Società Italiana delle Scienze Veterinarie

in collaborazione con

UNIVERSITÀ DEGLI STUDI DI TORINO

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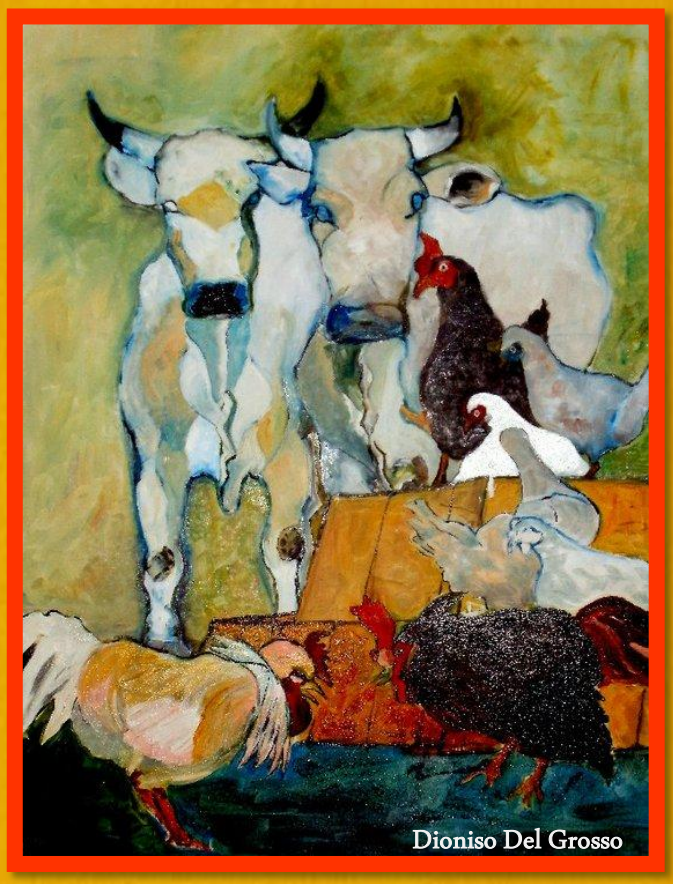

20-22 Giugno 2018

XVIII Convegno SICV

XVI Convegno SIRA

XV Convegno AIPVET

X Convegno ARNA

V Convegno RNIV

II Convegno ANIV

I Convegno SICLIM-Vet

Giornata Studio AIVI

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Sede:
MBC
Via Nizza 52
Torino

Con il patrocinio di

CITTA' DI TORINO

REGIONE PIEMONTE

72° CONVEGNO SISVET

Con il contributo di

Segreteria Organizzativa

SAFOOD srl
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72° CONVEGNO SISVET

20 – 22 Giugno 2018

Università degli Studi di Torino

XV Convegno AIPVet
II Convegno ANIV
X Convegno ARNA
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I Convegno SICLIM-Vet
XVIII Convegno SICV
XVI Convegno SIRA

Giornata Studio SOFIVET
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I contributi presenti negli Atti del
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SEDE CONGRESSUALE

Molecular Biotechnology Center (MBC)

Via Nizza, 52 – 10126
Torino

Relazione del Presidente

Care colleghe e cari colleghi,
il 72° Convegno della SISVet accoglie i Convegni e le Giornate scientifiche delle seguenti Società: **SICV** (Società Italiana di Chirurgia Veterinaria), **SICLIM-Vet** (Società Italiana di Clinica Medica Veterinaria), **SIRA** (Società Italiana di Riproduzione Animale), **AIPVet** (Associazione Italiana dei Patologi Veterinari), **AIVI** (Associazione Italiana Veterinari Igienisti), **ANIV** (Associazione Nazionale Infettivologi Veterinari), **ARNA** (Associazione Ricercatori Nutrizione Alimenti), **RNIV** (Rete Nazionale di Immunologia Veterinaria) e **SOFIVET** (Società Italiana di Fisiologia Veterinaria). Inoltre, l'Associazione Italiana di Storia della Medicina Veterinaria e della Mascalcia ha organizzato un Convegno internazionale sul "*Ruolo della medicina veterinaria nella prima guerra mondiale*" al quale interverranno i rappresentanti dei Servizi Veterinari degli Eserciti Alleati. Il Convegno include una mostra itinerante che, partita a Giugno 2017 da Napoli, giunge a Torino dopo essere transitata nelle Scuole di Medicina Veterinaria italiane.
Il programma scientifico del 72° Convegno SISVet include 350 comunicazioni scientifiche, distribuite in 9 sessioni scientifiche, numerose letture magistrali tenute da relatori internazionali, oltre a 5 *workshop* e 3 tavole rotonde su temi riguardanti la didattica, la patologia renale, la ricetta elettronica, le malattie da vettori, l'antimicrobico-resistenza, il latte nell'alimentazione del futuro, le nuove conoscenze sul ruolo della barriera emato-encefalica, la biodiversità e la resistenza alla mastite nelle bovine da latte. I "*Mistery Case*" proporranno casi curiosi di patologia, clinica e parassitologia. Il 19 giugno ci sarà un *pre-congress* sui risultati della ricerca corrente dell'Istituto Zooprofilattico di Torino. Anche quest'anno la partecipazione dei giovani non strutturati è stata agevolata con una quota di iscrizione ridotta del 50%. Il Consiglio Direttivo della SISVet ha inoltre deliberato di bandire, per il Convegno 2018, 9 premi da 1500 €, uno per ogni sessione scientifica. I premi sono destinati alle comunicazioni orali, presentate da autori under 40, che saranno pubblicate su riviste indicizzate, mentre il miglior poster di ogni sessione sarà premiato con l'iscrizione gratuita al convegno del 2019. Sarà anche l'anno di avvio della "Federazione delle Società Scientifiche Veterinarie Italiane" che darà vita alla nuova SISVet. Infatti, il prossimo autunno si procederà alla modifica di statuto e all'elezione dei nuovi Organi di Governo e del Presidente della Federazione, scelto dai Presidenti delle Società Scientifiche Veterinarie associate alla Federazione.
Il Convegno di Torino è stato organizzato in collaborazione con il Dipartimento di Scienze Veterinarie di Torino, l'Istituto Zooprofilattico del Piemonte, Liguria e Valle d'Aosta e la Federazione degli Ordini Veterinari del Piemonte e della Valle d'Aosta cui vanno i nostri ringraziamenti per il supporto e la fiducia dimostrati.
Ringrazio L'Università degli Studi di Torino, la Regione Piemonte e la Città di Torino per aver concesso il patrocinio al Convegno, la Fondazione CRT e la Fondazione CRC, da sempre amiche della Medicina Veterinaria, l'ENPAV, l'Huvepharma, l'Elanco e il CUS di Torino che ci ha accompagnati come sempre inserendo l'attività sportiva accanto alle iniziative scientifiche.
Infine, esprimo un caloroso ringraziamento a tutti i partecipanti ai quali auguro una proficua e gradevole permanenza, invitandovi a visitare nel tempo libero la città e i magnifici tesori d'arte da essa custoditi.

Benvenuti a Torino!

Presidente SISVet
Bartolomeo Biolatti

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PROGRAMMA GENERALE

PROGRAMMA SCIENTIFICO GENERALE

19 Giugno 2018	
14.00 **(Aula Aristotele)**	**PRE-Congress IZS PLV** L'attività di Ricerca Corrente dell'IZS PLV: nuove strategie a difesa della salute umana e animale

20 Giugno 2018	
8.15 **(MBC, via Nizza 52, Torino)**	**APERTURA DESK REGISTRAZIONE**
8.30 **(cfr. Comunicazioni orali)**	**Sessioni Scientifiche** Comunicazioni delle Società: SICLIM-Vet; SICV
8.45 -12.30 **(Aula Aristotele)**	**WS1-ECM:** Approccio clinico-patologico alle malattie renali del cane
10.00	***Coffee break***
10.30 **(cfr. Comunicazioni orali)**	**Sessioni Scientifiche** Comunicazioni delle Società: ANIV
12.30	***Lunch***
13.30 – 15.30 **(Aula Keplero)**	**Tavola Rotonda:** Efficacia didattica in medicina veterinaria: la sfida della modernità
14.00 - 18.15 **(Aula Leonardo)**	**WS2-ECM:** La ricetta elettronica: nuova sfida per la professione del medico veterinario
14.00 **(cfr. Comunicazioni orali)**	**Sessioni Scientifiche** Comunicazioni delle Società: AIPVet
15.30	**POSTER TOUR n. 01**
16.00	***Coffee break***
16.30 **(cfr. Comunicazioni orali)**	**Sessioni Scientifiche** Comunicazioni delle Società: AIPVet, SICLIM-Vet, SICV, ANIV, RNIV
20.00 ***Welcome Party*** (Rettorato dell'Università degli Studi di Torino - via Verdi 15, Torino)	

21 Giugno 2018	
8.30 (cfr. Comunicazioni orali)	**Sessioni Scientifiche** Comunicazioni e Letture Magistrali delle Società/Sessioni: AIPVet, SICLIM-Vet, SIRA, Parassitologia e Sanità Pubblica
9.00 (Aula Leonardo)	**Presentazione Mostra a cura dell'IPAM** "Scienza e Coscienza: viaggio all'interno delle 3R"
9.30	**Poster Tour n. 02**
10.00	***Coffee break***
10.30 (Aula Darwin - Aula Keplero)	***Inaugurazione 72° Convegno SISVet***
11.15 (Aula Darwin - Aula Keplero)	**WS3:** Piani d'Azione contro l'Antimicrobico Resistenza (AMR) nelle produzioni animali: responsabili e responsabilità
11.00 (Aula Aristotele)	***Teaching Course* AIPVet:** "Patologia Infiammatoria Renale"
13.00	***Lunch***
14.00 (cfr. Comunicazioni orali)	**Sessioni Scientifiche** Comunicazioni e Letture Magistrali delle Società/Sessioni SIRA, SICLIM-Vet, SOFIVET, SICV, AIPVet, Scienze di base e farmacologia, Antimicrobico Resistenza e riduzione del farmaco
14.15 (Aula Keplero)	***Federation of Veterinarians of Europe*:** Tomorrow's veterinary medicine: between science and profession
14.30 (Aula Keplero)	**OIE-Sanità Pubblica:** OIE PVS pathway and the Twinning on Veterinary Education
14.00-18.00 (Aula Copernico)	**WS ECM ARNA**: Il latte nell'alimentazione del futuro
14.00-16.30 (Aula Darwin)	**WS4:** Una per tutti, tutti per una: l'approccio *One Health* nella sorveglianza delle malattie trasmesse da vettori
14.00 (cfr. Comunicazioni orali)	**Sessioni Scientifiche** Comunicazioni delle Società: AIPVet
15.30	**POSTER TOUR n. 03**
16.00	***Coffe break***
16.30 (cfr. Comunicazioni orali)	**Sessioni Scientifiche** Comunicazioni delle Società: AIPVet, SICLIM-Vet, SICV, SOFIVET, RNIV, Scienze di base e farmacologia
17.00	**WS SOFIVET**: New insights in BBB
18.00	**Assemblea Società Scientifiche/SSD**
20.00 ***Cena Sociale*** (Il Ristoro del Priore, Superga – TO)	

22 Giugno 2018	
8.30 **(cfr. Comunicazioni orali)**	**Sessioni Scientifiche** Comunicazioni e Letture Magistrali delle Società/Sessioni: AIPVet, SICLIM-Vet, SIRA, SICV
8.30 **(Aula Keplero)**	**WS 5:** Prospettiva normativa applicata al controllo dei *food-borne parasites*
10.00	**Poster Tour n.04**
10.30	***Coffee break***
11.00 **(cfr. Comunicazioni orali)**	**Sessioni Scientifiche** Comunicazioni e Letture Magistrali delle Società: AIVI, SIRA, RNIV, SICLIM-Vet, SOFIVET
11.00 **(Aula Keplero)**	**Sessione *Mystery Case***
13.00	***Lunch***
14.00 **(cfr. Comunicazioni orali)**	**Sessioni Scientifiche** Comunicazioni e Letture Magistrali delle Società/Sessioni: SIRA, SICLIM-Vet, SOFIVET, SICV, AIPVet, Scienze di base e farmacologia, Antimicrobico Resistenza e riduzione del farmaco
14.00 **(Aula Eraclito)**	**WS RNIV-SOFIVET:** Biodiversità e resistenza alla mastite nelle bovine da latte
16.00	***Coffe break***
16.30	**ASSEMBLEA SISVET**
17.30	**Sessioni Scientifiche** Comunicazioni Società/Sessioni: AIVI
20.00 **Cena Rustica** (Circolo CUS, c.so Sicilia 50 – TO)	

ABSTRACT

WORKSHOPS

and

Main Lectures

DI SEGUITO VENGONO RIPORTATI PROGRAMMI DEI WS E I RELATIVI CONTRIBUTI PERVENTUTI

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

Claudio Brovida
ANUBI - Ospedale per animali da compagnia, Moncalieri (TO)

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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- International Renal Interest Society. Consensus Clinical Practice Guidelines for Glomerular Disease in Dogs. JVIM. Vol. 27, 2013.
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THE ROLE OF THE PATHOLOGIST IN THE DIAGNOSIS OF CANINE GLOMERULAR DISEASES

Luca Aresu
Dipartimento di Scienze Veterinarie, Università di Torino

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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Dipartimento di Medicina Veterinaria, Università di Milano

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

Francesco Dondi
Dipartimento di Scienze Mediche Veterinarie, Alma mater studiorum – Università di Bologna

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.

TAVOLA ROTONDA

Mercoledì 20 giugno 2018

Efficacia didattica in medicina veterinaria: la sfida della modernità

Moderatori:
Prof. Eugenio Scanziani (Università degli Studi di Milano)
Prof. Gualtiero Gandini (Università degli Studi di Bologna)

13.30	**Presentazione degli obiettivi del workshop e dei relatori a cura dei moderatori**
13.40	**Sono cambiati i nostri studenti? La Generazione Z alla prova dell'Università: il caso di Veterinaria** ***M. Franceschetti*** *Università di Bologna*
14.05	**Can we teach basic subjects in an interesting and effective way?** ***M. Kankofer*** *Università di Lublino*
14.30	**E-learning: come trasformarlo in una opportunità?** ***I. Perroteau*** *Università degli Studi di Torino*
14.55	**Discussione con domande all'*audience*** **Conclusione dei lavori**

Workshop 2-ECM

Mercoledì 20 giugno 2018

La ricetta elettronica: nuova sfida per la professione del medico veterinario

Con la collaborazione di:
Federazione Interregionale Ordini dei Medici Veterinari del Piemonte e Valle d'Aosta

Moderatore:
Prof. Giovanni Re *(Dipartimento di Scienze Veterinarie - Università di Torino)*

14.00	**Saluto delle Autorità** ***Dr. Gianfranco Corgiat*** *Dipartimento di Prevenzione e Veterinaria Regione Piemonte*
14.25	**Patto d'aula**
14.30	**La ricetta elettronica e sue basi normative. Modalità di prescrizione negli animali DPA** ***Dr.ssa Raffaella Barbero*** *Istituto Zooprofilattico di Torino*
15.45	**La ricetta elettronica negli animali da affezione e modalità di prescrizione** ***Dr. Thomas Bottello*** *Ordine Veterinari di Torino*
16.30	**La ricetta elettronica per i servizi veterinari e il veterinario pubblico** ***Dott. Stefano Gatto*** *ASL TO3*
11.55	**Discussione e Test Finale**

Workshop 3

Giovedì 21 giugno 2018

Piani d'azione contro l'Antimicrobico Resistenza (AMR) nelle produzioni animali: responsabili e responsabilità

Moderatori:

Dr. Antonio Saitta *(Assessore alla Sanità, Regione Piemonte)*

Dr. Silvio Borrello *(Capo dei Servizi Veterinari italiani, Direzione Generale della Sanità Animale e dei Farmaci Veterinari, Ministero della Salute)*

11.00	**The association between antimicrobials, vaccination, biosecurity and productivity in swine production – Danish experience** ***Lis Alban*** *Danish Agriculture & Food Council, University of Copenaghen*
11.20	**L'approccio condiviso delle istituzioni nazionali per il contrasto all'AMR. Verso un uso responsabile degli antibiotici** ***Loredana Candela*** *Ministero della Salute*
11.35	**Analisi del rischio di Antimicrobico Resitenza (AMR) negli allevatori** ***Alessandro Mannelli*** *Dipartimento di Scienze Veterinarie - UNITO*
11.45	**Il ruolo della GDO nella lotta all'AMR: l'esperienza di COOP Italia** ***Renata Pascarelli*** *Direttore Qualità - COOP Italia*
11.55	**Il ruolo della GDO nella lotta all'AMR: l'esperienza di Esselunga** ***Luca Magnani*** *Direttore Assicurazione Qualità - Esselunga S.p.A.*
12.05	**Il ruolo dell'industria farmaceutica nella battaglia all'antimicrobico resistenza** ***Arianna Bolla*** *Presidente Associazione nazionale imprese salute animale - AISA*
12.15	**Il ruolo degli allevatori** ***Rossella Pedicone*** *(UNA Italia)* ***Maurizio Gallo*** *(ANAS)* ***Alessandra Tropini*** *(ARAP)*
12.45	**Discussione**

ASSOCIATION BETWEEN ANTIMICROBIALS, VACCINATION, BIOSECURITY AND PRODUCTIVITY IN SWINE PRODUCTION - DANISH EXPERIENCE

Lis Alban
University of Copenhagen

There is concern about use of antimicrobials (AM). The fear is that the use of AM may lead to the development of resistance, and such resistance may lead to treatment failure in humans or animals. Therefore, risk mitigation is needed, not just in livestock, but also in pets and humans. In this presentation, focus will be on the use of AM in livestock and the experience in Denmark.

It is unrealistic to expect that farmers or vets will change their habits, unless official regulation takes place. First, because it is difficult for the individual farmer to perceive the responsibility related to his or her use of AM for the society. Next, because the framework conditions should be the same for all producers. If the conditions are not the same, competitiveness problems may arise. It is important to inform the sector about the usefulness of legislation to ensure compliance.

From society´s side, we have to accept that for a livestock producer, productivity is essential. This implies that we should identify cost-effective measures to apply to ensure responsible use. Such measures may be divided into measures at the national level and at the herd level. In the search for such measures one can be inspired by what works in other countries. But it is often necessary to adapt the identified measures to the individual country in which the measures are supposed to work and have an effect. Without that, there may be no or even a negative effect e.g. on productivity.

During the last two decades, several risk mitigating initiatives regarding use of AM in pigs have been implemented in Denmark at the national level: 1) DANMAP program monitoring the prevalence of resistance in livestock, food and humans, 2) Vetstat recording the use of AM, 3) ban on use of AM as growth promoters, 4) ban on use of flouroquinolones, 5) ban on using 3rd and 4th generation cephalosporins in pigs, and 6) the Yellow Card Scheme. These measures have ensured that the level of AM in pigs is low compared to other countries with a similar pig production.

The Yellow Card Scheme was adopted in July 2010 by the Danish Veterinary and Food Administration. It makes use of data recordings regarding AM use in Vetstat combined with information about the herd size originating from a register called the Central Husbandry Register. Herd size is divided into three different age groups. Originally, the Scheme implied that restrictions were imposed on pig farmers who used more than twice the average used in the age group of interest: 1) sows with piglets, 2) weaners or 3) finishers. The scheme was implemented to avoid very high use seen on individual farms. The use is calculated as animal daily doses (ADD) per 100 animal days for a 9-month moving average. Initially, the permit limits were: 5.2 ADD per 100 animal days for sows and piglets, 28 for weaners, and 8 for finishers, reflecting that the most vulnerable period is the weaning phase. Later, the limits have been reduced.

