.7%, n=18), and secondary Gram-positive bacterial infections were microscopically noticed (10.4%, n=10). Immunohistochemical (IHC) findings showed the presence of PPRV antigen in bronchiolar epithelial cells, alveolocytes, alveolar and interstitial macrophages and syncytial cells in lungs in 57% of the animals (n=55). In 59.4% of the total examined animals (n=57), foreign bodies were found in the gastrointestinal tract: these materials caused gastric impaction, obstruction and/or gastrointestinal impairment. Most of the foreign bodies were made of plastic material and a possible role of carrier for organic pollutants has been hypothesized since toxicological analysis carried out on hepatic tissue of 5 PPRV positive animals revealed the presence of Persistent Organic Pollutants (POPs). Therefore, organochlorine compounds found during these analyses could have played a role in the immune impairment as suggested by the direct relation between debris contents and IHC results: positive statistical correlation between a severe ruminal impaction due to plastic debris and the viral infection was reported in the present study (30% of the animals, n=29). Plastic materials found could act as a carrier for most of the POPs as already shown in wildlife and in marine organisms [3]: the mechanical action of the rumen on these contents could have enhanced a slow and constant release of chemical substances, subsequently absorbed by the animals [4].

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PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF CANCER STEM CELLS FROM CANINE AND FELINE MAMMARY GLAND TUMOR CELL LINES

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Many studies on human cancer support the idea that tumors are initiated by a small subpopulation of cells, namely cancer stem cells (CSCs), thought to be responsible for tumor heterogeneity, resistance to chemotherapy, relapses, and metastasis formation [1]. Thus, studying CSC biology would be extremely relevant for diagnosis, prognosis, and for the development of new effective treatments of several types of cancers [2]. The aim of this study was to isolate and characterize cancer stem cells from established canine and feline mammary cancer cell lines.

Canine (CYPp) and feline (FMCp) mammary cancer cell lines were cultured either as adherent (AD) (serum-containing medium) or as mammospheres (MS) (serum-free medium). Flow cytometry (FC) on adherent cells and mammospheres either enzymatically or mechanically harvested was performed after 7 days (p1), 28 days (p4), and 49 days (p7) for the following antibodies: CD45, CD44, CD24, CD34, and CD133. Additionally, quantitative real-time PCR (qPCR) on AD and on MS at p1, p4, and p7 for CD44, CD133, SOX2, and OCT4 was carried out. Flow cytometry on mechanically harvested cells showed almost 20% of dead cells and therefore was not included in FC analyses. At FC performed on enzymatically harvested cells, both CYPp and FMCp were negative for CD45 and CD34. CD44 in CYPp and FMCp did not show relevant differences between AD (100%-positive, mean fluorescence intensity (MFI) = 155) and MS (100%-positive, MFI = 175) throughout the passages. The expression of CD24 increased over time in CYPp MS (from 2% to 19%-positive, MFI = from 1.11 to 1.21) and FMCp MS (from 3% to 25%-positive, MFI=from 0.68 to 5.52) when compared to CYPp AD (from 2% to 6%-positive, MFI = from 0.7 to 0.4) and FMCp AD (from 5% to 4%-positive, MFI = from 0.7 to 0.4). Conversely, in CYPp and FMCp, the expression of CD133 was higher in CYPp MS (25-32%-positive, MFI = 0.42-0.59) and FMCp MS (54-80%-positive, MFI = 0.6-1.25) when compared to CYPp AD (6-18%, MFI = 0.35-0.46) and FMCp AD (44-47%, MFI = 0.65-0.80), respectively. In FMCp, the CD44+/CD133+ population was higher in MS (75%) than AD (40%). At the RNA level, CD44, CD133, and SOX2 expression was higher in MS than AD. The expression of OCT4 increased in FMCp MS p7 when compared to AD and MS p1 and p4. In summary, canine and feline mammospheres, which are known to be formed by CSCs, possessed cancer stem cell properties, such as an increased expression of CD133 at both proteins and RNA level, and an increased expression of CD44 and SOX2 at the RNA level. CD44 in mammospheres does not show a relevant increase at the protein level, presumably due to either post-transcriptional alterations or, most likely, to the enzymatic treatment used to harvest the cells, as previously reported. The application of CD44 as a CSC marker in animal cancer should be carefully evaluated.