Since 1995, Danish vet are only allowed to profit up to 5% from sales of medicine. Instead, the vet and the individual farmer draw up veterinary advisory service contracts at herd level. The contracts were introduced in 1995 and they became mandatory for large herds in 2010 implying ≥ 300 sows, ≥ 3,000 finishers or ≥ 6,000 weaners. The contract involves frequent visits, during

which the vet gives advice with a focus on disease prevention, production and responsible use of AM. Reports are written after each visit, and a quarterly report is giving details about the use of AM and productivity. Together, the farmer and the vet decides on which actions to initiate. Focus is on limiting the need for treatment, but the final decision and responsibility lies upon the farmer.

The need for treatment with AM can be lowered through use of targeted vaccination, eradication of infections, high biosecurity (also to avoid African swine fever), good management and housing, and correct feed. Application of these measures, will at the same time ensure productivity and hereby, the future of the pig producer. The pig vet is key in this work through collaboration with the pig producer. However, some challenges are beyond what the individual producer and vet can handle – such as eradication of infections requiring high amounts of AM. An example is dysentery. Here, a public-private partnership regarding eradication is needed.

NATIONAL ACTION PLAN ON ANTIMICROBIAL RESISTANCE
Implementation of PNCAR 2017-2020 Strategy in the veterinary sector

Loredana Candela
Ministero della Salute

Antimicrobials are one of the most important therapeutic discoveries in the history of medicine. Since the discovery of penicillin in the 1940s, they have played an essential role in the treatment of infections in humans and animals and have significantly improved public health, animal health and welfare, also guaranteeing increasingly higher standards of foodstuffs of animal origin. Seventy years later, we are threatened by the selection and spread of microorganisms resistant to commonly used antimicrobials. Although such resistance is a natural biological phenomenon caused by the genetic mutations affecting bacteria, and therefore it cannot be eliminated, it has been proven that the inappropriate use of antimicrobials in human and veterinary medicine and in agriculture actually accelerates the development and spread of resistant microorganisms and, consequently, their loss of effectiveness.

Antimicrobial resistance is a serious European and global threat for both humans and animals, since the loss of effectiveness of these valuable molecules in the control of bacterial infections involves:

- Greater likelihood and spread of the disease;
- Greater morbidity and mortality of the disease;
- Reduction of productivity and efficiency, even in the livestock sector.

According to the main European and international Organizations:

- each year in Europe, there are 4 million infections from antibiotic-resistant germs, causing 25,000 deaths per year in the European Union alone (1);
- 700,000 deaths a year worldwide are due to resistant infections (2);
- in 2050, *"superbugs"* will be responsible for at least 10 million annual deaths (3), largely exceeding deaths due to cancer (8.2 million), diabetes (1.5 million) or road accidents (1.2 million);
- the highest number of deaths will be recorded in Africa and Asia.

The epidemiological impact of AMR, related to the increase in morbidity and mortality associated with infections from antibiotic-resistant bacteria, directly affects the economy, with consequences in terms of loss of lives and working days, a greater use of health resources due to the prolonged duration of hospitalization, a greater use of diagnostic procedures and more expensive antibiotics, where available (4). In Europe, significant expenses for not only healthcare systems amount to approximately 1.5 billion euros per year. Worldwide, expected costs exceed 100 trillion dollars (2) as a result of the 10 million deaths estimated for 2050. The OECD estimates that hospitals spend on average 10.000 to 40.000 dollars to treat a patient infected with resistant bacteria (5). **In the light of the above, it is more necessary than ever to take actions to minimize the risks arising by the development and spread of AMR, which knows no boundaries, neither geographical nor sectorial.**

In veterinary medicine, antimicrobial resistance should be considered in terms of both public health and protection of animal health and welfare, irrevocably and closely liked, and in terms of the environment. In fact, a non-prudent or irresponsible use of antimicrobials in animals is a potential risk factor for the selection and spread of resistant microorganisms and determinants of antimicrobial resistance from animals to humans, also through the consumption of food of animal origin. This is why the fight against antimicrobial resistance needs a ***"One Health"*** **approach**,

that is to say *a joint effort of different professional disciplines operating at local, national and global level to achieve an optimal human, animal and environmental health* (6)(7).
In Italy, the multi-sectorial and collaborative approach to address potential or existing risks arising from the Human-Animal-Ecosystem interface, has been reflected in the **PNCAR 2017-2020** (4), approved with the Agreement of November 2, 2017, between the Government, the Regions and the Autonomous Provinces of Trento and Bolzano, containing the whole veterinary strategy. The Plan represents the tool to implement the Italian strategy through the identification of actual planned actions. Moreover, on November 3, 2017, a **multi-sectorial Table for the coordination and functional integration** between the different institutional levels and local competences was established. Its mandate is to encourage the achievement of the objectives set by the Plan.

http://www.salute.gov.it/portale/documentazione/p6_2_2_1.jsp?lingua=italiano&id=2660

http://www.salute.gov.it/portale/documentazione/p6_2_5_1.jsp?lingua=italiano&id=362

In the veterinary sector, there are several Authorities with specific competences having responsibility for prevention and control of animal diseases, animal health and welfare, authorization and prudent and responsible use of veterinary medicines, agricultural and agri-food policy. Consequently, strengthening the above-mentioned coordination and integration is a fundamental element to fully implement the veterinary strategy. To this end, the Directorate General for Animal Health and Veterinary Medicines (DGSAF) is constantly monitoring the implementation of the Plan. The roadmap of cross-sectorial actions started and/or planned, for the veterinary sector, will be published on the website of the Ministry of Health.

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PREVENTION OF THE EXPOSURE OF ANIMAL FARMERS TO ANTIMICROBIAL-RESISTANT AGENTS

Alessandro Mannelli (1), Lebana Bonfanti (2), Marta Bottino (1), Anna Rosa Favretto (3), Giorgio Franceschini (1), Elisa Martello (1), Ilary Millet (1), Paola Tomao (4),Nicoletta Vonesch (4), Francesca Zaltron (3)

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In this study, funded by the National Institute for Insurance against Accidents at Work (INAIL), we adopted a mixed methods research design, integrating qualitative, and quantitative techiques. During the first year of the study, we focussed on two main aspects: 1. the transmission dynamics of agents in the animal populations, resulting into varying levels of infection of animals and of the farming environment (release assessment); 2. the analysis of working practices, which may lead to contacts of farmers with animals and fomites, as potential sources of infection (exposure assessment). We carried out systematic literature reviews and meta analysis, to estimate prevalence of AMR in pig, and poultry farming , and to estimate associations between working practices and the transmission to farmers. We recontructed farming phases, as well as corresponding working practices, based upon semi structured interviews of public and private veterinarians, and of farmers in Northern Italy. Working practices were classified and ordered in terms of the probability of exposure of farmers to AMR, by a modified FMEA (Failure Modes and Effect Analysis). During the project's second year, antimicrobial usage will be estimated in a sample of farms. Results of FMEA and of farm distribution and drug usage will be used to produce predictive maps of AMR occurrence. Preventive measures will be based upon a participated approach, involving farmers and practicing veterinarians. Communication of preventive measures to farmers and other stakeholders will be implmented by means of a website.

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"ALLEVIAMO LA SALUTE" - "BREEDING HEALTH"

Renata Pascarelli
Direttore Qualità - COOP Italia

Thirty years ago, Coop launched meat products "**Prodotti con Amore**".
Even today, the expressed and unexpressed needs of the consumer are the same: **safety**, **values**, **good** products with a **fair price**. Nowadays an increasing number of people, the health and protection of farm animals are essential elements that cannot be renounced. We consider it right to guarantee a fair dignity to the animals while respecting the five freedoms defined by the European Convention for the Protection of Animals. Animal welfare is a fundamental theme of our policies for years and is one of the two main point of the "**Breeding Health**" campaign, this is why we have been collaborating for a long time both with the Animal Welfare Reference Center and with organizations such as LAV, CIWF (Compassion In World Farming). The second one is the fight against **antimicrobial resistance**. WHO, EFSA and the Ministry of Health have launched an alarm on antibiotic resistance of pathogenic microorganisms that may affect men and cause infections that sometimes cannot be cured. Coop wants to play an active role in increasing animal welfare and preventing antibiotic resistance supporting the institutions and promoting a substantial change in management of the animal supply chains. The reduction of the use of antibiotics must be properly managed and with a very clear idea: **the sick animals must be cured**. A Coop report has been made to support the campaign, which include the scientific positions on: The necessary change of approach on the breeding method to reduce the abuse of antibiotics; the necessary good practices; a list of antibiotics that can be used. The targets of the project are: a) Request and encourage good breeding practices based on principles of biosafety and animal welfare, so that the responsible and awareness use of drugs in zoo-technics becomes a consolidated practice; b) Control of the drugs through a selection of antibiotics avoiding the use of those that are particularly important for human medicine in order to prolong the effectiveness; c) Avoid mass treatment in any case, limit them to strictly necessary cases, in a targeted manner, following the diagnosis and prescription of a veterinarian. In order to achieve these goals we have selected the best partners able to provide adequate facilities and redefined the technical supply specifications with the new requirements and reprogrammed the control activity. We have also requested to our suppliers to define the company veterinarian for each farms belonging to our supply chains as required by the Italian Ministry of Health decree published in December 2017. Since the beginning of 2017 we have requested the adoption of a camera system at the slaughterhouse and in the various stages of breeding, bearing witness to the good practices adopted in order to minimize the suffering of animals in compliance with privacy procedures. The impact of the project has been on more than 1700 farms including bovines, pigs and poultry. It started in October 2016 with the poultry supply chain and, from April 2017, all the Coop poultry meat come from animals raised without the use of antibiotics. Then the project involved the pigs supply chain with the achievement of the goal "Raised without use of antibiotic in the last 4 months" for the pigs raised outdoors and, from January 2018, all the pigs in the Coop chain are raised with this item. Then it was the turn of the bovines and veals in October 2017, we reached the same goal, about the non-use of the antibiotics in the last 4 month, adding to this result also the CReNBA certificate regarding the animal welfare required for all farms included in the coop Bovine supply chain. Nowadays we have also all the eggs derived from hens breed on the

ground and without the use of antibiotics (project started in july 2017 on two eggs formats). Now we are working on other product like Mayonnaise, sauces and pasta produced with eggs derived from hens raised on the ground and without the use of antibiotics.
A major change was made to the pig industry that was the least structured.
Different requirements have been specified: castration in analgesia, no mutilation, presence of manipulable material and above all eliminating the practice of tail cutting. We are aware that achieving these standards implies investments and commitments and for this reason Coop recognizes a prize money directly to the breeder for each individual animal. To the activities required of suppliers, the activities of Coop Italia are added in order to direct the supply chain management and planning controls. To date, we have invested over 3 million of euros in this operation, which will become over 5 million when fully operational (once the targets set by the end of the year will have been completed).

THE ROLE OF GDO IN THE FIGHT AGAINST AMR: ESSELUNGA'S EXPERIENCE

Carlo Ferrari
Assicurazione Qualità Esselunga

Esselunga's production chains, already at the end of the '90s, are attentive to the theme of animal welfare, with production of extensive indoor chicken and extensive reared beef. In those years, due to the crises like BSE or dioxin, priority was food safety and providing the customer with the reassurance of the safety of the products.
In those years, the production systems were born as "closed" systems with a high level of traceability, constant laboratory analysis and controls of the various production steps (farm, plants, feed mills).
At the same time, there has been a rapid evolution of the regulatory framework, aimed at strengthening food safety and traceability, which, thanks to the efforts of all operators, has made it possible to increasingly consider food safety as a prerequisite.
Today the quality of the supply chain is a more multifaceted concept. Food safety, as mentioned rightly and dutifully increasingly a prerequisite, is also flanked by the need to provide customers with reassurance in an ethical, social, environmental sense.
In addition, a new emergency appears to emerge, that of resistance to antimicrobials, molecules used in the veterinary field, as well as in human medicine.
The Esselunga supply chains, today, maintaining a necessary control over the safety and hygiene of production, are also characterized towards animal welfare, biosafety and responsible use of the antimicrobials, animal nutrition, sustainability, communicating the characteristics of the animal products and their origin through increasingly complete and "talking" labels.
Biosafety, welfare and animal nutrition, play key roles to prevent the onset of diseases and to achieve a lower use of the antibiotic.

On the other hand, in case of need, the antibiotic is essential to cure diseases and to ensure the welfare of the animals; as such, it must be used correctly and responsibly in order to prevent antibiotic resistance.
At national and international level, numerous documents and guidelines have been published concerning welfare, biosafety and therefore the responsible use of the drug.
These documents have been used as a precious tool also by Esselunga in defining the supply chain requirements.
Among these, the Manual of " Biosicurezza e uso corretto e razionale degli antibiotici in zootecnia " (Ministero della Salute, 2012), the " Piano nazionale per l'uso responsabile del farmaco veterinario e per la lotta all'antibiotico-resistenza in avicoltura " elaborated from the Società Italiana di Patologia Aviare, from the UNAITALIA Association and the Ministero della Salute, the " Manuale per la valutazione del benessere e della biosicurezza nell'allevamento bovino da carne " developed by the Centro di Referenza Nazionale per il Benessere Animale (Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna, Brescia) and the " Piano Nazionale di Contrasto dell'Antimicrobico-Resistenza (PNCAR) 2017-2020" (Ministero della Salute, 2017), which for the first time

sets the objectives of total reduction of the use of antibiotics, as well as those considered most at risk for public health (eg Fluoroquinolones, Cephalosporins of 3rd and 4th generation, Colistin).

Esselunga products

Esselunga, first chain of large retailers, since 2016 has started the marketing of chicken reared without use of antibiotics.
Starting from April 2018, all the Naturama chicken production takes place without the use of antibiotics.
As for the Eggs, among those with the Esselunga brand there are barn eggs from hens raised without the use of antibiotics.
Esselunga in 2017 has started an important production of beef, Scottona (heifers) and Vitellone (Young Bull) Naturama, whose welfare is assessed and guaranteed according to the standard of the Centro di Referenza Nazionale per il Benessere Animale (CReNBA).
At the beginning of 2018, the range of beef with the application of the CReNBA standard, has expanded to a chain of Veal calves meat.
Also regarding pigs, Esselunga has developed a Naturama chain, based on the principles of animal welfare and the responsible use of antibiotics. The therapies are chosen based on the precise diagnosis by the Veterinarians, and laboratory analysis to establish the sensitivity of the bacteria to the chosen drugs and are carried out in compliance with the dosages and the appropriate duration. Drugs considered to be of critical importance to human medicine can only be used when, based on the selection criteria, there are no alternatives and there is therefore a risk of animal suffering.

AMR: THE ROLE OF BREEDERS

Nicolò Cinotti
UNA ITALIA

The debate on the use of antibiotics in livestock has become increasingly important worldwide in recent years. Unaitalia, the Italian association of poultry producers, and his associated Companies have voluntarily decided to play their role in this game, because the poultry sector, being organized in a highly integrated supply chain, has all the characteristics and sensitivity to give the answers that the consumer and the authorities expect from him.

Starting from the data of the 2013 ESVAC report on the sale of antibiotics per Country, which saw Italy in a far from positive position, Unaitalia and the veterinarians of the main Companies easily calculated how many of these antibiotics are used in the poultry sector (expressed in mg/kgpv), thanks to the integration of the sector. Then, with the collaboration of the Italian Society of Avian Pathology and the Ministry of Health, the "National Plan for Rational Use of Veterinary Medicine in Poultry Sector" was elaborated. Unaitalia had already done a similar experience for the rabbit sector, but in Italy this is the first intervention of this scale, based on the will of the Companies and that collects more than 80% of the national production of poultry meat.

The Plan aims to develop a preventive approach along the entire supply chain, starting from the breeders to hatcheries and farms. The companies are committed to respect and maximize the biosecurities measures, to pay attention to animal welfare standards, to take charge of continuously updating all the operators in the supply chain, to eliminate antibiotic prophylaxis in the hatchery, prophylaxis and metaphylaxis in farms, the use of 3rd and 4th generation cephalosporins, preferring the use of large spectrum and leaving critics as extrema ratio. The objective of the plan is the reduction of 15% by 2015 and 40% by 2018.

The results showed a reduction of 39.95% mg / kgpv in 2015 and of 50% mg / kgpv in 2016. In light of these excellent results in 2017 a new plan was developed that aims to result in a further decrease of 10 % in antibiotic consumption, a general reduction of CIAs and the total ban of colistin in broiler frming. The adoption of the electronic veterinary prescription, which will be mandatory in Italy starting from September 2018, is fundamental for the continuation of the actions undertaken with the plan. This tool will allow to have consumption data for all the livestock sectors. Furthermore, Unaitalia intends to continue on the path of a constant and progressive improvement of results, adding to the indicator used the new DDDAIt indicator, approved by EFSA, and participating in the new Classyfarm system of the Ministry of Health for the categorization of farms.

The reduction in the use of the drug has led to the possibility of carrying out production cycles without use of antibiotics and, consequently, to the desire of Companies and Retailers to label these products. The labeling of poultry meat with additional information (such as the raised without antibiotic use) is only possible thanks to the Standard of

which Unaitalia is owner and which is authorized by the Ministry of Agriculture. This Standard indicates the requirements to be able to use a specific claim on the label, the checks that must be performed and when a batch must be downgraded. A Certification body recognized by the Ministry of Agriculture supervises on the application of this regulation.

In conclusion, breeding animals without antibiotic use is not the goal of Unaitalia. Antibiotics are essential tools in the fight against diseases and in the protection of animal welfare. Unaitalia's objective is to protect animals and their health, preventing diseases and taking care of them when they occur, using antibiotics as little as possible, as much as necessary.

The Italian poultry sector is therefore at the forefront in the fight against antimicrobial resistance and intends to continue on the path already undertaken for years.

ACTION PLANS AGAINST AMR IN ANIMAL PRODUCTION: ROLE OF THE PIG FARMERS

Maurizio Gallo
ANAS

Italian pig industry is the seventh producer in the EU with 1,325 million tons of pig carcasses produced annually (around 6% of the EU total) and it is leader in EU for geographical indications with 22 PDO and 16 PGI pig meat products. The two most important products are PDO Parma and San Daniele Hams and around 80% of the pigs born in Italy are reared for PDO productions. Italian Pig Industry is unique in the world, because it is oriented to the production of traditional and quality products. However, the sustainability of the sector requires the maintenance of the distinctiveness of traditional products and the updating of some management practices and housing conditions to improve the welfare and the health of the animals. In this way, it will be possible to reduce also the use of antibiotics as requested by citizens and consumers. In the past years, in collaboration with the PDO Consortia of Parma and San Daniele and others farmer associations, ANAS has organized many activities to inform and train farmers. The dissemination activity has been organized paying particular attention to the Guidelines of the Ministry of Health "Biosecurity and rational and correct use of antibiotics Guidelines – 2012", … and reports of some research activities (as the project "*Filiera Verde Suino*" funded by AGER Foundation).

In the 2014, ANAS and the Consortium of Prosciutto San Daniele DOP developed a pilot project, called "*Italico Filiera San Daniele"* to reach a superior standard of pig welfare and the reduction in the use of antimicrobials. IZSLER Brescia is the technical and scientific body responsible for the farms classification activity concerning welfare, biosecurity and the use of antibiotic.

The first results are encouraging: the cooperation between IZSLER technicians and pig producers is improving some management practices and a more responsible use of antibiotics and this is confirmed by the trend of the *DDD (Defined Daily Dose)* data. The genetic improvement of pigs in their resistance to disease is another important way–to contrast Antimicrobial resistance (AMR). ANAS manages breeding programmes of the Italian breeds for traditional heavy pig and some autochthonous breeds. The breeding programme of Italian breeds for PDO traditional heavy pig develops carcasses with a balanced relationship between lean meat and fat. Fast growing of lean meat has negative effects on the hypothalamic–pituitary–adrenal axis (HPA) and on the robustness and resilience of the animals. So, concerning "robustness and resilience", Italian selection is more sustainable than other selections are.

Furthermore, the progress in genomics is opening new and interesting scenarios. "*Suinicoltura Italiana Sostenibile - SUIS*", is an ANAS project funded by PSRN 2014-2020 that has the aim to develop pilot genetic scheme for the improvement of resilience-resistance of pigs. The activity started in 2017 recording new phenotypes (morbidity, data of treatments, data of analysis) and genotyping many animals (boars, sows, gilts, fatteners). The new available information are used for Genome Wide Association studies. For instance, the improvement of the resistance to enteric diseases is pursued using

some polymorphisms in linkage disequilibrium with causative genes or markers (*MUC4* and *FUT1*), and the resistance to *PRRSv* using QTLs responsible of an immunity reaction. The project results will give new knowledge to update the breeding programmes of Italian breeds. This way in the medium/long term it will be possible to rear more resilient and healthier pigs. The better health status of the animal will reduce the need of antibiotics. This can be the most effective contribution of ANAS and its breeders to achieve the objectives of the "*Piano Nazionale di Contrasto all'Antibiotico Resistenza 2017-2020*" and contrast Antimicrobial resistance (AMR).

ACTION PLANS AGAINST ANTIMICROBIAL RESISTANCE (AMR) IN ANIMAL PRODUCTION: ACCOUNTABLE AND ACCOUNTABILITY: THE ROLE OF FARMERS

Alessandra Tropini
Regional Association Breeders Piedmont

The Advice Office of the Regional Association Breeders of Piedmont confirms its important role of interlocutor between the primary production of farm, supervisors and the processing industry, in the performance of all those activities relating to good practice of processing and more specifically related to food safety. In detail the path with the breeder requires first of all the transmission of knowledge of being a major ring of Agri-Food Chain and be Food Business Operator. The aim is to deepen the link between primary production of farm and finished product, in a journey that involved all stakeholders in the supply chain, primarily farmers, first link of production, up to the end customers, not only because they ask the quality of the finished product, but also they Know the consumer voice and its perceptions.

The relationship between the consumption of animal foods, animal husbandry and environmental impact is today one of the topics of greatest cry not only at scientific communities, but also at the heart of the communications media, so much so that the consumer tends to turn to make more informed purchasing decisions, avoid waste and opt for certified foods that meet certain levels of animal welfare and environmental parameters, including the recent One Health concept.

From the perspective of the management of antimicrobial resistance, the issue of the use of antibiotics in animal husbandry, it proves to be very timely, considering the subject of attention in public health, national and European level. The actuality animal husbandry is considered a central ring as the resistances created in breeding can spread in the community and in terms of environmental spread of antibiotic molecules and farmers, with the support of their veterinary are called forcefully for a rational and prudent use of the antibiotic.

Therefore, the Advice Office on one side keeps firmly in its role in promoting the implementation of procedures in the stable to prevent the possible entry of residues in the primary product and analytical activity in self-control exercised before placing on the market but, on the other hand, supports farmers in identifying and applying all those preventive practices that aim to reduce or eliminate the use of antibiotics.

Because of the privileged position compared to livestock farms, accompanies the breeder to understand their role and responsibilities with respect to this issue: it thus passes from a food safety problem to a theme of public health.

In particular the Ministry of Health Manual "Biosafety and correct and rational use of antibiotics in animal husbandry" underlines the active role entrusted to the breeder, who is called to prevent the disease of their animals and to ensure the correct use of medication; should regularly monitor the health and wellbeing of their animals; take note of any changes in their health status.

Our Office is the promoter of this concept, fielding some useful tools to achieve evaluation of the level of animal welfare and biosecurity by using CReNBA Protocol, useful to bring

out any critical points and to share with the breeder to develop improvement plans. We progress through discussions and dialogue with farmers at their companies, as well as classroom training, recalling the collaboration of supervisory bodies and other links in the supply chain.

The breeder is encouraged to identify with its veterinarian any corporate actions to replace-reduce and rethink antibiotic treatments on farms, but we think that only through a deep work of alignment of information and requests aimed at livestock farms will be possible this awareness and this path towards the fight against AMR.

Starting from the evaluation of the practices adopted in the farm and the detection of possible areas for improvement, the Advice Office promotes primary production protocols companies which may make the increasingly sustainable companies, both in terms of regularity at regulations, both in terms of marginalization and competitiveness, both in terms of sharing with the market requirements, responding to consumer expectations, qualitative and not, in which sense the struggle against AMR is emerging as a new challenge to be achieved.

Workshop–ECM

Giovedì 21 giugno 2018

Con la collaborazione di:
Associazione Ricercatori Nutrizione Alimenti (ARNA)

Il latte nell'alimentazione del futuro

Moderatore:
Prof. Giuseppe Bertoni *(Presidente ARNA)*

14.00	**Caratteristiche nutrizionali del latte vaccino e dei suoi derivati: le evidenze scientifiche** ***Franca Marangoni*** *Nutrition Foundation of Italy*
14.30	**Prospettive mondiali della zootecnia da latte** ***Roberto Pretolani*** *Università degli Studi di Milano*
15.00	**Problematiche di tipo sanitario, anche zoonosi, connesse alla produzione di latte** ***Andrea Serraino*** *Università degli Studi di Bologna*
15.30	**Sostenibilità ambientale dell'allevamento bovino da latte** ***Maddalena Zucali*** *Università degli Studi di Milano*
16.00	***Coffee break***
16.30	**Effetti del latte sul profilo del microbioma intestinale nell'era della NGS** ***Patrizia Brigidi*** *Università degli Studi di Bologna*
17.00	**Human malnutrition and animal husbandry in developing countries. Milk and egg production and consumption in Meghalaya State (India)** *André Ndereyimana, Andrea Minuti, Andrea Minardi, Serena Annese, Giuseppe Bertoni*
17.15	**Analysis of the chemokine receptor genes variability and association with SCS in italian Holstein** *Dominga Soglia, Stefano Sartore, Francesca Tiziana Cannizzo, Sandra Maione, Fabio Operti, Roberto Rasero, Paola Sacchi*
17.30	**Dynamics of volatile molecules in raw milk for the production of parmigiano reggiano and grana padano cheeses** *Massimo Faustini, Curone Giulio, Carla Colombani, Luca Maria Chiesa, Sara Panseri*
17.45	***Discussione***
18.00	***Test finale***

NUTRITIONAL ROLE OF COW'S MILK AND ITS DERIVATIVES: THE SCIENTIFIC EVIDENCE

Franca Marangoni
NFI – Nutrition Foundation of Italy, Milan

The scientific literature focussing on cow's milk in human nutrition highlights its nutrient composition, the impact of its consumption on overall diet quality, and the link between its regular consumption and health status. However, the increasing available scientific information on this topic is often misleading or incorrectly communicated.
In order to shed light on the topic, NFI –Nutrition Foundation of Italy, organised a symposium which included a group of experts and representatives from several Italian medical/scientific societies and Institutions. Participants discussed and evaluated the role of cow's milk in the human diet across different age groups and health conditions using a strictly evidence-based approach. They also addressed the relationship between milk consumption and specific diseases or disease risk factors.
A shared document summarising the main themes discussed throughout the debate is presented in a question and answer format, to be used by health professionals, whose challenging task is to explain the diet-health relationship to their patients[1].
The main findings concern cow's milk nutritional role in reaching the nutritional goals for essential macro and micronutrients across the entire lifespan, if consumed according to the relevant guidelines and within a balanced diet. Milk's nutritional role is particularly relevant for specific physiological conditions such as pregnancy and breastfeeding as well during physical exercise for active individuals. Moreover, regular milk consumption is associated with regular breakfast intake, and the latter is known for its positive effects on general health and well-being.
Furthermore, the vast majority of associations between milk consumption and health appear favourable according to the available scientific evidence. The relationship between milk and milk-derived products and bone mass has been established as crucial during the initial life stages (as well as throughout the entire lifespan). The available evidence also confirms a neutral or favourable association between milk consumption and risk of overweight, obesity, diabetes or of developing cardiovascular diseases, with a potentially protective effect on stroke risk. Finally, milk consumption does not appear to affect overall cancer risk, however small positive effects have been found on colon and prostate cancer.
Overall, the Authors of the document agree that, except for subjects with allergies and symptomatic lactose intolerances (which can be overcome by consuming lactose-free milk), there is currently no reason to limit the consumption of cow's milk in the healthy general population.

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GLOBAL PERSPECTIVES OF DAIRY

Roberto Pretolani
Department of Environmental Science and Policy, Università degli Studi di Milano

According to the 2016 FAOSTAT data, world milk production is around 800 million tonnes. Over 80% consist of cow's milk, 14% from buffalo milk and 4% from milk of other species.
Production is concentrated in Southern Asia (27%), followed by the EU (20%) and Northern America (13%).
Cows yields are very different between the world areas: compared to the world average of 2,400 kg / cow, in Northern America it exceeds 10,000 kg / cow, while in Africa the yields are less than 1,000 kg / cow.
In the last 10 years overall production has grown by 17.5%, largely due to the increase in cows (+12%) while yields have increased by only 5%.
Over the last 50 years, total production has increased 2.3 times, with large differences between geographical areas: while in Europe the increase was only 15% and in Northern America 57%, production increased 450% in Southern America and Africa and more than 700% in Asia.
Production variations followed those of per capita consumption and population increases. Currently the average consumption per capita (milk equivalent) is about 90 kg / year. However, there are still big differences: in Europe and Northern America consumption is about 250 kg / year per capita, while in many areas of Africa and Asia consumption is less than 50 kg / year per capita. In addition, milk consumption grew only 15% more than the population increase, while consumption of other foods increased significantly higher (cereals 50%, meat 80%, eggs 130%).
Currently around 55% of milk is consumed fresh, 30% is used to produce processed milk products and 11% for animal feed. In the last 50 years, consumption per capita of fresh milk has increased by only 13%, while cheese consumption has increased by 56%.
Currently, just over 15% of the milk produced in the world is exported, with a growing trend. International dairy trade has increased 9 times since the ‘60s to today. However, excluding intra-EU trade (which accounts for almost half of the total), the share of trade in production is only 9%.
Oceania holds the largest share of exports (33%), followed by the EU (26%). The majority of imports are instead directed in Asia (60%).
Mid-term estimates (2030) indicate that global milk supply is expected to grow by about a third compared to 2015. Supply should remain stable in Northern America and Oceania, decrease in Europe, weakly grow in Africa and increase strongly throughout all Asia.
The expected increase in consumption could easily be covered with a production increase. However, contrary to the recent past, the increase will have to be achieved by limiting the growth of herd and improving yields. International trade will also have to increase and, in order to prepare for this increase, in 2030 EU expects to increase production by over 20 million tonnes (+15%) compared to 2015 and to double its production surplus and the extra-EU export.

ENVIRONMENTAL SUSTAINABILITY OF MILK PRODUCTION

Zucali Maddalena
Dipartimento di Scienze agrarie e ambientali, Università degli Studi di Milano

The future of livestock sector has to face both the increasing demand of animal food products and the need for reducing the environmental impact. At a global level the livestock sector accounts for approximately 14.5% of total human-induced global warming potential (GWP); within the livestock impact on climate change, milk sector contributes 20%. At Italian level, environmental impact as GWP from agricultural sector is estimated as 7% of the human activities. Animal feeding is a critical point in terms of both production efficiency and environmental impact for the livestock sector and farmer choices about home-grown feed can have great influence on environmental impact of milk production.
The aim of the current study was to assess the environmental impacts of dairy production by a life cycle approach and to identify relations between farming management choices and environmental performances expressed on milk. Feed efficiency was identified as one of the most important parameters controlling GWP intensity; from a study of [1] reducing feed efficiency by 10% causes an increase of 6.6-8.5% in GWP emissions per kg milk. Dairy efficiency is the result of many factors: genetic traits, diet formulation, reproductive performances, health and welfare.
From a study from [2] decreasing the clinical mastitis rate from 25 to 18% and the subclinical mastitis rate from 33 to 15% reduced the GWP of milk unit by 2.5%. This reduction is mainly due to increased input-use efficiency; decreased losses of milk production and a decreased amount of waste milk. Results from [3] show that there is a potential to reduce the farm GHG emissions intensity by 3.7% if the milk production was improved through reducing the level of SCC to 50,000 cells/mL in relation to SCC level 800,000 cells/mL. In a study of [4], life cycle assessment was used to compare a typical baseline farm with scenarios assuming increased lameness severity and prevalence. It was found that lameness could increase the farm level global warming potential, acidification potential, eutrophication potential and fossil fuel depletion by 7-9%.
In conclusion it is important to remember that all the animals in dairy farms have an environmental impact (heifers, calves, dry cows), so it is useful to improve: reproduction efficiency, calves' health, dry period length and management. Poor health and welfare in the dairy herd will affect overall systems emissions of GWP, as more replacement animals will be required to maintain the herd size as more dairy cows may be culled involuntarily.

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MILK EFFECTS ON THE INTESTINAL MICROBIOMA IN THE NGS ERA.

Patrizia Brigidi
Department of Pharmacy and Biotechnology, University of Bologna, Italy

The human intestinal microbiome is an adaptive entity, capable of being reconfigured in response to diet, lifestyle and environment. This plasticity provides extra metabolic flexibility to the host and functional traits that humans have not evolved by their own. In particular, the intense microbiota-host transgenomic metabolism deeply influences our energy, metabolic and immune homeostasis, impacting on host weight gain, obesity and related metabolic disorders.

Even if present in a lower total concentration respect to the human milk, bovine milk contains oligosaccharides (BMOs), many identical to those found in human milk (e.g. 3'-sialyllactose, 6'-sialyllactose and lacto-n-neotetraose), with higher proportions of acidic and sialylated oligosaccharides, small total amount of fucosylation as well as glycoconjugates similar to human milk (lactoferrin, glycoproteins and gangliosides) but generally highly sialylated. The goal of the study is to analyze the role of BMOs in modifying the human intestinal microbiome.

In the last few years metagenomics has emerged as one of the most powerful sequence-driven approaches (Next Generation Sequencing, NGS) to study the phylogenetic and functional changes of the microbiome associated to a shift from a mutualistic profile toward a dysbiosis configuration, impacting on several metabolic and physiological activities of the host.

Different studies, based on the NGS characterization of the gut microbiome profile, demonstrated that BMOs support intestinal microbial signatures associated with various health-enhancing functional roles, similar to those associated with HMOs, including prebiotic (mainly bifidogenic) activity, host protection from pathogens and infection, decreased gut permeability and reduced inflammation.

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Workshop 4

Giovedì 21 giugno 2018

Una per tutti, tutti per una: l'approccio *One Health* nella sorveglianza delle malattie trasmesse da vettori

Moderatori:
Prof.ssa Gabriella Elia *(Università degli Studi di Bari)*
Dr. Michele Dottori *(IZS Lombardia-Emilia Romagna)*

14.00	**Saluto delle Autorità e introduzione** ***Silvio Borrello*** *Direzione Generale della Sanità Animale e dei Farmaci veterinari, Ministero della Salute*
14.20	**Chikungunya and Zika: what's going on?** ***Alfonso J. Rodriguez-Morales*** *Universidad Tecnológica de Pereira*
14.50	**Sorveglianza e controllo delle zanzare invasive in Trentino** ***Roberto Rosà*** *Centro Ricerca e Innovazione FEM*
15.15	**Sorveglianza integrata di West Nile Virus in Italia** ***Federica Monaco*** *CESME_IZS*
15.40	**Approccio One Health nelle Regioni del Bacino Padano: l'esperienza dell'IZSPLVA** ***Cristina Casalone*** *IZS Piemonte Liguria e Valle d'Aosta*
16.05	**Il ruolo della sorveglianza entomologica nella prevenzione delle malattie trasmesse da vettore** ***Gioia Capelli*** *IZS delle Venezie*

SURVEILLANCE, MONITORING AND MATHEMATICAL MODELS TO ESTIMATE THE TRANSMISSION RISK OF INVASIVE MOSQUITOES-BORNE INFECTIONS IN TRENTINO (NORTHERN ITALY)

Roberto Rosà (1), Annapaola Rizzoli (1)
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The rapid invasion and the spread of some alien invasive mosquito species and in particular of the tiger mosquito (Aedes albopictus) in our territories, combined with the effects linked to climate change, created the suitable conditions for the emergence of tropical arbovirosis in the new areas of invasion of the vector.

The Autonomous Province of Trento (northern Italy) has recently funded a research project (LExEM - www.lexem.eu), coordinated by the Fondazione Edmund Mach of San Michele all'Adige (TN), in order to define the best strategies for the mitigation and control of invasive alien mosquitoes of medical-veterinary interest recently introduced to national and provincial territories.

This work summarizes the main results obtained within the LExEM project concerning the monitoring and surveillance programs for tiger mosquito (Ae. albopictus) in relation to the territorial, urban and climatic conditions of Trento province.

In particular, the results obtained from predictive mathematical models are presented. These quantitative models, which are fed by mosquito abundance data collected in Trentino during the project (2014-2016), permit to evaluate the seasonal dynamics of tiger mosquito density in Trentino, as well as the potential risk of transmission of tropical arboviruses transmitted by this vector such as Chikungunya, Dengue and Zika.

ONE-HEALTH SURVEILLANCE OF WEST NILE VIRUS IN ITALY

Federica Monaco
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise G. Caporale, Centro di Referenza Nazionale per le malattie esotiche degli animali

In Italy, the first outbreak of WNV infection was reported in 1998 in Tuscany and since 2002, a national veterinary surveillance plan based on wild bird mortality, entomological and sentinel animal (horses and chickens) surveillance has been in place. The plan aims at monitoring areas at risk for WNV introduction and circulation [1]. The surveillance systems did not detect any local circulation of WNV until 2008, when the virus was identified in mosquitoes, birds, horses and humans in an area close to the Po river delta [2]. Since the re-introduction of the virus in 2008, an endemic [3] and intensified WNV circulation across the whole country was observed with a geographical spread of WNV west and southward. Moreover, from 2008 to 2011, WNV lineage 1 was responsible for both human and veterinary cases, but, since 2011, evidence of circulation of lineage 2 closely related to strains belonging to the Central European clades was extensively reported [4]. In 2011, strains of WNV belonging to lineage 1 spread for the first time in Sardinia region. In contrast to the previous WNV Italian incursions the virus was able to cause severe clinical signs in the affected birds [5]. Viruses detected from carcasses of wild birds (2008-2017) confirmed differences in susceptibility to WNV. Whatever the viral lineage detected, carcasses of birds belonging to the order of Passeriformes were more frequently infected (21%) followed by Columbiformes (20%), Strigiformes (15%), Accipitriformes (14%) and Charadriiformes (8%). Entomological surveillance demonstrated its capability to early detect the viral circulation in the infected areas [6] and identified Culex pipiens as the major WNV vector in the Italian scenario [7]. Since 2016, a unique integrated surveillance, targeting mosquitoes, birds, and humans is active in Italy and has been crucial to timely set up preventive measures such as the screening of blood donors and the vector control activities in affected areas thus reducing the risk of transmission of the WNV to humans. Most of the data collected during the 10 year period of surveillance have been essential to refine and implement the surveillance itself demonstrating the importance of interdisciplinary approach.