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DIAGNOSIS OF DROWNING IN VETERINARY FORENSIC PATHOLOGY

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A diagnosis of drowning is a challenge in veterinary forensic pathology. Although characteristic macroscopic and microscopic injuries of drowning are reported in human medicine, none is conclusive. For these reasons, this diagnosis is often one of exclusion. In human forensic pathology, diatom analysis is considered very supportive for a diagnosis of drowning. However, its sensitivity and specificity is still controversial for some investigators [1]. Although diatoms were identified in the tissues of animals recovered from aquatic environments, application of the diatom test in veterinary medicine requires additional rigorous validation studies [1-2]. The aims of this study were: 1) evaluate the macroscopic and microscopic findings in animals dead in drowning conditions; 2) investigate the differences in number and location of diatoms between animals dead in drowning and non-drowning conditions. To these aims, nine dead adult animals were employed for the study, subdivided into three groups of three animals each. The group A comprised cadavers (1 lemur, 2 dogs) recovered from aquatic environments, the group B comprised animals (2 dogs, 1 cat) dead for causes other than drowning and subsequently immersed in water for 24 hours, while the group C comprised control animals (dead for causes other than drowning). For each animal, a complete macroscopic and microscopic examination was performed. Furthermore, five grams of lung, liver, kidney and brain and the drowning medium were recovered for diatom test performed with standard acid digestion method. Finally, diatoms were counted and measured. Macroscopic and histological findings of the animals of the Group A showed pulmonary congestion, edema and hemorrhages. However, similar injuries were also observed in the animals of the group B and C. Diatoms of the family *Stephanodiscaceae* and *Bacillariaceae* were detected in lung, liver and kidney of all animals of the group A. Furthermore, diatoms were detected in the lungs of two dogs of the group B. In contrast, in the control group, diatom test was negative. Finally, a significant difference was found in diatoms number between group A and B for all tissues examined ($p<0.05$). It was notable that all diatoms in organs matched with the respective drowning media. The macroscopic and microscopic findings observed in animals of the group A showed some classical signs of drowning [1]. However, these findings, although characteristic of drowning, were not specific, as they were also observed in non-drowning animals. As well documented in human medicine, the detection of diatoms in drowning cases was due to the ability of the diatoms to percolate the alveolar wall and enter the bloodstream [1-2]. In contrast, in non-drowning animals, the detection of diatoms could be due to the post-mortem passive movement of the water into the lung. However, in these cases, the absence of cardiac function prevented diatoms spread to other tissues. This study demonstrated that the diatom test was a reliable method to support the diagnosis of drowning. However, a complete multi-organ panel should be examined to obtain a more reliable interpretation and to increase the sensitivity of the technique.