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THE ONE-HEALTH APPROACH IN THE REGIONS OF THE PO VALLEY: THE EXPERIENCE OF IZSPLVA

Cristina Casalone
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (IZSPLVA)

Mosquito-borne diseases (MBDs) are rapidly increasing in prevalence, geographic distribution and severity, threatening both human and animal health worldwide. Given their complex epidemiology, a multidisciplinary approach is needed to ensure early detection and control and prevent human infection. Most of MBDs are caused by flaviviruses, including West Nile virus (WNV) and Usutu virus (USUV), both vectored by mosquitoes of the genus Culex. Following an epidemics in 2008, WNV has become endemic to Northern Italy, progressively extending its range from the East to the West along the Po valley. In the Piedmont Region (NW Italy), a risk-based approach was applied to the surveillance of West Nile fever since 2011 and a regional surveillance network was established in 2014, under the "One-Health" perspective, integrating veterinary and human surveillance, in order to early detect viral circulation and quickly apply control measures to reduce the risk of transmission through blood and blood components. Such approach, including: i) passive surveillance through the detection of neurological signs in equines and increased mortality among wild birds; ii) active surveillance on adult mosquitoes (June-October) and sentinel horses, and virological screening in migratory and resident wild birds, mainly of the Corvidae family, was later extended to the Liguria and Valle d'Aosta Regions. In 2015, WNV detection in mosquitoes and wild birds or detection of WNV-IgM in horses were introduced as triggers for the implementation of NAT testing for human blood donor screening. In 2011-2017, 111,676 and 33,244 adult mosquitoes were trapped in Piedmont and Liguria, respectively; 4,620 and 1,255 mosquito pools were analyzed for flaviviruses. USUV was first reported in mosquitoes in Piedmont in 2011 and in Liguria in 2014 [1], where it continued to circulate. WNV Lineage 2 was first detected in both Regions in 2014 and again in Piedmont in 2015-17, but no more detected in Liguria [2]. Passive and active surveillance on horses allowed to confirm WNV circulation in Piedmont in 2014-17. Surveillance on birds still allowed WNV detection as well as WNV-USUV coinfections in Piedmont, while a single case of infection in a long-range migrating raptor was recorded in Liguria in 2013. In 2015-2017, four autochthonous human cases of West Nile Neuroinvasive Disease were detected in Piedmont [3]. Since 2014, a total of 99,882 blood bags were screened by NAT; no infected blood bags were detected. In conclusion, the creation of regional working groups composed by public and animal health authorities, together with the bodies in charge of vector surveillance and control, is crucial for an integrated and cost-effective surveillance, under the One-Health perspective. The approach adopted in NW Italy showed to be effective for the prompt implementation of control measures and for saving resources, reducing the risk of MBDs transmission to humans.

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THE ROLE OF THE ENTOMOLOGICAL SURVEILLANCE IN THE PREVENTION OF MOSQUITO-BORNE DISEASES

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The aim of this presentation is to highlight the role of the entomological surveillance in the prevention of mosquito-borne diseases (MBD), using mainly, but not only, the long term mosquito-West Nile virus (WNV) monitoring in north-eastern Italy as a paradigmatic example. Going through the objectives of the entomological surveillance and using other targeted surveys we show how the results obtained were used to better target surveillance, to drive methods of mosquito control and risk communication and to prevent human transmission by modulating the screening of blood donors. Since 2010 an entomological monitoring was set up in north-eastern Italy using CDC-CO2 traps and screening of mosquitoes by RT-PCR and sequencing for Flaviviruses. Other surveys included captures of mosquitoes every 2hrs for 24hrs, pre- and post-disinfestation captures, PCR blood meal analysis of fed Culex pipiens, retrospective analysis for other MBDs using stored mosquito DNA, and WNV complete genome. Over 1 million of mosquitoes were collected in 158 sites, with Cx.pipiens the most abundant (88%) and the only vector of WNV and USUV. Tahyna, Marisma and mosquito-only Flaviviruses were isolated for the first time. The retrospective analysis on zoonotic filariae indicated the presence and distribution of Dirofilaria immitis and D.repens. Cx.pipiens fed preferentially on birds, mainly blackbird, magpie, sparrow and collared dove. Cx.pipiens changed its host searching activity according to the season, showing a night/evening peak in early/late summer, respectively. The disinfestation in rural areas did not reduce Cx.pipiens density. The entomological monitoring proved to early detect the viral circulation, before human cases. In conclusion the following aspects were explored and better defined: i) the entomological monitoring defined the risk areas for WNV; ii) new viruses could also be detected; iii) the blood-meal analyses indicated possible bird targets for surveillance; iv) the determination of Cx.pipiens peak of activity defined the highest risk of human bite and WNV transmission; v) the control of the efficacy of disinfestations highlighted its poor efficacy in rural areas. The early detection of WNV in mosquitoes is now one of the triggers for the starting of WNV human blood screening, as regulated by the National Integrated Surveillance and Control Plan for WNV and USUV, in a perfect One Health perspective.

Workshop SOFIVET

Giovedì 21 giugno 2018
(Aula ERACLITO)

New insights in BBB

Moderatore:
Prof. Baratta Mario *(Università degli Studi di Torino)*
Prof.ssa Bacci Maria Laura *(Università degli Studi di Bologna)*

17.00	**Physiology and physiopathology of the blood-brain barrier: implications for microbial pathogenesis and neurodegenerative disorders** ***Dennis Grab*** *Uniformed Services University of the Health science, Maryland-USA*
17.45	**Development of BBB and biotechnological application in a swine neonatal model** ***Domenico Ventrella*** *Università degli Studi di Bologna*
18.30	**Discussione e chiusura dei lavori**

Workshop 5

Venerdì 22 giugno 2018

AGGIORNAMENTI IN MATERIA DI *FOOD BORNE PARASITES*

Parte I

Prospettiva normativa applicata al controllo dei *food-borne parasites*

Moderatore:
Prof. Enrico De Santis *(Università degli Studi di Sassari)*

8.30	**Parassiti dei pesci quali causa di malattia alimentare umana: evidenze scientifiche ed aspetti normativi** ***Antonio Panebianco*** *(Università degli Studi di Messina)* ***Aniello Anastasio*** *(Università di Studi di Napoli)*
8.50	**Analisi del rischio per il controllo dei parassiti di interesse alimentare nella filiera carne** ***Tiziana Civera*** *(Università degli Studi di Torino)* ***Anna Rita Loschi*** *(Università degli Studi di Camerino)*

Parte II

Aggiornamenti epidemiologici basati su tecnologie biomolecolari

Moderatore:
Prof. Fabrizio Bruschi *(Università di Pisa)*

9.10	**Studio di *outbreaks* di giardiosi e criptosporidiosi attraverso metodi di *subtyping*** ***Simone Cacciò*** *Istituto Superiore di Sanità*
9.30	**Tecniche di amplificazione isotermica per l'identificazione di parassiti trasmessi da alimenti** ***Marco Lalle*** *Istituto Superiore di Sanità*
9.50	**L'utilizzo dei microsatelliti nell'epidemiologia molecolare di *Trichinella*** ***Edoardo Pozio*** *Istituto Superiore di Sanità*
10.10	**Discussione e chiusura dei lavori**

FISHERY PRODUCT-BORNE PARASITIC DISEASES: SCIENTIFIC EVIDENCE AND EU RULES

Antonio Panebianco (1), Aniello Anastasio (2)

(1) Department of Veterinary Science, University of Messina; (2) Department of Veterinary Medicine and Animal Production, University of Naples "Federico II"

Fishery product-borne parasitic diseases is an old problem that increase in relation to the new knowledge. Risk analysis, new instrument for food hazard reduction, is connected to the difficulty to identify "dose-reponse" evaluation, necessary for risk evaluation. This is related to the large diffusion (sea and freshwater fish) of zoonotic parasites responsible also of indirectly damage in consumers. EFSA, in its opinion of 2010 [1] deriving from a risk analysis approach, among parasites of public health importance in fishery products refer to cestodes, trematodes, nematodes and microsporidia also. Among these parasites, Anisakidae nematodes represent the most important problem and their complete risk evaluation is very difficult;it is not easy define a "dose-response" relationship as that human anisakidosis is related to ingestion of viable parasites with their penetration in human tissue but also to allergic (hypersensitivity) reaction against parasite antigens (dead or alive). While the first problem is related only to the ingestion of raw or undercooked fish containing third larval stages (L3) of Anisakis, the allergic reactions can appear also after the consumption of dead larvae following freezing, cooking and other biocide treatments. This aspect is complicated also from the probable cross sensibilization with other fish parasites (i.e. Ghymonorychus gigas) and parasites of different animal species (Ascaris, Toxocara, etc.). Furthemore an element not still well known is the probable relation between human anisakidosis and neoplasms, of wich several cases are reported. In regards, it was showed that exposures to Anisakis extracts, obtained from live and devitalized larvae, could induce inflammation on in vitro cultured human colonic cells with possible apoptosis inhibition [2]. EU rules appears to be suitable for the prevention of transmission of Anisakis larvae and of all the other parasites reported in EFSA opinion, but are not able to prevent allergic reaction. EU rules (i.e. Hygiene package) follows different approaches; Reg. EC 2074/05 states that fish business operators must ensure the application of an accurate visual inspection on fishery products avoiding that obviously infested fish were released for human consumption. Reg. EC 853/04 states that fishery products, derived from finfish or cephalopod molluscs, to be consumed raw or almost raw and fishery products marinated and/or salted require freezing (-20° for 24 h or -35° for 15h) if the ripening process is insufficient to kill nematode larvae. This treatment affect organoleptic quality of marinated or salted product. For this reason several alternative methods were studied in order to obtain an equivalent effect such as use of natural extracts like allyl isothiocyanate [3] or R(+) limonene [4] on marinated anchovies and salting process on ripened anchovies [5] and baccalà [6]. Moreover because no sea fishing grounds can be considered anisakids free [1], surveillance studies are of great interest to determine the parasite identification and the risk exposure, but at now is no possible to identify hot-spot geographic areas without no parasite. Therefore it is desirable that derogations from the treatments referred to Reg 853/04 are not allowed without the necessary certainty. Furthemore , it is

important to remember that fish parasites with muscle localization could, occasionally, induce uncontrolled bacterial contamination, as bacteria can be present in the body and on the cuticle parasites. This is a real risk in particular for predatory fish species whose infesting with L3 larvae of Anisakis often occurs by "re-training". Finally, it should be remembered that insidious sanitary hygienic compromises may derive from changes in the muscle-fish substrate induced by microparasites, in particular mixosporidia,that could interfere with chemical fish freshness indicators (TMA-N and ABVT).

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RISK ANALYSIS FOR THE CONTROL OF PARASITES OF FOOD INTEREST IN MEAT SUPPLY CHAIN

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According to the scientific opinion of the EFSA, meat inspection must be supported by risk analysis. To this end, it is essential to identify Harmonised Epidemiological Indicators (HEI) for each animal species. HEI are defined as the prevalence or incidence of the hazard at a certain stage of the food chain or an indirect measure of the hazards (such as audits of farms) that correlates to a human health risk caused by the hazard. The principles applied for the identification of appropriate indicators are the following: for each biological hazard the prevalence is considered according to the risk factor considered in each phase of the production chain, evaluating the critical points for the origin of the danger and possible reservoirs; to be considered a "key" epidemiological indicator, its prevalence in the animal population or in food must be confirmed; the identification of a list of risk factors is not sufficient in itself. An estimate of the impact that such risk factors may have on public health is required, so as to consider any need to make changes in current methods of inspection. This is more easily accomplished by estimating the prevalence of the agent considered within populations subject to different levels of exposure. For each defined epidemiological indicator, the minimum monitoring and control requirements, the methods and the sampling strategy at a given stage of the food chain must be outlined, as well as the type of sample and the diagnostic methods to be used. For foodborne parasitic hazards, it is important to develop a global list of importance, identify the most at-risk foods and provide an overview of risk management options and approaches. This entails having information on the impact of foodborne parasitic diseases on public health and socio-economic context, defining precise monitoring and inspection systems, control and management, and carrying out a correct assessment and definition of the risk classes of each agent. For example, among the HEIs identified for *Toxoplasma gondii* in pigs, sheeps and goats, are scheduled auditing and serological tests, respectively, in farm and slaughterhouse. For *Trichinella* spp. in pigs it must be differentiated if they come from farms under controlled housing or not. In the first case, farm audit is carried out, with the aim to confirm the controlled housing standards without proceeding with the systematic search for the parasite at the slaughterhouse. In the second one and in the wild boar, as usual, we proceed to the direct search of the parasite to the slaughterhouse. In farmed game (deer and wild boar) HEI for *Toxoplasma gondii* are identified only at the slaughterhouse with serological investigations. Risk factors and potential epidemiological indicators for cysticercosis (*T. saginata*) in cattle, at farm and slaughterhouse, were also analyzed. Suggested HEI for farm include audit and prevalence of animals positive for cysticercosis at slaughter (serology and/or lesions) as well as audit of the inspection procedure. Further risk factors as eating raw or undercooked meat was not indexed. The Piedmont Region has a long tradition of consumption of raw meat, an element likely favoring the maintenance of the parasitic cycle. The positive animals at slaughter on the total of the slaughtered animals (apparent prevalence) were 0.13% in the period 1999-2004, falling to 0.058 in the period

2005-2010 and to 0.04 in 2014-2017, but with significant increases (2014: 0.10%) caused by single large cases, (over 100 animals/year/farm). Sporadic cases, however, prevail: farms with a single case between 2012 and 2017 account for the 70% of all the farms with at least one case in the same period. Although cases are notified by the slaughterhouse, no audits are required on farms, and the available evaluations are provided by research projects, carried out in collaboration with the University, applying a case-control approach with audits at the farm. In a study conducted in 2012, the bivariate analysis showed significant factors such as the type of feeding and the supply of fresh fodder; type of production (milk>meat); housing; proximity of the farm to water streams within a radius of 1 km, proximity of the farm to “nomads settlements” within a radius of 1 km; position and type of dunghills, factors mostly already present in the literature. The suggested serological tests as epidemiological indicators are not commercially available. In another study, we joined the traditional inspection on suspected lesions to a biomolecular assay targeting the mitochondrial cytochrome c oxidase subunit I gene (COI). The proposed test, amplifying a short DNA fragment, confirmed the diagnosis at *postmortem* inspection in 95% (162/171) of cases, both in viable and degenerated *T. saginata* cysticerci, yielding an unequivocal diagnosis. Human taeniosis is not notifiable, implying the absence of systematic data collection and reporting,also in areas where a frequent consumption of raw beef represent a significant risk factor. It should be acknowledged that the economic impact of *T. saginata* at farm level is significant and even more so, that, despite the low public health impact, acquiring *T. saginata* should not be acceptable from a food safety perspective. With this in mind, a greater integration of the activities of the public veterinary services along the supply chain is necessary, as already proposed by EFSA in 2013.

In general, the recommendations applicable to all species with regard to biological hazards are to introduce an integrated system to guarantee meat safety that includes clear objectives in relation to the main hazards. To support the achievement of these objectives, it may be necessary to acquire new data on biological hazards, and make use of available control options relating to the main hazards, both at rearing and slaughter level.

USEFULNESS OF MICROSATELLITES FOR MOLECULAR EPIDEMIOLOGY OF *TRICHINELLA*

Edoardo Pozio
Laboratorio di Referenza per i Parassiti dell'Unione Europea, Istituto Superiore di Sanità, Roma

Until the 70s, nematodes of the genus *Trichinella* were considered monospecific, i.e. all of them were identified as belonging to *Trichinella spiralis*. The increase of biological and epidemiological knowledge on these pathogens and the use of biochemical and molecular techniques, resulted in the identification of 12 taxa. These nematodes are primarily parasites of wildlife and only some taxa can be transmitted by a domestic cycle. The molecular identification of *Trichinella* larvae at the species level supports epidemiological investigations in the course of animal and human outbreaks, suggests the possible natural cycle of the parasite, the infection source, the risk for domestic animals and the severity of the clinical pattern in humans. However, it is difficult to uniquely identify the source of infection 'from fork to farm'. Microsatellites present in the *Trichinella* genoma were investigated, to trace 'from fork to farm' the source of infection.
Microsatellites are short tandem repeats (simple sequence repeats) of DNA consisting of very short repeating units (about 2-6 bp) useful as molecular markers. Microsatellites show a high level of polymorphism and, consequently, are highly informative. The simultaneous reading of multiple microsatellites, i.e. the multilocus genotype (MLG) analysis, allows the creation of a DNA profile (genetic fingerprint) by which identify a specific strain of the pathogen. *T. spiralis* and *T. britovi* genomes were screened and 12 and 6 microsatellites were identified in *T. spiralis* and in *T. britovi*, respectively. The study of microsatellites of *T. spiralis* isolates collected globally shows that the non-Asian isolates from Europe, North and South America, are rather similar to one another, suggesting a common recent origin, whereas each Asian isolate appears comparatively distinct.
Investigation on microsatellites of *T. britovi* isolates from two Mediterranean islands (Corsica and Sardinia) and from three continental areas (Italy, France and Spain) showed different genetic structures between the two island populations. Furthermore, two geographically separate genetic groups were identified among Corsican isolates. Lastly, pork delicatessen marketed in Nice (France), where they had caused an human outbreak of trichinellosis, was linked to a breederbutcher in Corsica. The low level of genetic admixture of the insular *T. britovi* isolates suggests that this pathogen colonized the two islands by separate events. The MLG analysis is a suitable method in supporting epidemiological investigations to trace "from fork to farm" insular populations of *T. britovi*.

Workshop RNIV-SOFIVET

Venerdì 22 giugno 2018

Biodiversità e resistenza alla mastite nelle bovine da latte

Moderatori:
Prof. Nicola Lacetera *(Università della Tuscia)*
Prof. Daniele Vigo *(Università degli Studi di Milano)*

14.00	**Introduzione dei moderatori**
14.10	**Biodiversità delle razze bovine italiane e resistenza alle malattie** ***Giulio Curone*** *(Università degli Studi di Milano)* ***Daniele Vigo*** *(Università degli Studi di Milano)*
14.25	**I fondamenti della risposta immunitaria agli agenti di mastite** ***Massimo Amadori*** *IZS Lombardia-Emilia Romagna*
14.40	**Risposta immunitaria innata nella ghiandola mammaria e biodiversità** ***Joel Filipe*** *Università degli Studi di Milano*
15.00	**Microbioma del latte e biodiversità** ***Paola Cremonesi*** *CNR, Lodi*
15.20	**Stress metabolico, risposta infiammatoria e biodiversità delle bovine da latte** ***Erminio Trevisi*** *Università Cattolica del Sacro Cuore*
15.40	**Discussione e chiusura dei lavori**

BIODIVERSITY OF MILK ITALIAN BOVINE BREEDS AND DISEASE RESISTANCE

Giulio Curone and Daniele Vigo
Università di Milano, Dipartimento di Medicina Veterinaria

The anamnesis in the field of clinical and practical procedures has been submitted in the last years a great re-evaluation, including these aspects in the group of the diagnostic tools needed to obtain quality results, reducing laboratory expenditures and waiting times. The term “history”, either individual or collective, physiological or pathological, is no longer used, although this mus be in part known. In 1988, Luigi Antonio Chierico, a breeder in Pavia province, argued that local breeds, especially the “Varzese-Ottonese-Tortonese (VOT)”, were on the way of extinction; he recovered some VOT heads, followed by other Northern Italy breeds, along with Holstein and Brown Swiss of his farm. Mr. Chierico foreseen several years before the indications included in the “Carta di Milano, and the Encylical “Laudato Si’” by Pope Francesco (2015), understanding and spreading the idea that the decline of biodiversity in local bovine breeds was exclusively linked to the yield of a few milk liter more by Holstein and Brown (cosmopolite breeds), subject for over 60 years to intense selection criteria towards these aims. This selection led to a decline of several aspects, as the resistance/resilience to pathologies and reproductive performances, maintained until nowadays in autochtonous breeds. The possibility to conduct researches comparing autochtonous and cosmopolite breeds in the same environment, with same feeding allowed to develop new unexplored fields (Felipe et 2017) The continuous analysis and evaluation of several populations of dairy cows led to express a new field in Veterinary Medicine: the Clinical and Applied Veterinary Medicine, which does not contemplate an operative standard between physiology, pathology and clinical condition with sharp distinctions. Each subject and each breed express completely different characteristics inherent to adaption to environment, growth and development, production and storing of energy, omeostasis/allostasis-resistance-resilience-recovery, reproduction and lactation (Curone et al 2016; Curone et al 2017). This plethora of information is paramount in terms of diagnosis efficiency and quality, but also prevention, therapy and prognosis, as well as management, feeding, nutrition, and genetics, remembering that all these activities are tightly linked and regulated by physiological, endocrine and immunitary mechanisms (Addis et al 2017; Curone et al 2018)

Phylogenetically and by secular selection, the cow should deliver one calf per year: the functional consequence is a short-term lactation, below 300 days. A loner lactation period deriving from a late pregnancy (over 100 days in milk) is considered normal: nowadays the Clinical and Applied Veterinary Physiology tells us that a problem less detectable by an ordinary operator is the depletion of mammary stem cells for tissue regeneration and defense systems.

The comparative evaluation of metabolism between local and cosmopolite breeds, especially the energy balance, put in evidence that milk ketone bodies are deeply

different taking into account age, days in milk ,and number of lactations breed in the same farm.

Two cow breeds, a cosmopolite (Holstein), a local (Bianca Val Padana or Modenese), and the F1 (Modenese x Holstein) and F2 Modenese x F1) have been submitted to the determination of the three ketone bodies in milk at 20, 40, and 90 days of lactation. Each specimen was analyzed by GC, evidencing that the concentration of ketone bodies in milk is significantly higher in Holstein milk with respect to Modenese ($p<0.001$). In Modenese the calving to conception period was physiological (about 90 days); F1 and F2 cows showed a longer calving to conception period (100 and 115 days, respectively) (Curone et al 2016)

Another aspect in dairy cows biodiversity in several local breeds involves also the fatty acid profile in milk. Breeds as Cabannina, Valdostana and VOT show higher MUFA percentages with respect to Holstein ($p<0.001$); PUFAs evidence higher values in Cabannina and Valdostana, with respect to VOT and Holstein ($p<0.001$). Similar differences have been detected for alpha-linolenic acid (C18:3n3) (Faustini et al 2016). The importance of fatty acids in milk is to be highly considered for nutritional aspects in newborns, but also for protective activities towards udder. These activities include the formation of a biofilm on the tubulo-alveolar structures and the Furstenberg's rosette. Biofilms protects against microorganisms, oxidation, substances absorption, dehydration. Some unsaturated fatty acids are endowed of anti-inflammatory properties, opposed to some saturated acids, with pro-inflammatory activities.The structural and molecular activity of intracanalicular biofilms is enhanced by the presence of lysozyme produced during the first 10 days of lactation (Curone et al. 2018).

Starting from fatty acids concentrations calculated on the considered breeds, is possible to derivate the desaturase and the atherogenic indices for each cow group. The milk from Cabannina, Valdostana and Varzese cows have higher desaturase indices ($p<0.001$) and lower atherogenic indices ($p<0.001$) than Holstein (Faustini et al 2016).

Metabolomic analyses conducted by Tomassini et al. (2018) by NRM put in evidence that between 100-200 and 200-300 days in milk, the attitude between Holsteins and local breed cows are sharply different; the presence of small molecules populations that can act as molecules promoting resistance/resilience towards udder pathologies. These molecules could also modulate defense activities in the newborn, although at 200-300 days a calf is almost completely weaned.

Introductory studies related to resistance and resilience in milk cows is facilitated by the possibility to conduct comparative analyses between local and spread breeds; the knowledge of the resistance and resilience dynamics between breeds is an anamnestic tool of paramount relevance, aiming to perform a correct diagnostic process, as requested by the European Community in order to correct the procedures of antibiotic therapy and metaphylaxis, no longer admitted by supranational agencies.

The Clinical and Applied Veterinary Medicine finds a full position in complex researches about resistance and resilience in bovine specie, particularly in dairy breeds. From

functional comparative studies between local and cosmopolite breeds on cows' personalities, it must be underlined that every breed has a precise personality expressed on breeders or conspecifics. Thus, behavioral signs must be carefully detected and interpreted from the physiological point of view before acting on the clinical side of the problem paying attention to the breed differences, and the collection of anamnestical data.

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THE IMMUNE RESPONSE TO THE ETIOLOGICAL AGENTS OF BOVINE MASTITIS

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Bovine mastitis still causes huge losses in dairy farms and represents one of the main causes of early cull for dairy cows. There is in fact a correlation between prevalence of mastitis and high milk yield (Ingvartsen, 2003), despite the great advances in farm hygiene and management achieved over many years. The correlation between high milk yield and mastitis is accounted for by metabolic stress, which is sensed by the innate immune system. In a continuous trade-off, the host offsets the elimination of microbial stressors against the need to avoid major tissue damages and waste of metabolic energy. Therefore, two major options, not one, are available to the immune system:

- To develop a protective immune response aimed at the elimination of the microbial pathogen.
- To develop a "protective tolerance" aimed at avoiding tissue damage and waste of feed energy.

In this scenario, the control of intramammary infections takes place at 3 distinct levels:

1. The exposure to an array of antimicrobial, humoral and cellular factors in the milk (Florisa et al., 2003).
2. The direct contact with mammary gland epithelial cells, able to further secrete antimicrobial peptides (Addis et al., 2013).
3. The influx of granulocytes following major chemotactic stimuli for an overt inflammatory response, often underlying clinical mastitis.

The above scheme outlines a primary role of epithelial cells and their innate immune mechanisms in the control of bacterial infections in the mammary gland. Granulocytes go into action in case of failure of epithelial cells or in the presence of strong inflammatory signals conveyed by bacterial agents like *E. coli*.

In this respect, *S. aureus* and *E.coli* represent as many models of "too weak" and "too strong" innate immune responses (Petzl et al., 2018), underlying chronic and acute types of infection, respectively.

The role of adaptive immunity in the mammary gland is dubious. On the one hand, the available concentrations of complement fraction C'1q do not probably allow for antibody-mediated lysis of bacterial cells (Rainard, 2003). Instead, antibody to bacterial toxins and/or bacterial secretion products (biofilms) should work. Yet, they should be validated for activity in milk and shown to reach adequate protective titers in the mammary gland.

Tolerance to bacterial endotoxin (lipopolysaccharides, LPS) is likely to play a fundamental role with important clinical repercussions. This is the case of bovine mastitis sustained by *E. coli*, which can be effectively checked following inoculation of minute doses of bacterial LPS in the mammary gland (Petzl et al., 2012). This is in line with memory, epigenetic mechanisms in the innate immune response.

Biodiversity of cattle breeds can teach us a lot about mastitis resistance. Crucial priming events of the innate immune system are observed in autochtonous cows in the colostral period, as opposed to Holstein Friesian cattle (Curone et al., 2018). This can be nicely

correlated with different levels of metabolic stress after calving. Interestingly, crucial effector functions of innate immunity in Holstein Friesian cattle could be conveniently boosted in the colostral period by local injections of low-dose cytokines like interleukin-2 (Zecconi et al., 2009).

In conclusion, innate immunity plays a major role in udder defense. This should be re-appraised in disease control programs, and proper correlates of protection should be defined accordingly.

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INNATE IMMUNE RESPONSE IN THE MAMMARY GLAND AND BIODIVERSITY

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The genetic ability to increase milk production seen during the last years has been associated to a huge number of difficulties of cows to adapt to the environment. This translates into increased use of veterinary drugs, a higher risk of metabolic and infectious diseases, and a reduction of life expectancy as well as reduced fertility. It is well known that specially during the calving period, high-yielding dairy cattle are more susceptible to common environmental stressors, affecting disease occurrence and milk production levels. However, less is known about the biological mechanisms behind these relationships.

In a preliminary field work with 10 animals (6 Holstein and 4 Rendena cows) reared in the same farm and under the same management conditions, some innate immune response patterns have been studied in milk samples (inflammation marker cathelicidin and innate immune-related mediators such as lysozyme, CD45, IL-1β, TNF-α, PTX3, IL-1R8).

The expression of innate immune related genes such as PTX-3, IL-1β, TNF-α, as well as the CD45/KRT5 expression ratio in milk cells, indicating the epithelial and leukocyte components, was lower in Holstein Friesian compared with Rendena in the colostrum. Similar results were obtained with the lysozyme concentration in the colostrum, with a higher concentration at this time point for the rendena cows when compared with Holstein. Rendena cows have also demonstrated the ability to maintain lower levels of the mastitis markers cathelicidin and somatic cell count, and therefore of mammary tissue inflammation; this ability is of significant interest especially in the post-partum period and it appears to be combined with the capability of Rendena cows to release in colostrum a higher amount of other immune-related proteins, such as lysozyme and PTX3, that efficiently protects the mammary gland against pathogen infections. Interestingly, the major differences found between the two breeds occur in colostrum, leading to the belief that there is a critical role of the innate immune response in the colostral period. The observations reported in this work present some hints on the factors that may provide autochthonous, more rustic breeds with a higher resistance to mastitis.

MILK MICROBIOME AND BIODIVERSITY

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The evolution of “omics” technologies made us aware of the varied and complex assortments of microbes that inhabit living animals and of the reciprocal interactions they entertain among themselves and with their hosts [1]. Among these technologies, metagenomics enables the characterization of a microbial population in a culture-independent manner [2], providing a powerful means for identifying dominant and subdominant microbes and their dynamics in highly complex ecosystems. Animals host on their skin, gut, rumen, oro-pharyngeal, urinary and genital tracts a variety of microbial communities that have evolved with them because of mutualistic interactions, playing crucial roles in their biology and health [3]. Recently, the mammary gland has also been included among the sites colonized by microorganisms [1]. The milk microbiota composition depends on both the composition of microbial ecosystems in direct contact with the milk and on various environmental microbial sources not directly in contact with the milk. Other studies also support the hypothesis that the presence of bacteria in milk is not due only to an external colonization, because bacterial isolates present in the mammary gland are genotipically different from those found on skin within the same host and the same bacterial species [4]. Most studies on milk microbiota focused on its changes during food production, on milk microbiota changes during mastitis or following antimicrobial treatment, and on the effects on milk microbiota of different therapy conditions during the dry period [5-6], that represents the most critical phases for udder health, especially for highly productive breeds. The milk microbiota in 6 Holstein Friesian (HF) and 3 Rendena (REN) cows, indigenous Italian dual-purpose alpine breed, reared in the same farm and under the same management conditions, with a special focus on the transition period to define bacterial groups prevalence with a plausible effect on mammary gland health, was compared. Four time points (dry-off, 1 d, 7-10 d and 30 d after calving) were considered, characterizing the microbiome for 117 milk samples with a somatic cell count lower than 200,000 cell/ml, to focus on physiological microbiome changes avoiding shifts due to suspected diseases. Microbial populations were deeply different in the two breeds along all the time points, with REN milk showing a significantly lower microbial biodiversity. The taxonomic profiles of both cosmopolitan and local breeds were dominated by Firmicutes, mostly represented by the Streptococcus genus, although in very different proportions (HF 27.5%, REN 68.6%). Profound differences in HF and REN cows were, also, evident from the metabolic predictive analysis from microbiome data. Lastly, only HF milk displayed significant changes in the microbial composition along the transition period, while REN maintained a more stable microbiota. In conclusion, in addition to the influence on the final characteristics of dairy products obtained from milk of the two breeds, differences in the milk microbiome might, also, have an impact on their mammary gland health.

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BIODIVERSITY OF DAIRY COWS: CONSEQUENCES ON METABOLIC STRESS AND INFLAMMATORY RESPONSE.

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The pressure to increase milk production forced dairy farms to adopt higly selected breeds to replace local ones. Even though less productive, local breeds were highly adapted to overcome challenges of diseases and harsh climates. On the other hands, intense selection for milk traits leaded cosmopolitan breeds to develop peculiar physiological adaptations, which have increased the incidence of infectious and metabolic diseases and reduced the fertility. Holstein friesian (HF) is the most widespread and highly selected modern breed. HF often shows relevant physiological dysfunctions (i.e. reduction of the immune competence, severe negative energy balance, inflammatory like status, oxidative stress and hypocalcemia [1]) that are associated with the high milk production. Most of these dysfunctions occur during the transition period, that is known as the most stressful phase of the cow carreer. The role of the immune system seems crucial, even in healthy subjects. Relevant varations on immune functions have been reported in late gestation (i.e. raise of proinflammatory cytokines or PIC, reduction of diapedesis and phagocytosis), with a high variability within the population. Rarely those conditions lead to the development of clinical diseases before calving but, interestingly, immune dysfunctions in the dry period are associated with poor health and metabolic status (e.g. reduction of appetite, increased ketogenesis, severe inflammation) in the early lactation and with a low milk yield. In this context, the comparison of breeds with different selective pressure (e.g. contemporary vs unselected HF; HF vs local breeds or other selected breeds) can improve the comprehension of adaptative mechanisms in cattle. Literature is limited and with some limitations (e.g. comparisons occur in different environments, diet, management; mainly in extensive conditions; rarely in stressfull conditions as the transition period), but highlights important differences. In comparison to high selected HF, unselected HF show lower plasma NEFA, for the lower milk production and better energy balance in early lactation; lower ketogenesis (lower plasma BOHB) and less prolonged acute phase reaction after calving. Under stressful conditions (i.e. endotoxin treatment), selected HF showed a less robust response, with a lower production of PIC and chemoattractans. Macrophages when challenged with pathogens showed a longer activity and a higher production of Reactive Oxygen Substrates in Brown Suisse in comparison to HF. Despite the fragmented nature of the available data, studies on cattle biodiversity are a promising tool to clarify the biology of lactation and the adaptive strategies in critical phases. Differences in innate immune responses among breeds suggest the utility of the inflammation and oxidative stress parameters as biomarkers to elucidate the tolerance mechanisms involved in the adaptation of the animals.

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Main Lecture

TOMORROW'S VETERINARY MEDICINE: BETWEEN SCIENCE AND PROFESSION

Arianna Russo

Training, communication and discussion represent the priorities of all the actors involved with veterinary medicine, nationally and at an European level.
During the last few decades, veterinary science and therefore veterinary medicine, have experienced an exponential advance in the scientific knowledge, which lead to the need for dynamic, more differentiated, as well as interchangeable, forms of training. Such a need aims at promoting post-graduate education, an aspect that is at present fulfilled mostly by veterinary cultural societies. This particular aspect is also associated with a high pressure, in term of number of available jobs in some sectors, and a decrease in university funding with consequent decrease in the amount and quality of research with respect to international trends.
There is a need for a reduction of the total number of veterinary medicine programs, now redundant and widespread on the territory (by centralizing the universities with more funds and adequate infrastructures), and freshmen (saturated job market) and for an expansion of the horizons of veterinary medicine, profession and research.
Nowadays the gap between the expectations of new graduates, the job market needs and the level of academic education/training has increased. For this reason the adaptation of university courses with the most recent scientific evidences and the integration of such courses with fundamental skills (not only from a practical point of view but also as regards communication, business and management, scientific method, ethic and animal welfare), in the view of the modern veterinary medicine, do represent a priority. It's also fundamental to create synergies between different professional and research figures, avoiding competition and promoting the role of veterinary medicine and veterinary sciences within the society. In conclusion, there is an urgency for changing the veterinary medicine from a rather “closed” system to a key player in the future development of biosciences. To achieve such goal, the veterinary sciences should captivate new research figures, bringing in new ideas and interdisciplinary expertises capable of leading the veterinary medicine in a more global vision than that considered in the past and present times.

Main Lecture

HERD HEALTH MANAGEMENT AND SELECTED PREVENTIVE MEDICINE PROGRAMS IN SMALL RUMINANTS IN SWITZERLAND

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The official sanitary status of countries is of great significance for international trade. A country may either lose or enhance its commercial attractiveness in the eyes of potential or existing importing partners, depending on the official recognition of its sanitary status. By acquiring and maintaining its status, a country demonstrates transparency and helps to promote animal and public health worldwide, thereby gaining the trust of its partners and the international community.

In order to maintain animal health at a high standard, disease awareness and biosecurity measures are important. Therefore, breeders and veterinarians bear a great responsibility in the prevention of the spread of diseases among animals, from animals to humans, and from humans to animals. Over the past ten years biosecurity has become a more commonly used word. Biosecurity increases awareness and focus of attention to deal with diseases for which, for example, there are no vaccines or only vaccines with serious limitations. Biosecurity involves efforts designed to prevent introduction and spread of disease within populations, herds, or groups of animals.[1] Biosecurity programs are a critical element in the control of infectious diseases. Such programs should be prioritized to address factors that pose the greatest risk of disease introduction.

Producers, farmers or pet owners should be educated about key biosecurity practices for their animals. Animals need to stay at home on the farm until they have recovered if contagious diseases like ecthyma, caseous lymphadenitis, keratoconjunctivitis, ringworm, footrot, or mites are diagnosed. It is also important to always isolate newly-purchased animals for at least 2 to 4 weeks. These animals should have no contact with the resident farm herd during isolation. One way to accomplish this is to prevent introduction onto the farm by keeping a closed herd or flock, and to purchase animals only from known sources. If the animal has a disease without, or with less clinical, manifestations such as toxoplasmosis, chlamydiosis and coxiellosis, all of which could cause abortion, it is recommended to restrict its access to the farm and always wear different shoes and clothes. Regular animal traffic and displacement of animals to other farms is often practiced; this increases the risk of delaying the disease to animals on other farms significantly. Switzerland does not have many livestock compared to other countries. In 2017, cattle populations were the largest contingent (1’528’234; -0.1% compared to 2016). The decline in dairy cows (562’172; -0.5%) is largely offset by the increase in "other cows" (124’683; +2.3%). The sheep stock remained stable for the first time after several years of decline (343’618). The increasing popularity of goat and sheep's milk products lead to an increase in goats (75’720; +3.4%) and dairy sheep (14’567; +4.9%). South American camelids as exotics and relatives of the native ruminants also increased in numbers (6824; +4.5%).[2] In any case, animal traffic in Switzerland is intense, , so the

Swiss animal diseases ordinance introduced on 25 April 2018, stronger regulations. Specifically all births, arrivals and departures, imports and exports as well as the death of sheep and goats now need to be reported to the animal traffic database, as is the case with cattle. As a further innovation, sheep and goats will in future have to be labeled with two ear tags. Thus, the traceability is guaranteed at the outbreaks of infectious diseases.
In Switzerland, there is a counseling service for small ruminants, South American camelids and deers (Swiss Consulting and Health Service for small ruminants (SCSR); Servizio consultivo e sanitario per piccoli ruminanti http://bgk.caprovis.ch/cms02/showlinx.asp?id=1&lang=3). This institution helps farmers with questions about health management, disease awareness, feeding and animal welfare. Farmers or pet owners can become members and benefit from many services. The SCSR provides an important link between farmers, pet owners, veterinarians, agricultural advisory services, the Confederation and the Cantons, research and animal hospitals. The SCSR wants to disseminate easily understandable and accessible information, especially for livestock owners. This is in form of courses or leaflets, and offers various health programs, of which most are for free for members or are strongly subsidized. This includes support for any kind of herd health problem (e.g. paid autopsy and subsequent help on the farm in collaboration with the referring veterinarian) and advice on milk quality problems. Other control programs that can be claimed include

- *Dichelobacter nodosus* infections: these are analyzed by controlling the claws for disease symptoms. Necessary management and hygiene measures are discussed with the farmer and the claws are checked at intervals of around 4 weeks until no animals with foot-rot signs are detected. Animals are also PCR tested with swab samples. A national fight against foot rot is being planned and is expected to start in the next two years.
- *Maedi-Visna* controlling: at the beginning, all sheep older than 6 months, are annually serologically examined. If they are found to be still negative after 3 years, the farm receives the status "*Maedi-Visna*-free". If positive reactors are detected, they have to be slaughtered and in females the (off)-offsprings (2 generations). The SCSR prepares an annual certificate with the operating status per farm. This indicates the health of the herd and is therefore very important for the purchase and sale of *Maedi-Visna*-free sheep.
- *Parasites* cause massive losses in small ruminant farming and so parasitic problems are constantly a topic. Targeted deworming at the right time with suitable worming agents can improve animal health and prevent performance losses.
- The SCSR also offers help in the fight against *pseudotuberculosis* with different programs.