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GUT MICROBIOME AND FELINE INFECTIOUS PERITONITIS

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Feline coronaviruses are found in the intestinal tract, however, they can spread sistemically and, due to some unclear mutations, may cause feline infectious peritonitis (FIP) [1]. In people, gut microbiota is involved in the development of systemic disorders and could influence the immune response. In feline medicine, there is a small number of studies reported [2]. The aim of this study is to provide preliminary data about the correlation between the composition of fecal microbiota in healthy cats compared to Coronavirus infected cats, with and without FIP. To correctly group each cat, screening clinico-pathological analyses were performed. After the application of strict inclusion criteria (cats younger than 2.5 years, living indoor and not treated with antibiotics for at least 2 months), 15 cats were selected and equally grouped (Healthy, Coronavirus positive - COR, FIP). A fecal sample was collected and frozen, to evaluate the microbiota composition using Next Generation Sequencing (Metabarcoding) and also to perform traditional faecal bacteriology. For the bioinformatic analysis, the sequences quality (using FastQC v0.11.2), alpha rarefaction and beta diversity were evaluated. Statistical analyses were performed with "R" statistical software. A total of 3,231,916 sequences were analyzed. The samples' alpha diversity curves did not reach a proper plateau (only the most abundant bacteria were identified) and, for the beta-diversity, the samples seemed not to group perfectly by category, but the Coronavirus positive group showed a hybrid microbial composition between Healthy and FIP. Unfortunately, there is no taxa significantly linked to the different conditions. However, some peculiar patterns were recognised: *Firmicutes* was the most represented Phylum, followed by *Bacteroidetes* and *Actinobacteria*. In Coronavirus positive group *Firmicutes* and *Bacteroidetes* were respectively over- and under-represented, compared to the other groups (*Bacteroidetes:Firmicutes* ratio: 0,16 COR; 1,11 FIP; 0,9 Healthy). In FIP group three subjects shared a similar microbiome, while one showed a different microbial profile and the other one had a lower number of diverse Phyla. The same pattern was observed in relative class and order abundance. Despite the limited number of animals, some differences in the fecal microbiome between the groups were observed, even if not statistically significant. This was not surprising due to the peculiar enteric tropism of feline Coronavirus. The different microbiota composition observed in the present study, with respect of the literature, might be related with different sampling or technique but also with the high individual variability. Nevertheless, there is concordance about the three main Phyla [3].

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PRELIMINARY INVESTIGATIONS IN FORENSIC PATHOLOGY OF EASTERN GRAY SQUIRREL'S (*Sciurus carolinensis*) SKIN LESIONS

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Forensic pathology is a discipline that focuses on determining the cause of death by examining a corpse. One of the most challenging tasks of forensic pathology is to determine the post-mortem interval (PMI), i.e. the time that passes from the death of the animal to the finding of the corpse. Various methods have been proposed to establish the PMI, including liver temperature, gastric emptying time, rigor mortis, etc. However, none of these methods is applicable to each scenario. To date only few studies, especially in veterinary medicine, relate to the post-mortem changes in the skin. In a previous study performed at the Department of Veterinary Sciences of the University of Turin, numerous fungal elements were observed in the skin of healthy Eastern gray squirrels (*Sciurus carolinensis*). Aims of this study were to:

• evaluate post-mortem skin changes in Eastern gray squirrels, with particular attention to fungal growth, bacterial colonization and cell conservation status;
• determine if in the previous study the fungal skin colonization started intra-vitam or post-mortem;
• clarify if (and how) a post-mortem growth of fungi is possible.

The gross and histopathological alterations of the skin of 5 Eastern gray squirrels (3 males and 2 females), obtained from a regional containment program, were evaluated. The corpses were storage at constant temperature (26°C) and humidity (80%) and skin was sampled at 5, 51 and 111 hours after death in six different body areas (underarms base of the nape, base of the tail). For each sample, hematoxylin-eosin, PAS and Grocott histological stainings were performed, and possible alterations were evaluated by two independent double blind operators, using a semi-quantitative scale from 0 to 3 for the following parameters: presence of crusts, inflammatory infiltration, presence of hyphae, fungal elements and bacteria, and cell conservation status.

The cells appeared to be preserved, apart from some slight alterations, up to 51 hours, while at 111 hours cell degeneration and absence of nuclei were detected. Histological examination showed the growth of hyphae and fungal elements at 111 hours after death as well as an increase of bacterial colonies, as already reported in literature [1]. The hyphae may belong to soil keratinophilic fungi, which are commensal organisms in healthy individuals and are not normally able to cause disease in living organism [2]. Then, according to our results, we can state that the fungi (already found in our previous study) colonized the animal's skin intra-vitam; this finding raises interesting clinical considerations, as for the first time fungal elements were found in the stratum corneum of the skin in asymptomatic animals. The detection of skin changes, including an effective fungal growth, after 111 hours from the death can be considered an important finding in forensic pathology, and may have practical implications in determining the PMI.

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Linguatula SPP. IN OVINE LYMPH NODES: A SURVEY IN SICILIAN ABATTOIRS

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Linguatula is an obligate arthropod parasite which inhabit the upper respiratory tract of canids [1]. *Linguatula serrata* is the most important species reported in european countries. Panebianco described its presence in sicilian cattle in 1957 [2]. Most herbivores, including ruminants such as sheep, cattle and camels may serve as intermediate hosts for *Linguatula* species [3]. In these intermediate hosts larvae penetrate the intestinal wall and encyst in visceral tissues such as the mesenteric lymph nodes, liver, spleen and lungs. Zoonotic cases of infection with *L. serrata* have been reported from several countries [4]. Aim of this study was to describe six cases of *Linguatula* infestation in ovine mesenteric lymph nodes observed at slaugthterhouse in Sicily during a paratuberculosis survey. A total of 474 adult sheep of both sexes, regularly slaughtered in different abattoirs of Sicilia region (south of Italy) was examined between 2014 and 2015. Almost 3 lymph nodes were collected from each animal, macroscopically examined and 10% formalin fixed for histological investigation. Parasite macroscopically detected was submitted to detailed morphological evaluation and was identified as the nymphal stage of *L. serrata*. Nymphs are observed encapsulated in mesenteric lymph nodes (MLNs) of six animals. No parasitic lesions were detected in other organs. The affected MLNs were grossly enlarged, sometimes edematous and red in colour. In one case a live larva was appreciable cutting the organ. Microscopically moderate to severe lymphoid depletion, hemorrhages, edema and hemosiderosis were detected. Larvae were elongated, slightly triangular surrounded by an evident cuticle. In some cases a granulomatous reaction composed by macrophages, lymphocytes, plasma cells and eosinophils was detected around the parasite. All animals were affected by paratuberculosis. Studies on the prevalence of *Linguatula* spp. in small ruminants in Italy are not available. The role of this parasite to predispose to other diseases is well reported and particularly concurrent occurrence of visceral linguatulosis with paratuberculosis, polymorphic bacteria and yeast infections has been reported [4,5]. Because of the veterinary and human medical importance of linguatulosis, further investigations in both domestic and wild herbivores and carnivores together with more detailed studies on the occurrence of this infection in humans are suggested.

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CHROMOGENIC IN SITU HYBRIDIZATION FOR THE DIAGNOSIS OF FELINE HERPESVIRUS-1 ASSOCIATED DERMATITIS

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Felid herpesvirus type 1 (FHV-1) is a worldwide pathogen mainly responsible of upper respiratory tract infection, ocular disease and dermatitis in felids [1]. The FHV-1-associated dermatitis is a facial and nasal dermatitis commonly seen on the dorsal and lateral muzzle, nasal planum and periorbital areas. These lesions overlaps with other feline dermatoses including hypersensitivity disorders, granuloma complex and cutaneous adverse food reaction [2]. Positive FHV-1 PCR results cannot guarantee an active role of FHV-1 in development of skin lesion because of latent infection, widely spread in cats and therefore conventional PCR possess limited clinical values [3]. The aim of this study was to correlate the presence and the amounts of FHV-1 viral genomes on feline tissues, assessed by conventional and qPCR assays, to the visualization of FHV specific nuclear signal of infected cells by chromogenic in situ hybridization (CISH).