Through the support of the SCSR and other institutions such as the animal hospitals, an attempt is made to offer the owners of small ruminants the best possible support for the health of their animals.

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Main Lecture

SARCOCYSTOSIS AND BOVINE CATTLE CONTAMINATION IN FARM: AN ORIGINAL APPROACH

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Sarcocystiosis is a parasitic disease caused by protozoan coccidian parasites, whose cycle includes two hosts. In the intermediate host the parasite typically occurs in the muscle whereas the definitive host is affected by an intestinal coccidiosis. There are six species of *Sarcocystis* at least in bovine cattle (intermediate host), named *S. cruzi*, *S. hirsuta* and *S. bovifelis*, *S. hominis* and *S. heydorni*, and *S. rommelli* (or *S. bovini*). For the first five species, canids, felids, primates and humans are respectively definitive hosts. The last one is still unknown. Bovine eosinophilic myositis (BEM) is a striated muscle eosinophilic inflammation, leading to meat condemnation because of numerous little grey-green muscle lesions. It is responsible for important economic losses. Several experimental evidences have suggested that *Sarcocystis* species are responsible for BEM lesions, in particular thick walled sarcocysts, such as *S. hominis*.

Although studies have shown a very high prevalence of sarcocystiosis in the muscles of cattle, on the contrary, BEM prevalence is very low. Its occurrence is not yet understood, but seems to have a multifactorial origin.

This study has an innovative and exploratory nature and focuses on sources of cattle contamination in farms. The objectives are to identify and quantify the different species of oocysts of *Sarcocystis* in the environment of cattle, in relation with farming systems. A better knowledge of the risk factors of BEM lesions, via a more important exposure of cattle to the parasite, would allow to identify some preventive measures to reduce the economic impact of the disease.
A case-control study has been conducted on 60 farms in six different areas of France. The investigations were mainly targeted on *S. hominis*. Five groups with breed / area matched criteria were formed, each with the same number of case farms (farms with bovine condemnations for BEM at slaughter) and control farms (farm with no condemnation for BEM for the past ten years).

The first step consisted in a qualitative investigation based on a detailed questionnaire, to describe precisely the breeding management system in the selected farms. The objective was to assess existing practices, in particular those that can lead to faecal contamination in the farm environment.

The second line of action consisted in taking environmental samples (pasture grass and soil) to identify and quantify environmental *Sarcocystis* species in each farm on risk and control zones. Risk zones of presence of effluent discharge were determined on the basis of a risk analysis produced by the answers to the questionnaire. Drinking water was

sampled : a specific risk scale was applied in order to select the source most likely to contain *Sarcocystis* oocysts.

For each sample, a Multiplex PCR protocol targeting the 18s RNA gene was applied in order to determine and quantify the different *Sarcocystis* species.
A statistical analysis using multivariate models has been carried out to identify risk factors which significantly (at a 5% threshold) increase the probability for a farm to be a case.
The results have highlighted several significant risk factors, mostly breeding factors, in particular factors linked with grazing. The risk for the farm to be a case is higher when surface of pastures increases (which leads to the increase of the probability of presence of risk zones), and when flood lands exist on the farm (that could be linked with a more favourable habitat for the persistence of *Sarcocystis* in wetlands). The risk increases too when the mean number of calving per cow increases (which may signify that cows are older, and have probably been more exposed to the parasite during successive grazing periods), and when duration of grazing of cows decreases (which seems counterintuitive, but may be explained by a role played by the stall period, possibly via a more important stimulation of the immune system, which has an impact on the development of BEM lesions).
The species difference found in the environmental samples did not show to have effects to determine the status of a farm. *S. hominis* was rarely found (6 positive samples), while *S. hirsuta* and *S. bovifelis* were found only once. *S. cruzi* was the most frequent (58/60 farms had at least one positive sample). The presence of *Sarcocystis* in pasture grass (irrespective to the species), howerver, emerged as a significant risk factor.

Main Lecture

THE DIAGNOSTIC APPROACH AND TECHNIQUES FOR THE WHO CLASSIFICATION OF LYMPHOMA

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Hematopoietic neoplasia is a large and diverse group of clonal proliferative disorders of hematopoietic cells. Historically, numerous systems have been used to classify hematopoietic neoplasms in human medicine, some of which have been applied inconsistently to veterinary species. The World Health Organization (WHO) classification of hematopoietic neoplasia was first published in 2001 and is considered the first true worldwide consensus on the classification of hematopoietic malignancies and currently accepted as the method of choice in both human and veterinary medicine. The WHO classification integrates information on tumor topography, cell morphology, immunophenotype, genetic features, and clinical presentation and course. It broadly categorizes neoplasms primarily according to cell lineage: myeloid, lymphoid, and histiocytic.

The WHO classification of lymphoma postulates a normal cell counterpart for each type of lymphoma, which acknowledges that lymphoma can arise at any stage in the maturation of a lymphocyte. The features used in histopathologic classification of biopsy samples are tumor architecture (nodular or diffuse); cell size (small-intermediate-large) based on comparison with a red blood cell; and grade based on the number of mitotic figures in a single high-power field (indolent-low-mid-high). Although there are numerous subtypes of lymphoma recognized under the WHO system, a select number of subtypes are more commonly seen and clinically range from slow-growing indolent tumors to highly aggressive tumors.

There are numerous tests for the diagnosis of lymphoma, some of which can aid in prognostication. Fine needle aspiration and cytology is a quick and less invasive technique for diagnosis and clinical staging, and samples can be used for immunocytochemistry (ICC), flow cytometry, and PCR for antigen receptor rearrangement (PARR). Indolent, early-stage, and non-large cell lymphomas are more difficult to diagnose on cytopathology. Routine histopathology is required for proper assessment of architecture and to employ the WHO classification system. Formalin fixed paraffin embedded tissues (FFPE) can be used for immunohistochemistry (IHC) and PARR. Both ICC and IHC are important diagnostic tools for immunophenotyping; in IHC, a pan T-cell marker (e.g. CD3) and ideally two B-cell markers to cover the entire B lymphocyte maturation (e.g. CD79a or b, pax5, CD20) are utilized.

Flow cytometry (FC) is now routinely used in clinical oncology. FC is optimal for cell identification and enumeration, and fluorescent immunolabels are applied for the diagnosis, staging, immunophenotyping, and, in some subtypes, can provide prognostic information. PARR is a powerful test for clonality to distinguish between a reactive versus

neoplastic population of lymphocytes and can be performed on many samples types (blood, fluids, slides, FFPE tissues). Reported sensitivity and specificity in dogs is 70-80% and 96-100%, respectively (60-65%->90% in cats). False positives have been noted with E. canis infection in the dog; false negatives can occur with poor sampling, oligoclonal or cross-lineage neoplastic populations, or when primers fail to amplify every hypervariable region. As a genotyping test, PARR is not used to immunophenotype lymphoma; ICC, IHC, or FC should always be used in conjunction with PARR. New on the horizon is the use of genetic profiling techniques in lymphoma. Next Generation Sequencing studies may provide insight into mutations that drive lymphomagenesis and provide prognostic information and actionable (druggable) targets for individualized cancer therapy.

ORAL COMMUNICATIONS

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MITIGATING THE EFFECT OF SHEEP RUMEN KERATINIZATION DEGREE ON THE FARM INCOME LOSS

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Grazing is a main tool for biodiversity conservation, however, there is a risk of pasture abandonment due to the climate changes leading to an increasing summer aridity that negatively affect forage features; worsening in forage composition resulted in decline of sheep body status, mainly due to the rise of fiber content, which causes the increase of rumen keratinization degree [1]. During summer, the deterioration of the body condition of sheep is the main cause of the declining milk yield and quality; this can have serious impacts on farm household income, enhancing the risk of pasture abandonment with consequent environmental and territorial degradation. This work presents the food supplementation as an efficient way to contrast the rapid rumen keratinization in sheep fed on the pasture during the summer. A flock of 45 adult female sheep were conducted on pasture at the beginning of June 2016, where they were free to grazing until the moment of pasture maximum flowering. From this moment until the maximum pasture dryness the animals were divided in two groups: the control group (Cnt) fed only on the pasture, while the experimental group (Exp) was also supplemented with 600g/day/animal of corn and barley (1:1). Only animals destined to the slaughter house for human feeding were used. Samples of rumen ventral sac were removed from rumen visceral surface [1] of 5 subjects to evaluate the keratinization degree of rumen epithelial lining [2] at the beginning and at the end of the trial. Body Condition Score and milk production were monitored at the beginning at the middle and at the end of the period of differentiated diets. The food supplementation didn't affect the animal body state, but it slow down the growth of the rumen keratinization degree (Exp 32.86% vs Cnt 45.59%; P= 0.00123), allowing a better absorption of nourishing principles and then bringing to a significant increase of the milk production (P=0.0013 at the middle of the trial; P=0.03 at the end of the trial). The cost-benefit analysis (CBA) was applied to assess the economic impact of feed supplementation during the trial. Thus, the break-even point (BEP) expressed in liters of milk for 10 ewes was achieved both for the Cnt and Exp sample. Among the conventional measures of business performance, BEP defines the volume of sales at which revenues just equal its costs. The CBA, attempted also considering the newborn numerousness, indicated that food supplementation could mitigate the productive loss induced by summer aridity preserving the economic sustainability of sheep milk production, thus avoiding land abandonment.

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MODULATION OF ARYLHYDROCARBON RECEPTOR ACTIVITY DURING IN VITRO MATURATION OF BOVINE OOCYTES

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Exploring the molecular pathways involved in mammalian oocyte maturation process is critical to understand the mechanisms leading the oocyte to acquire full developmental potential. In this context, the Aryl hydrocarbon receptor (AhR), traditionally considered as an intermediate of the toxic response to dioxin through transcriptional activation of drug-metabolizing enzymes (e.g. CYP1A1), has proven to be also an important regulator of the ovarian physiology and homeostasis. We previously demonstrated that AhR signaling is activated during in vitro maturation (IVM) of mammalian cumulus-oocyte complexes (COCs) and that treatment with specific AhR antagonists negatively affected ability of oocytes to reach the metaphase II [1,2]. This suggests that the AhR may play a major role during oocyte resumption of meiosis. However, little is known on the molecular mechanisms underlying this phenomenon. Aim of this study was to unravel biological networks regulating AhR signaling during in vitro resumption of meiosis of bovine oocytes. Since a complex cascade of phosphorylation events is involved in molecular control of oocyte maturation [3], and several reports point to a critical role of phosphorylation in the regulation of AhR activity, we focused our study on the role of p42/44 Extracellular Regulated Kinase (ERKs). Bovine COCs were obtained from ovaries of slaughtered animals and classified as healthy. Treatments: 24h in control medium (bMM); 24h in bMM + 4mM 6-DMAP; 3- 15- and 24h in bMM + 10µM PD98059; 24h in bMM + 10 µM cycloheximide; 24h bMM + 10 µM AG1478 or AG825. Nuclear morphology was assessed by lacmoid staining. COCs were analyzed by RT-PCR and Western Blot. Treatment with both the broad-spectrum ser/thr kinase inhibitor 6-DMAP and the specific ERKs inhibitor PD98059 induced reversible oocyte meiotic arrest and downregulation of the AhR main target gene CYP1A1. Conversely, AhR activation was not affected upon exposure to cycloheximide, a protein synthesis inhibitor also able to induce meiotic arrest, confirming the role of ERKs in the regulation of AhR activity in bovine COCs. To investigate the upstream activators of ERKs within the AhR pathway during IVM, we analysed the effects of exposure to inhibitors of Epidermal Growth Factor Receptor isoforms (EGFR - erbBs), known to activate ERKs. In presence of AG1478, an erbB1/erbB4 inhibitor, the phosphorylation level of ERKs and the expression level of CYP1A1 were significantly down-regulated, whereas no effects were observed in presence of AG825, a selective erbB2 inhibitor. These results suggest that erbB1/erbB4 signaling is, at least partially, regulating the AhR activity in bovine COCs through ERKs cascade. In conclusion, our data indicate a specific role of ERKs in the regulation of AhR signaling during bovine oocyte IVM and suggest the EGFR pathway as a possible upstream regulator. This study supplies new insights into mammalian oocyte biology and, in turn, might improve strategies in assisted reproduction.

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PRELIMINARY STUDIES ON ENVIRONMENTAL POLLUTANTS IN GAME ANIMALS FROM EAST PIEDMONT: INTERSPECIES COMPARISON

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Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are synthetic chlorinated compounds classified as POPs whereas only the penta- e tetra-brominated polybromodiphenyl ethers (PBDEs) are so defined by the Stockholm Convention. Their log Kows, makes them accumulate in the fatty tissues of living organisms, bioconcentrate, and biomagnify in the animals at the higher trophic levels, including humans, possibly causing, through chronic exposition, endocrine disruption and cancer (1,2). Among polyciclic aromatic hydrocarbons (PAHs), benzo[a]pirene is classified by IARC as cancerogen and benzofluorantene as possible cancerogen. The aim of this study was to determine the contamination status of four game animal species (chamois, red deer, roe deer and wild boar) from East Piedmont by these substances. The chemical analysis for the detection of six non-dioxin like indicator PCBs, seven indicator PBDEs, fifteen OCPs and metabolites, and four PHAs was performed by GC-MS/MS on muscle samples purified and extracted using a QuEChERS method, validated according to SANTE 2017 (3). The preliminary data collected on four animals for each species show the occurrence of the studied compounds in almost all samples and the scattered presence at concentration around 1 ng/g of the organohalogenated compounds and of PAHs in the chamois, red deer, roe deer muscle whereas quantitable PCBs in wild boars were more frequent. In the chamois, particularly, a mixture of α- and β-hexachlorocyclohexane, hesachlorobenzene, lindane and aldrin at concentrations above LOQ were found in three out of four animals. These preliminary results seem to show the ubiquitary presence of the studied contaminants, with a preferential localization of PCBs at lower altitudes and in wild-boar than other animals. Based on the similar habitat of the three ungulate species, the cause of the difference in the above mentioned OCPs is unclear. Further studies on the distribution of animals in the territory are underway in order to better understand the causes that influence the presence of environmental contaminants in the various species.

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THE CASE STUDY OF NESFATIN-1 IN PANCREAS: *TURSIOPS* AS NEW ANIMAL MODEL

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In 2006 a new regulating molecule involved in food behavior called nesfatin–1 was identified [1]. Besides its central action as anorexigenic peptide, current data suggest that nesfatin-1 is a pleiotropic molecule involved in several regulatory processes in peripheral organs and tissues [2]. In particular, it has been shown that in pancreas of rodents and human, nesfatin-1 is localized in the β-cells and promotes the release of insulin. This raises the possibility that the dysfunction of the nesfatin-1 could be implicated in metabolic disorders, particularly in type 2 diabetes mellitus (T2D) [3]. Although various animal models have been used to study diabetes, a single species that fully complements T2D in humans has not been identified [4]. Recently, it has been discovered that dolphins have a prolonged glucose tolerance curve and during fasting maintain a state of hyperglycemia similar to human T2D [5]. The similarities between dolphins and human have led to the possibility of being able to consider dolphins as models for the studies at both physiological and pathological human behaviors. To this aim we decided to characterize nesfatin-1 distribution in pancreas of bottlenose dolphin (*Tursiops truncatus*) and evaluate pre and post-prandial blood levels of this molecule. Pancreas samples were receveid from the Mediterranean marine mammal tissue bank of the University of Padova while blood samples were provided by different Aquariums during routine veterinary controls. We carried out immunohistochemistry against nesfatin-1. We found that nesfatin-ImmunoReactive (IR) cells were distributed in pancreatic islets of Langerhans. In addition, by double immunofluorescence we discovered that nesfatin-1 never co-localizes with insulin-IR cells but with some glucagon-IR cells in the pancreatic islet. The specificity of nesfatin-1 antibody was confirmed by western-blot on homogenates of pancreas. We also evaluated plasma nesfatin-1 levels through ELISA analysis: we didn't find a significant difference among samples comparing fasting and post-prandial states. We hypothesized that in dolphins nesfatin-1, due to its localization in α-cells, can stimulate glucagon secretion in order to maintain high blood sugar levels. In conclusion, our report adds novel information on the presence and distribution of nesfatin-1 in the pancreas of bottlenose dolphins. These results emphasize some common features between bottlenose dolphin and terrestrial mammals. More research is needed to fully understand the role that research on dolphins can play in understanding T2D in humans.

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APELIN EXPRESSION IN THE UTERUS OF SHEEP GRAZING ON SEMI-NATURAL PASTURE, PRELIMINARY RESULTS

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Adipokines, mainly produced by adipose tissue, are molecules involved in energy metabolism and represent important links between nutritional status, and neuro-endocrine axis. Physiological levels of adipokines are necessary for the regular performance of ovarian activity, the correct embryo implantation and a healthy pregnancy. Apelin (APLN) expression in adipose tissue is regulated by factors such as fasting and refeeding and this molecule plays a role in the regulation of adiposity, food intake and body weight. However, investigation on this topic has yielded disparate results [1]. APLN and its receptor have a widespread distribution throughout the CNS and periphery. The apelinergic system is also active in the female reproductive tissues such as placenta, ovary and uterus [2] even if, differences emerged among species. APLN causes obesity increasing body weight but also leads to infertility modifying reproductive hormones in mouse [3]. In this work, the expression and localization of APLN was analyzed in the uterus of the sheep in an attempt to shed light on those cells and structures that might locally produce and secrete this peptide. A group of 15 Comisana x Appenninica adult female sheep in dry stage were fed with fresh hay from June to the pasture maximum flowering (MxF group). From this period to maximum dryness, the sheep were split into two groups: the control group (Cnt group) was fed with fresh hay while, the experimental group (Exp group) was fed with fresh hay supplemented with 600g/day/head of barely and corn (1:1). Samples of uterus were collected for each group and processed to perform PCR and immunohistochemistry. Samples for molecular biology were immediately frozen in liquid nitrogen, then stored at −80°C. Samples for morphology procedures were fixed in 10% neutral-buffered formalin solution and included in paraffin wax. Immunohistochemistry for APLN was performed with a polyclonal rabbit anti-apelin antibody. In all uterus samples analysed, PCR demonstrated the presence of the transcript for APLN. By immunohistochemical investigation, a positive staining for APLN was observed in the apical membrane of the endometrial epithelial cells and in the glands located in the connective tissue. APLN staining appeared stronger in Cnt and Exp groups respect to MxF one. To the authors' knowledge, no studies describe APLN expression in the uterus of domestic animals. Its identification in the sheep suggests that this peptide may play a role in the activity of this organ and is involved in the reproduction. This is a preliminary report that introduces APLN investigation in the sheep female genital system however, the exact role of APLN and the influence of diet needs further elucidation.

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KISS1 AND GPR54 EXPRESSION IN THE CAT OVARIES DURING DIFFERENT REPRODUCTIVE STAGES

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Kisspeptin system has a key role in the central regulation of reproduction. Kisspeptin (KISS1) is a neuropeptide involved in the hypothalamic regulation of reproduction in many species [1]. Nevertheless, KISS1 and its G-protein-coupled receptor (GPR54) are expressed in various tracts of female genital system of different species, such as in human and rat ovary and placenta [2,3], in cotyledons of bovine placenta [4] and in canine ovary, uterus and placenta [5], suggesting also a local role in reproduction [1].