Twenty-two formalin fixed, paraffin embedded skin samples from cats with facial dermatitis were retrieved, and divided in four groups: 1) samples with a diagnosis of herpesvirus dermatitis (n=5); 2) samples with non-herpetic facial dermatitis (n=6); 3) samples with facial dermatitis of ambiguous nature (n=7); 4) samples from healthy cats (n=4). Data on conventional PCR and qPCR by the ΔΔCq method were available for all the cases. DNA extraction was performed using DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) and the extracted DNAs were amplified using specific set of primers amplifying two viral gene targets: glycoprotein B (gB) and thymidine kinase (TK). The probe synthesis was performed amplifying an 80 bp fragment of gB gene using DIG DNA labelling mixture (Roche) HotStartTaq plus PCR kit (Qiagen). CISH was performed in automation on Ventana BenchMarck ULTRA (Roche, USA). All the cases of group 1 and 2/7 of group 3 were positive by both qPCR and CISH; all samples of group 2 and 4 were negative by both methods. Some of the cases that were negative by both qPCR and CISH, scored positive to conventional PCR (2/6 group 2; 6/7 group 3; and 1/4 group 4).

To the authors' knowledge this is the first time that conventional PCR, qPCR assay by the ΔΔCq method and CISH are simultaneously applied for the diagnosis of FHV-1 associated dermatitis in cats. Both qPCR and CISH methods, resulted to be more specific than conventional PCR, and sensitive to provide a correct diagnosis for FHV-1 associated dermatitis, particularly when histological features are not conclusive.

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ELECTROPHORETICAL ALBUMIN CONCENTRATION MEASUREMENT IS NOT ALWAYS INTERCHANGEABLE WITH THAT OBTAINED USING BROMOCRESOL GREEN: METHOD COMPARISON IN FIVE SPECIES

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Analysis of albumin concentration is routinely performed for clinical purposes. Reduced concentration may be due to decreased synthesis, increased loss or hemodilution, conversely the increases may be caused by dehydration or increased albumin production and life span as consequence of drugs administration. The correlation between bromocresol green (BCG) and agarose gel serum protein electrophoresis (AGE), the most adopted methods for albumin determination [1], have been investigated in different species [2] but no report was performed in accordance with modern procedures [3] and comparisons were often made on low number of samples. The serum concentration of albumin determined with BCG (BT3500) and with AGE (Sebia Hydrasis, with total proteins measured by biuret) were compared to each other on samples from 98 dogs, 81 cats, 78 horses, 123 cows and 76 goats. Data were analyzed using Wilcoxon t-test, Spearman's correlation, Passing–Bablok analysis and Bland–Altman plots. The possible influence of globulin fractions on the bias between the two methods was also investigated with multiple linear regression analysis. Spearman's correlation was moderate in cows (rs=0.42) and dogs (rs=0.58), strong in horses (rs=0.67) and goats (rs=0.68) and very strong in cats (rs=0.81). In all species (and in particular in cows), a proportional and constant error was found. A negative mean bias was present with lower values in goats (-0.58 mg/dl) followed by horses (-0.44 mg/dL), cows (-0.41 mg/dL), cats (-0.36 mg/dL) and dogs (-0.34 mg/dL). The biases between the two methods were always positively correlated with the percentages of total globulins in all species whereas the correlation with percentages of specific globulin fractions varied according to species. Differences between the two methods may be due to different species specific affinity of BCG to albumin or other proteins and to interferences with other proteins in case of hyperglobulinemia [1] or, not investigated here, to analytical errors associated to biuret dye [4] that may affect the conversion of AGE fractions in absolute values. In conclusion, BCG method underestimates albumin compared to AGE at normal values but overestimation occurs at low albumin values, except for goats where a negative bias was always present. The study showed acceptable correlation in dogs, cats, horses and goats and thus both BCG and AGE are reliable at clinical settings. In cows a wider discrepancy was found and reference intervals specific for the two methods should be adopted.