The aim of the present work was to verify the presence of KISS1 and GPR54 in the cat ovary during different reproductive stages. To the best of our knowledge, no studies have yet been performed on the kisspeptin system in the cat ovary, and the results might provide new data useful for a better understanding of the involvement of this peptides in the local control of ovarian function in this species. The experiment was conducted using six cats admitted to the day-hospital service at the Veterinary Hospital of Perugia and subjected to the ovariohysterectomy. The ovaries were collected during oestrus, luteal phase and anoestrus, fixed in formaldehyde and embedded in paraffin. Immunohistochemical analyses were performed using a mouse monoclonal anti-Kisspeptin antibody (Santa Cruz Biotechnology Inc.) and a rabbit poyclonal anti-Kisspeptin receptor antibody (Alomone Labs), polyclonal antibodies, avidin-biotin-complex (ABC) and DAB as the chromogen. All the sections were counterstained with haematoxylin. The immunohistochemical analyses showed a strong positivity for KISS1 in primordial follicles, oocytes, granulosa cells and in the muscular layer of vessels in all stages of the oestrous cycle and in cells of the corpora lutea during the luteal phase. Immunoreactivity for GPR54 was localized in the same cells. In general, the immunopositivity for KISS1 appeared localized in the cytoplasm, and for GPR54 in the cellular membrane.
In conclusion, this is the first study showing the presence KISS1 and GPR54 in queen ovaries. The presence of these molecules in different structures of the ovary suggests the involvement of the kisspeptin system in the local control of ovarian functions in the cat, likely including follicular development, oocyte maturation, steroidogenesis and ovulation.

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VETERINARY TOXICOLOGICAL ASSISTANCE (ATV): RETROSPECTIVE ANALYSIS OF CALLS REGISTERED IN THE PERIOD JANUARY 2017 - DECEMBER 2017

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The Veterinary Toxicological Assistance (ATV) is a service sponsored by Nestlé Purina with the collaboration of the Veterinary Italian Cultural Society for Companion Animals (SCIVAC) and the Department VESPA by the University of Milan (Italy). The ATV is managed entirely by veterinarians and offers free 24-hour telephone consulting, exclusively to colleagues who are facing toxicological emergencies in dogs, cats and non-conventional animals.
The aim of this work is to provide a critical review of data regarding toxicity/poisoning reports on the Italian territory in the period January-December 2017, with particular attention to reports concerning medicinal products.
All reports referred to cases of intoxication/poisoning in the main species of pets (dog, cat and non-conventional animals). Archiving of phone calls was performed through a toxicology record, filled in by the operator who entered the data related to the origin of the call (geographic area, region and province), the animal intoxicated (species, breed, sex, age and living environment), the active substance and toxicosis (intoxication, symptoms, anatomo-pathological findings). All these data were processed by our Department directly on a recording sheet and sorted in different combinations, according to the selected parameters (species and number of calls, species and active ingredient, number of calls and geographical area, etc.). In 2017, the ATV collected a total of 957 reports. In particular, 49% of calls came from Northern Italy (Lombardy, 28%), 27% from the Central Italy (Tuscany, 56%) and 24% from the South of the country (Sicily, 22%). Regarding the intoxicated/poisoned animal species, most of the calls concerned the canine species (759 cases out of 957, 79%), followed by the feline species (178 cases out of 957, 19%) and non-conventional species (6 cases out of 957, 0.6%). Three hundred thirty eight calls out of 957 (35%), were about veterinary and human medicinal products. Their geographical distribution overlapped that reported in the overall database (Northern Italy 49%, Central Italy 29% and Southern Italy 22%). Even the distribution of calls according to species reflected that of the overall database: 80% for the canine species (270 cases out of 338), followed by the feline species with 19% (65 cases out of 338). Reports of intoxication for non-conventional animals were reconfirmed as infrequent (2 cases out of 338, 0.6%). Among the main pharmacological classes involved in pet intoxication/poisoning, at the first place we found the veterinary antiparasitic drugs with 92 cases out of 338 (27.2%), followed by the human non-steroidal anti-inflammatory drugs with 64 cases on 338 (18.9%), then human anxiolytics and antidepressants (8.9%) and human contraceptives (5%). All these pharmacological categories, together, account for more than half (60%) of the reports for the year 2017.
Most episodes of intoxication/poisoning caused by drugs, were related to an incorrect administration of the veterinary product by the owner. For the rest of the episodes, however, the main cause was the incidental ingestion of the human drug by the animals.

AMNION DERIVED EPITHELIAL STEM CELLS FOR THE TREATMENT OF A CASE OF BILATERAL OSTEOCHONDRITIS OF THE STIFLE IN A HORSE

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Amnion-derived epithelial stem cells (AECs) are considered a promising source for the treatment of orthopedic diseases. AECs demonstrated to be very effective in tendon tissue regeneration both in experimental (1) and clinical (2,3) studies, but a direct response of the synovial joint to intra-articular injection of eterologous AECs has never been performed. Furthermore, recent studies demonstrated the low immunogenicity of these cells and their immunomodulatory effect (3).

The objective of this clinical case was to assess the clinical response to repeated intra-articular injection of AEC in a 3 year-old saddle horse with spontaneous bilateral Osteochondritis Dissecans (OCD) of the knee. Clinical signs included joint swelling and light bilateral lameness; radiographs showed a typical ODC picture with involvement of the femoral troclear ridges and of the patella. AECs were collected from amniotic sac of slaughtered 3-months pregnant sheep, then isolated and cultured-expanded as previously reported (4). After aseptic preparation of the knees, an aliquot of 10^6 AECs in 500µl of αMEM was injected into each joint. The procedure was repeated after two months. Neither adverse reactions nor signs of discomfort were noted following the injection. Clinical and radiographic details showed a significant improvement during the year after the treatment. Actually the horse is used for pleasure and jumping activities with satisfaction of the owner. The repeated injection of ovine AECs into the joints of a horse did not cause any negative reaction but rather a clinical improvement and this confirms the immunomodulatory properties of these cells. The clinical and radiographic data suggest that repeated intra-articular injection of AECs could help the recovery of OCD affected joints.

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DETERMINATION OF OCHRATOXIN A IN FARMED FISH BY ENZYMATIC DIGESTION (ED) COUPLED TO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH A FLUORESCENCE DETECTOR (HPLC-FLD)

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Several studies have demonstrated that fish feeds contain significant concentrations of chemical contaminants, many of which can bioaccumulate and bioconcentrate in fish tissues [1]. The serious concern regarding the use of fish meal and fish oil in the aquaculture industry has led to extensive search of alternative raw materials for aquafeeds. The most obvious alternatives are oils and proteins of plant origin. The use of these alternative feed ingredients can introduce contaminants that were previously not associated with fish farming such as mycotoxins [2]. Ochratoxin A (OTA) is a mycotoxin produced as a secondary metabolite by various *Aspergillus* and *Penicillium* species with nephrotoxic, carcinogenic, immunotoxic and teratogenic potential [3]. OTA has been found in several food commodities, including cereals and can also be present in food of animal origin as a result of carryover from contaminated feed [3]. The aim of the present study was to determine OTA concentrations in muscle, kidney and liver of 10 seabream and 10 seabass of farmed origin collected on the market. Analysis will be performed by using an enzymatic digestion (ED) method coupled to high-performance liquid chromatography with a fluorescence detector (HPLC-FLD).

Fish tissues were digested for 1 hour at 37°C with a 1% pancreatin solution in a phosphate buffer and then cleaned up with ethylacetate. After being evaporated to dryness and re-dissolved, the sample was processed using HPLC-FLD. The method was validated for: specificity, recovery, trueness, selectivity, linearity, limit of detection (LOD) and limit of quantification (LOQ), repeatability and reproducibility.

Recoveries of analytical method were higher than 85% for all the matrices. Intra- and inter-day repeatability expressed as relative standard deviation were less than 9%. The LOD and LOQ for liver and muscles samples were 0.001 and 0.002 µg/kg, respectively. The LOD and LOQ for kidney samples were 0.01 and 0.02 µg/kg, respectively.

The highest concentrations of OTA were found in the kidney of the 20 fish analyzed (range <LOD-0.91 µg/kg, mean 0.32 ± 0.30 µg/kg). The concentrations found in the liver ranged between <LOD-0.74 µg/kg, (mean 0.53 ± 0.22 µg/kg). The lowest concentrations were found in muscle (<LOD-0.28 µg/kg, mean 0.12 ± 0.11 µg/kg). No differences were found between the two analysed species.

The present results are in agreement with previous studies [4] suggesting that an high OTA amount could be present in feed administered to fish sampled in this study.

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DOGS AND CATS POISONING IN SICILY: THE 7 YEARS EXPERIENCE OF THE ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLA SICILIA "A. MIRRI"

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The poisoning by toxic substances were documented in dogs and cats [1]. Carbamates, pesticides, molluscicides and rodenticides are commonly involved in poisoning events and, despite it is long time banned in Europe, also Endosulfan was less frequently reported [2,3]. Considering the risk for human population and the environment, in order to control these events in Italy, Ministerial Ordinances were issued from 2008 (O.M. 18 december 2008 - "Divieto di utilizzo e di detenzione di esche o bocconi avvelenati"). Aim of this study is to describe the seven year experience on death dogs and cats suspected to be poisoned in Sicily by the Istituto Zooprofilattico Sperimentale (IZS) della Sicilia "A.Mirri". Between 2010 and 2017 suspected poisoned baits (493 baits) and owned or stray domestic animals (1165 dogs and 263 cats) were submitted to the IZS della Sicilia (official control laboratory of the Sicilia region). Baits were subjected to a visual inspection while animals were subjected to necropsy. Baits and tissue samples (liver, kidneys and gastric content) were submitted for toxicological assays, by HPLC, GC-MSD, GC-ECD methods. Necropsy and laboratory data, including the number of tested positive animals, autoptic and poison findings, were collected and reported in the present study. During the study period, 452 dog (38.8 %), 52 cats (19.8 %) and 348 baits (70.5 %) tested positive for toxic substances. The highest number of suspected baits (n=85) and animals (n=218) was submitted in 2014. The highest number of tested positive animals was reported in 2010 (42.2%) while the lowest one in 2017 (23.4 %). The highest number of tested positive baits was reported in 2013 (94.3 %). Despite most cases not showed characteristic autoptic lesions, some findings (such as the colour of gastric and intestinal content) were related with specific toxic substance (metaldehyde). Ingestion of toxic substances was detected as the most common cause of poisoning. Carbamates (43.8-29.8%) and organochlorines (46.2-28.8%) pesticides were the most frequently involved while rodenticides (0.07-0.01%) were less frequently detected. Among organichlorine pesticides, Endosulfan was also detected. Other causes (such as traumatic lesions, infectious diseases, gunshot wounds) were observed in negative tested animals. Despite the appropriate Ministerial Ordinances contribute to the control of poisoning events, inappropriate or careless use of toxic substances and scattering of poisoned baits still remain a continuous phenomenon. Moreover, some recent dog poisonings events in Sicily reached a great emphasis on newspapers and social media, also due to the increased sensibility on the social role of pets. The continuous monitoring activity of the IZS della Sicilia and the described related data play a key role to better elucidate poisoning events. In conclusion, this monitoring activity could contribute to control the abuse of toxic or banned substances and to the contrast of these phenomena, in consideration also of their social impact.

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MEASUREMENTS OF OREXIN LEVELS, OREXINS AND COGNATE RECEPTORS IN THE EQUINE CENTRAL NERVOUS SYSTEM

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Orexins, also known as hypocretins, are neurotransmitters which are spread throughout the whole body regulating important metabolic processes in mammals. Their primary role lies in the regulation of sleep rhythm, digestion and other endocrinological processes. Orexin-A and -B are known to be the main focus of research into both human and animal sleeping disorders. A lack of these neurotransmitters or their receptors is believed to be responsible for developing narcolepsy in humans, and also causes signs of narcolepsy in mice, rats and dogs, animal species in which orexins have previously been measured [1-3]. To the author's knowledge, no studies have yet been performed on orexins presence and distribution in the nervous system of the horse. The goal of this study was to obtain an overview of physiological concentrations of orexin in the equine central nervous system. Determining Orexin-A concentrations in the cerebrospinal fluid (CSF), as well as the measurement of Orexin-A and -B neurons and receptors in the central nervous system (brain and spinal cord), of horses could be a first step towards understanding equine narcolepsy. Eleven horses, which died at the hospital of the University of Veterinary Medicine in Vienna, for reasons other than a neurological disorder, were included in this study. The CSF was collected immediately postmortem from the atlanto-occipital-approach and the brains as well as part of the spinal cord were removed and fixed in 4% formalin. In total, ten different regions of the brain (frontal, parietal, occipital, temporal, basal ganglion, thalamus and hypothalamus, hippocampus, brain stem, cerebellum, spinal cord) were prepared for histopathological examination. The brain samples were analyzed by immunohistochemistry, utilizing antibodies for Orexin-A, Orexin-B, Orexin-A-receptor and Orexin-B-receptor. Each sample was observed at light microscope and the amount of neurons positive for orexins and receptors evaluated. CSF was frozen directly after collection at -20°C and later examined for its Orexin-A concentration with a specific ELISA test in the laboratory. In immunohistochemical semiquantitative assessment, positive neuron responses for orexin and receptors were determined, not only in the hypothalamus, but in all 10 regions of the central nervous system. Measured Orexin-A levels in the CSF appeared to be significantly higher in this study than previously reported in horses. However, these results differ to studies of orexin concentrations in other mammals. Based on these clinical findings, the horse might be unusual in its sleeping physiology and its orexin system.

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ALLOGENEIC MESENCHYMAL STEM CELLS IMPROVE THE WOUND HEALING PROCESS OF SHEEP SKIN

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Skin wound healing includes a system of biological processes, collectively restoring the integrity of the skin after injury. Healing by second intention refers to repair of large and deep wounds where the tissue edges cannot be approximated and substantial scarring is often observed [1]. The objective of this study was to evaluate the therapeutic effects of allogeneic mesenchymal stem cells (MSCs) in second intention healing using a surgical wound model performed on the back of six Bergamasca sheep. MSCs are known to contribute to the inflammatory, proliferative, and remodeling phases of the skin regeneration process in rodent models, but data are lacking for large animal models [2]. This study used three different approaches (clinical, histopathological, and molecular analysis) to assess the putative action of allogeneic MSCs at 15 and 42 days after lesion creation. At 15 days post-lesion, the wounds treated with MSCs showed a higher degree of wound closure, a higher percentage of re-epithelialization, and increased contraction in comparison to a control group. At 42 days, the wounds treated with MSCs had more mature and denser cutaneous adnexa compared to the control group. The MSCs-treated group showed a complete absence of inflammation and expression of CD3+ and CD20+. Moreover, the mRNA expression of hair-keratine (hKER) was observed in the MSCs-treated group 15 days after wound creation and had increased significantly by 42 days post-wound creation. Collagen1 gene (Col1α1) expression was also greater in the MSCs-treated group compared to the control group at both days 15 and 42. Peripheral blood-derived MSCs may improve the quality of wound healing not only for superficial injuries, but also for deep lesions. MSCs did not induce an inflammatory response and sped up the appearance of granulation tissue, neovascularization, structural proteins, and skin adnexa.

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SOFIVET

PARATHION POISONING IN BEEF HERD IN SICILY

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Organophosphorus (OP) and carbamate (C) insecticides are used commonly in agriculture to control pests of crops and animals [1]. Aim of this paper was to describe an outbreak of poisoning due to parathion in a beef herd in Sicily. In February 2018, veterinary surgeons were asked for an acute outbreak of suspected poisoning in a herd of about 300 crossbreeds beef calves. Anamnestic data were collected, and clinical, hematological and biochemical exams were performed. Anatomopathological, microbiological and toxicological investigations on selected organs of died animals were made. The animals, 6-24 months-old, were kept in several pens and fed with two types of unifeed made in farm using straw (same for growing and finishing groups) and feedingstuff (different for the two groups of animals). Water ad libitum was the same for all the boxes. Only the finishing beef (95 animals), showed marked or slight clinical signs with tremors, seizures, hypersalivation, recumbency, profuse sweating, lowing, depression and gastrointestinal atony with few feces covered with mucus. Within 7 days, 28 finishing beef calves died. Morbidity and mortality were 100% and 29% respectively. Increase of CPK, LDH, AST, ALT and K was detected in both markedly symptomatic finishing animals (G1) and slightly symptomatic finishing animals (G2). Increased urea values were found in G1 only. Growing beef groups showed no symptoms or alterations of hematological and biochemical profiles. Necropsies, performed on 6 calves, showed diffuse petechial hemorrhages on subcutis, mesentery, epicardium and heart, edema of mesentery, hemorrhagic enteritis and sometimes diarrheal material in rectum, renal and hepatic congestion, pulmonary congestion and edema with froth in the airways, lungs and nostrils. Main histological findings were marked congestion of lungs and spleens and marked/moderate pulmonary edema. Toxicological exams performed in a calf samples detected parathion in liver and spleen at 0.4 mg/kg and 0.64 mg/kg respectively. OP and C pesticides are often implicated in poisoning in mammals, fishes and birds. The poison inhibits the enzyme acetylcholinesterase at the muscarinic, nicotinic and central nervous system synapses [2]. Acute toxicity develops within minutes to a few hours and includes signs of muscarinic (salivation, excessive lacrimation, frequent urination, and diarrhea), nicotinic (weakness and tremors) and central nervous (depression and seizures) toxicity. Tremors and seizures can cause muscle damage and increase of muscle enzymes and potassium [1]. In fatal cases, death is often caused by respiratory failure and/or bradycardia and heart block. Furthermore, hemorrhagic lesions may be caused by prothrombin deficiency due to the hepatic toxicity of parathion derivatives [3]. The use of parathion is forbidden in Italy, but illicit purchase is not excluded. In this case a malicious poisoning was suspected.

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CONCENTRATION DEPENDENT EFFECTS OF *Melaleuca alternifolia* ESSENTIAL OIL ON SWINE SPERMATOZOA

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Melaleuca alternifolia (Ma) essential oil (EO), commonly known as tea tree oil, is a natural compound highly employed in medicine and in the pharmaceutical, food and cosmetic industries due to several biological properties (antimicrobial, antioxidant, antitumor, insecticidal, etc.) [1]. Such properties may suggest interesting applications in the reproduction field, both human and animal, as cryo-preservatives or substitutes for antibiotics, but studies regarding their direct effects on spermatozoa are lacking [2,3]. The aim of the study was to evaluate the effects of Ma EO on the principal morpho-functional parameters of swine spermatozoa. Nine ejaculates (n=9) of good quality (inclusion criteria: viability >80 %, objective total motility >80%) from three boars were used in the present work. The experimental protocol was previously validated and described by the authors [2]: briefly, after Gas Chromatography characterization of the EO, experimental samples were prepared by suspending a fixed number of spermatozoa in 5 mL of swine fertilization medium (SFM) with 10 different concentrations of EO (from 0.2 to 2mg/mL, at intervals of 0.2) with emulsifiers (DMSO and Tween80). After 3 hours of incubation at 16°C (±1°C), the samples were evaluated for Viability (Eosine-Nigrosine staining), Objective Motility (CASA), Acrosome Status (Comassie blue staining), Mitochondrial Membrane Potential (MMP, JC-1 staining) and pH. Moreover for 2, 1 and 0.2 mg/mL concentrations, the spermatic morphology was evaluated by Scanning Electron Microscopy (SEM). To evaluate differences between the control doses and the one added with Ma EO, a non-parametric one-way ANOVA (Kruskal-Wallis test) was performed with the significance level set at 0.05. Post hoc analyses were performed by means of Dunnett's test ($p<0.05$). The results showed that the effects of the Ma EO are concentration-dependent, as previously reported for other EOs [2]. All parameters, except for pH, resulted statically significant ($p<0.001$) at variance analysis. Acrosome status was significantly altered starting from 1.4 mg/mL ($p< 0.0001$), MMP from 1.2 mg/mL ($p<0.0001$) and Viability from 1 mg/mL ($p=0.0370$). Total motility, the most sensitive parameter as already reported by literature, was altered starting from 0.8 mg/mL ($p=0.0003$). The morphological alterations were confirmed by the SEM images, especially for the samples treated with 2 mg/mL, which displayed severe outer alterations on the entire cell. Since the characterization of this EO showed terpinen-4-ol as its main component (41.49%), the effects of this single compound using the same methodology will be analysed in further studies. Overall, this EO seems to be suitable for future applications in the reproductive fields at concentrations lower than 0.8 mg/mL.