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NEW INSIGHTS INTO THE PATHOGENESIS OF *Leishmania* ASSOCIATED MYOPATHY IN DOG

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Inflammatory myopathy (IM) associated with *Leishmania* infection has been documented in several species such as dogs [1] and syrian hamster. [2] *Leishmania* spp. should also be considered as a possible cause of human myositis. [1] The pathogenesis of this myopathy is still unclear but the latest evidence depicts an autoimmune etiology. [1,2] The aim of this study was to investigate the presence of circulating autoantibodies against skeletal muscle in affected dogs. For this purpose, 50 sera from leishmaniotic dogs with no evident signs of neuromuscular diseases were grouped in 5 pools, purified and tested with an indirect immunofluorescence (IIF) on muscle sections of 5 normal dogs, 3 normal sheep and 3 normal mice. As controls, 10 sera from normal dogs were pooled, purified and processed in the same way. All pools from leishmaniotic dogs show positivity up to a dilution of 1:10,000 on sarcolemma of muscle sections of all selected species, while, no positivity was seen using sera from controls dogs. The muscle antigen partially colocalizes with alpha-sarcoglycan, beta-sarcoglycan, beta-dystroglican, alpha-2-laminin and dystrophin proteins. The partial colocalization with dystrophin associated proteins suggests that our target protein may be expressed at or close to the sarcolemma. Furthermore, immunoblot analysis was performed using the same pools and normal muscle proteins extract to check the molecular weight of the unknown antigen. A band to about 100 kDA was identified. This finding suggest that the major antigen is a non species-specific sarcolemma associated protein of about 100 kDa. A sarcolemmal location would expose it to the immune system and perhaps even be a trigger for an autoimmune reaction. In conclusion, we have identified a *Leishmania* associated myositis- and muscle-specific protein that is associated with the sarcolemma. Further investigations on this unknown protein may shed some light on possible mechanisms of the development of autoimmunity in inflammatory muscle disease not only in dogs but also in humans.

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MICRORNAS ASSOCIATED TO *Mycobacterium avium* SUBSP. *paratuberculosis* INFECTION IN CATTLE.

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Paratuberculosis is a chronic granulomatous infection caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Its control is hampered by the lack of effective and accurate assays for its diagnosis. In particular, the time-gap between infection occurrence and clinical signs manifestation and the low sensitivity of current diagnostic assays make difficult to identify MAP during the sub-clinical phase of infection [1]. Circulating microRNAs (miRNAs) have been shown to have significant potential as novel biomarkers for a range of human and animal diseases [2]. This study aimed to improve early diagnosis of MAP infection through the identification of miRNA associated to the infected and infectious status of the disease.

We followed 5 Holstein-Fresian herds in a 3-year prospective study where each animal was periodically tested for MAP infection by MAP-faecal culture, PCR and ELISA (734 samples from 478 animals). We investigated 40 samples from heifers and cows for miRNA identification by deep sequencing with the next-generation sequencer NextSeq500/550 and profiling using mirDeep2 software. For the differential expression analysis, 26 out of 40 samples were divided into five groups, selected based on animal age – young (10-15 months) or adult (>=23 months) – and disease status – infected, infectious and control.

Overall, we identified 408 known and 620 novel miRNAs among all samples analyzed [3]. We found 6 known and 2 novel miRNAs as differentially expressed (DE). Specifically, all DE miRNAs were identified from the comparison adult-infectious vs adult control groups, 6 from adult-infected vs adult-control groups and 3 from young-infected vs young-control groups. All DE miRNAs showed decreased expression levels in control respect to infectious/infected animals and were involved in biological functions related to cancer, hematopoiesis, B-cells proliferation and generic immunology.

We finally set up Quantitative Realtime PCR to quantify the 4 most interesting miRNAs (Bta-miR-15b, bta-miR-150, bta-miR-342 and bta-miR-505) in order to extend NGS results to a large field sampling in infected herds. This will allow to determine the diagnostic potential of paratuberculosis-associated miRNAs, in particular of those identified in young infected animals, by a simple laboratory protocol. Once established this approach may complement the current MAP diagnostic tests to detect latently infected animals.

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