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AQUAPORIN-1 (AQP1) ROLE IN THE PHYSIOLOGY OF OVINE MESENCHIMAL STEM CELLS (MSCs)

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Mesenchymal stem cell (MSC)–based regenerative medicine is a promising approach in tissue regeneration due to the multipotency of the cells, that are able to differentiate into various cell types (bone, cartilage, fat, etc.) under appropriate conditions. On this regard, sheep are considered a “perfect” model for bone tissue engineering [1] and have been proposed for wide-ranging applications in biomedical investigations [2,3].

Limited literature regarding the physiological properties of ovine MSCs (oMSCs) and their possible use in tissue repair suggests the opportunity to amplify the studies concerning the morphological and biochemical characterization of such cells.

More recently, an increasing number of papers have reported that aquaporins (AQPs), a family of channel proteins, known for their key implications in fluid transport in various tissues, play an important role in different biological processes related to cell physiology (migration, proliferation, and cell volume regulation) [4].

The aim of this study was to characterize oMSCs physiology and behaviour, with main focus toward the possible role of AQP1 in migration and proliferation. In addition, in consideration of several advantages (rapid collection, freeze-dried, packaged, and transported more easily) of the conditioned medium (CM) for possible application in tissue regeneration, the second aim was to investigate whether CM obtained from oMSCs could affect, through autocrine signals, their capacity to migrate and proliferate.

For these purposes, ovine MSCs isolated from bone marrow (BM) were cultivated in □-MEM medium [5]. In addition, CM was obtained from oMSCs (80-90% confluence) after for 24 h growth. AQP1 expression was evaluated by Western blotting, while its possible involvement in migration and proliferation was analyzed by proliferation, wound healing and Transwell migration assays. Ovine MSCs showed a good capacity to migrate and proliferate similar to human MSCs, and CM caused an enhancement of oMSC migration and proliferation. Moreover, oMSCs express AQP1 with increasing levels when cells were grown in presence of 10% FBS or in presence of CM respect to 1% FBS. Our results demonstrate the importance of AQP1 in mediating important physiologic roles in the biology of oMSCs, and, for the first time, that the autocrine loop of CM produced from oMSCs on oMSCs themselves causes an enhancement of migration, wound healing closure, as well as, expression of AQP1.

Further studies will be needed to better characterize aquaporins’ role in ovine MSCs, as well as, the autocrine activity of oMSC-CM, by accurate characterization of its composition.

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PRE AND POST RUT TESTOSTERONE AND CORTISOL HAIR CONCENTRATIONS IN ROE DEER BUCKS: CORRELATIONS WITH BLOOD LEVELS AND TESTICULAR MORPHOMETRIC PARAMETERS

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The roe deer (*Capreolus capreolus*) is a seasonal breeder, whose reproduction shows a distinct seasonal pattern with the breeding season (rut) lasting from mid-July to mid-August. Mature bucks show synchronized testicular cycles and transitions between totally arrested and activated spermatogenesis thanks to the peak of testosterone (TEST) between June and July [2]. Recently, hair has proven to be a good non-invasive matrix for endocrinological analyses, providing long-term information [1]. The aims of the present work were, firstly, to quantify hair concentrations of TEST and cortisol (CORT) in wild roe deer bucks hunted during pre and post rut period, using a radioimmunoassay (RIA) methodology, and to look for relevant differences between the two periods, and then to evaluate any possible correlation of such hair concentrations with blood ones and morphometric parameters of the testes. Twenty-eight mature roe deer bucks, were sampled during the hunting season between June 1st and July 15th 2017 (pre rut, n=14) and August 15th and September 30th 2017 (post rut, n=14) in the South-Western Bologna Apennines (Italy). All the animals analysed were killed and immediately transferred to the pertinent biometrical centre, where the personnel collected blood upon jugulation, hair from the dorsal caudal region of the animals and scrotums, including testis and epididymis. For the testicular analysis each testis was isolated, weighed and sectioned longitudinally. Major and minor axis were calculated and for each roe deer the measures were averaged between the two testis, in order to get a final mean volume. CORT and TEST were previously extracted from hair [1] and blood, and their concentrations were determined using a RIA technique. The results show that the testicular volume and weight significantly decreased in the post rut group ($p=0.0089$ and $p=0.0056$ respectively); the same happened for blood TEST concentrations ($p=0.0008$). The blood CORT levels did not show any statistical difference between the two groups ($p=0.8336$). The hair results showed an increase in TEST levels in the post-rut ($p=0.00003$), almost double the pre-rut ones. TEST reaches its blood peak in the pre-rut period, therefore it is likely that high hair post rut values are the direct reflection of high blood pre rut values. Differently, CORT levels decrease in the post-rut ($p=0.0014$), and this may be due to different environmental factors, leading to higher concentrations of hair CORT in the pre-rut. The correlations found by the statistical analyses were in agreement with the existing literature about the fluctuating concentrations of the two hormones in the blood throughout the year and the cyclic growth and reduction of the testis [2]. Moreover, this study represents a first validation for the use of a RIA technique to quantify both TEST and CORT in roe deer hair, confirming the use of this matrix as a good long-term information provider, and giving interesting insights on the physiology of reproduction of this species.

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A NOVEL EX VIVO PORCINE MODEL FOR PRELIMINARY EVALUATIONS OF ANTI-GERD MEDICAL DEVICES

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Gastroesophageal reflux disease (GERD) is a motility disorder resulting from the reflux into the esophagus of stomach contents [1]. The prevalence, especially for the Western world, reaches levels as high as 30% [2]. The medical management of the pathology mainly relies on proton-pump inhibitors (PPIs), but long-term use of such molecules can lead to moderate to severe side effects [3]. Since it was proved that the defensive properties of the esophagus are impaired in most patients with GERD, new therapeutic approaches with medical devices aimed to protect the membrane are currently being developed [4]. Ex-vivo models represent a pivotal step in the refinement/ reduction of experimental protocols as they provide preliminary gross screening data exploitable for preclinical in vivo trials. The aim of the present work was the validation and optimization of an ex vivo porcine model of gastroesophageal reflux to evaluate the efficacy of a new mucosal membrane protectant medical device (MD) by means of pH, impedenzometric and histological analyses. Impedenzometry was chosen as an analytical technique as it is the most used clinical approach for the diagnosis of GERD in the light of its high sensitivity, while histology provides the most reliable data regarding mucosal damage. Esophagi collected at a local slaughterhouse were ablated of the cardiac sphincter, cut for a total length of 30 cm, and fixed on an inclined support in a 37°C thermostatic hood. A 6 channel ISFET catheter was inserted (25 cm) for the pH and impedenzometric analysis after calibration, and registration immediately started. Organs were washed with NaCl 0.9 % and either treated with 10ml of MD (MD group) or not (CTR group). Afterwards a 0.1 M solution of HCl was infused for 30 minutes (10 ml/min) to mimic the acid-induced mucosal damage and registration was continued for additional 120 minutes to evaluate any effect of the MD. At the end of the protocol, biopsies were performed and fixed in 4% Formaldehyde solution for histology. Moreover, to quantify the eventual damage, Evans Blue was used to stain pieces of esophagus, extracted and quantified by spectrophotometry (620 nm). The results showed how the MD determined a better recovery of both impedenzometry and pH after the acid infusion, suggesting an effective protectant action. The hypothesis was confirmed by the histological evaluations that highlighted a more compact inner mucosal layer and lower quantities of penetrated Evans Blue in the MD group. Overall, the proposed ex vivo model seems to be reliable and reproducible and may represent a good preliminary screening method in order to reduce the number of animals enrolled in preclinical pharmacological studies.

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SOY PHYTOESTROGENS NEONATAL ADMINISTRATION DECREASES FERTILITY IN MICE

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Soy is rich in phytoestrogens, including genistein (GEN), which may interfere with the endocrine system. In particular, GEN administration during developmental critical periods raises concern [1] since even at low doses, it may interfere with the formation of specific steroid-sensitive neuronal circuits [2], leading to irreversible alterations in adults including fertility impairment [3]. Since soy is the most used protein source for livestock feed, it may, thus, be involved in the “idiopathic” hypofertility, which often affects many breeding farms [4]. Here, we analyzed the effect of pre-weaning administration of GEN on specific hallmarks of the gonadal state (estrous cycle, gonadal hormonal levels, and gonads and mammary gland development) and on Kisspeptin (KISS) neurons which controls them through GnRH neurons. Moreover, we have recently shown that this treatment affects the expression of neuronal specific nitric oxide synthase (nNOS) in specific hypothalamic circuits [2]. Since this system controls many behaviors by interactions with other neurotransmitters such as catecholamines, we investigated whether GEN treatment may affect tyrosine hydroxylase (TH, a specific marker for catecholaminergic neurons) expression in selected hypothalamic and mesencephalic populations.CD1 mice (Harlan) received orally GEN (50mg/kg), or vehicle, from birth (P0) to P8. Females (in diestrus) and males were, then, sacrificed at different ages (P12, P22, P30, P60). Histological analyses were performed on mammary glands and gonads. Hormonal levels were evaluated in feces sampled at P30 and P60. Coronal serial cryosections of the brains were prepared and processed for immunohistochemistry against TH or KISS. The density of labeled cells was counted, in regions of interest (ROI), with ImageJ software. Statistical analysis was performed with GraphPad software. Fertility features were affected in both males and females. Males displayed alteration in gonadal hormonal levels and in testis development. The effect of GEN on females was evident in both KISS and TH circuits, involved in the hypothalamic-pituitary–gonadal axis, as well as in estrous cycle and puberty onset, while it was more variable on uteri and ovaries. Present and past results indicate that GEN exposure in early postnatal life may result in permanent alteration of several widely diffused neurotransmitters’ systems, which may decrease fertility.

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ROLE OF THE SMALL GTP-BINDING PROTEIN RHES IN THE STRIATAL PATHOPHYSIOLOGY

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Ras homolog enriched in striatum (Rhes) is predominantly expressed in the corpus striatum. Within the striatum, Rhes mRNA is localized in virtually all dopamine D1 and D2 receptor-bearing medium-sized spiny neurons (MSNs) [1], and cholinergic interneurons. Early studies in rodents showed that Rhes is developmentally regulated by thyroid hormone, as well as by dopamine innervation in adult rat, monkey and human brains. At cellular level, Rhes interferes with adenosine A2A- and dopamine D1 receptor-dependent cAMP/PKA pathway, upstream the activation of the heterotrimeric G-protein complex. At a cellular level, Rhes is able to negatively modulate dopamine D1 and D2-dependent transmission, by influencing cAMP/PKA signaling, and AKT pathway as well. Interestingly, we have recently documented that this small molecule binds to and activates mTORC1, one of the key players able to modulate L-DOPA-induced dyskinesia in the Parkinson's disease animal model [2]. In this respect, we found that the lack of Rhes caused a significant reduction of such motor disturbances in mutant mice, while the beneficial anti-akinetic effect of L-DOPA was preserved. Therefore, in order to better shed light on the putative role of Rhes in the striatal dopamine-dependent transmission, in we analyzed the expression levels of Rhes mRNA in the striatum of post mortem Parkinson's disease patients, as well as MPTP-treated *Macaca mulatta*, with or without L-DOPA supplementation. Taking advantage of quantitative Real Time PCR approach, results showed significant reduction of Rhes transcript levels in the post-mortem putamen of PD patients. Noteworthy, consistent with PD patient observations, a similar Rhes mRNA decrease was found also in the putamen of MPTP-treated *Macaca mulatta*. The overall downregulation of Rhes mRNA levels in the putamen of both patients and the non-human primate model is in agreement with previous findings obtained in the striatum of adult 6-OHDA-lesioned rats. In conclusion, together with previous findings, the present data obtained in both PD patients and experimental non-human primate model of PD suggest that the regulation of Rhes mRNA levels by dopaminergic innervation might play a functional role under physiological and pathological conditions.

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ATTITUDES TOWARDS ANIMALS AND THEIR WELFARE AMONG ITALIAN VETERINARY STUDENTS

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Human attitudes towards animals are becoming of increasing importance in the areas of conservation and welfare. This is particularly true among veterinarians, who are a source of information about animal care for owners. The aim of this study was to investigate the attitude towards non-human animals and their welfare in a sample of Italian veterinary students. A questionnaire eliciting information on respondents' demographics, including the Animal Attitude Scale (AAS) published by Herzog et al. [1] was administered to students of the Veterinary Medicine Course in three Italian Universities. In total 878 questionnaires were completed, 173.6 ± 60.1 per year; female students (75.1%) were overrepresented in all years of the course. Data were analysed using non parametric tests (IBM SPSS 25.0) and a value of $p<0.05$ was deemed statistically significant. Results suggested that although the attitude towards animals is generally quite positive among veterinary students (mean score= 3.58±1.31 SE), it becomes exponentially more negative over time (R2 of 0.803, fitting equation Y = 66,666e-0,0125x), the fifth year presenting the lowest total score (TS) of AAS (62.53±0.70, Kruskal-Wallis test, $p<0.05$). Geographical location also influenced students' attitude towards animals, as students coming from the Northern part of Italy and attending a Northern University showed a lower TS than students from either the South or Centre of Italy (Kruskal-Wallis test, $p<0.05$). In addition, females were more supportive of animal welfare than males (Female TS=66.43±0.28 vs Male TS=58.53±0.25, Mann–Whitney U test, $p<0.05$). Considering the 4 subscales of AAS suggested by Gazzano et al. [3], respondents were significantly less favourable to human moral dominance (TS=80.72±0.50) and to negative attitudes to dogs (TS=72.65±0.59) than to the use of animals in research (TS=45.73±0.71) and food of animal origin (TS=50.30±0.37). However, students at the beginning of their academic career showed lower levels of support for animal research, as did females and participants from the Centre and South of Italy (Kruskal-Wallis Test, $p<0.05$). The effect of gender was also detected within the other three subscales, with females showing significantly more positive attitudes towards animals than males. The differences in attitudes witnessed in this study expand what observed in previous research; specifically, gender and prior experiences influence both veterinary students [2] and professionals [3] in their attitude towards nonhuman animals. Overall, our findings suggest that other variables, such as the geographical location and year of the university curriculum, may also act as important influencing factors among veterinary students, who generally have positive attitudes towards animals. The veterinary curricula should take into consideration all this emerging knowledge, covering topics that allow future veterinarians to have a more positive attitude towards non-human animals and their welfare.

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REPRODUCTIVE PATTERNS IN A POPULATION OF GRAY SQUIRREL (*Sciurus carolinensis*) IN CENTRAL ITALY: LITTER SIZE AND GENITAL TRACT MORPHOLOGICAL SEASONAL CHANGES

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Invasive alien species are a major threat to long-term survival of local native species [1]. Deployment of control programs to limit the expansion of alien populations is one of the goals of the EC [2]. Gray squirrel is one of the 100 worst invasive alien species (IUCN list). This work, being part of the European project LIFE13 BIO/IT/000204 U-SAVEREDS, aimed at safeguarding the native red squirrel (*Sciurus vulgaris*) in central Italy. To this end, we studied a gray squirrel alien population using 45 female carcasses, 18 ovariectomized females and 24 orchidectomized males obtained from the 2016-2018 management activities. Traps were baited with hazelnuts and check two times per day, to minimize stress to captured animals [4]. Female animals were euthanized by CO_2 inhalation, following EC and AVMA guidelines [4], while the males and females gonadectomized were anesthetized with dexmedetomidine ketamine and, as needed, maintained with sevoflurane in 100% O_2. Individual fecundity, in terms of seasonal (spring, summer) and total (annual = spring + summer) litter size, was determined by counting the number of uterine-placental scars which are formed by the detachment of each embryo's placenta at parturition, thus allowing to estimate the total number of young born from a single female during the entire reproductive season (spring, summer) [4]. Uterine scars were identified after staining which reveals dark pigments of macrophages involved in processes of repairing the endometrium after detachment of the hemochorial placenta [4]. Sixty-nine % of examined females produced litters (37.8 % a single litter, 31.2% two litters). Spring litters (3.55 ± 1.57 scars/female) were larger ($P<0.007$) than summer ones (2.14 ± 1.46 scars/female); annual fecundity was 4.52 ± 1.88 scars/female, ranging from 2 to 7 births. On ovaries collected from ovariectomized squirrels during a whole year, we always found graafian follicles. Spermatozoa were observed in the germinal epithelium of the seminiferous tubules along with a great number of sperm cells in the epididymis, with testes in scrotal position, attesting an active spermatogenesis. Taken together, our findings confirm that this gray squirrel population, having a high reproductive success due to a potential non-seasonal ovarian cyclic activity, is well adapted to the new environment and thus is greatly dangerous for the native red squirrel.

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FIRST DESCRIPTION OF PATTERN ELECTRORETINOGRAM IN THE BIOMEDICAL PIG

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The pattern electroretinogram (PERG) provides an objective measure of central retinal function and can be used to detect and monitor dysfunction of retinal ganglion cells caused by conditions such as glaucoma, optic neuropathies and primary ganglion cell diseases [1,2]. The biomedical pig is largely used in ophthalmology research due to its anatomical and physiological similarities with humans [3,4]. Nevertheless PERG and spatial frequency using PERG were never assess in the pig, probably due to the fact that PERG is a very small signal and it is difficult to obtaining reliable results [5,6]. The aim of this study was to characterize PERG and spatial resolution visual acuity in the biomedical pig. Seven commercial hybrid pigs, neuterated males and femals of 85±5 days of age, were enrolled in the study approved by the Italian Ministry of Health (D.lgs 26/2014). The animals were anesthetized with tiletamine/zolazepam-dexmedetomidine (3mg/kg-20µg/kg) and the anaesthesia was maintained with sevoflurane. The animals were positioned at 30cm from the pattern screen, an erg jet electrode was used to recorder from the corneal surface of left eye, Retimax instrument was used to produce the stimuli and analyzed the responses (C.S.O. Srl. Scandicci, Firenze). The PERG stimuli were presented as alternating white and black vertical grating, with a screen luminostity of 45 cd/m2, 100% of contrast and temporal frequency of 2 reversals per second (rps). 7 different spatial frequencies were presented: 0.1, 0.5, 0.7, 1, 1.2, 2.5 and 5 cy/deg (cycles per degree). For the first time, reliable and reproducible PERG waves were recorded in the pig. The waves were characterized, as for humans, by tree peaks N35-P50-N95, two negative peaks (N35 and N95) arising at 28.2±4.7 and 123.2±9 ms post stimulus and a positive one (P50) arising 57.7±4.9 ms post stimulus. According to our findings, the N35-P50 and P50-N95 amplitudes for 0.1 cy/deg were 9.7±1.4 and 11.3±1.8 respectively. The waves were linearly flattened up to 5 cy/deg, characterized by 0.6±0.2 (N35-P50) and 0.4±0.1 (P50-N95) amplitudes. Exceeded 5 cy/deg the peaks were no longer detactable. This study provides an important tool for the use of the biomedical pig in ophthalmology, leading both to a refinement and to a better understanding and application of diseases models.

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SUMATRAN TIGERS (*Panthera tigris sumatrae*) BEHAVIOR DURING LATE ZOO EVENTS

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Several researchers have reported significant effects of visitor density (number) and intensity (noise level) on captive animal behavior [1]. Studies correlating human effects to animal behavior have been conducted - with varying results - on several species. Chamove, Hosey, and Schaetzel [2] found that visitors increased the activity level and aggressive behavior of primates, whilst decreasing non-violent social behavior. Margulis, Hoyos, and Anderson [3] studied 6 different species of felids (lion, amur leopard, amur tiger, snow leopard, clouded leopard, and fishing cat) in captive environments and found that the presence of visitors had no effect on any of the species studied. However, Mallapur and Chellam [4] found that visitors affected the behavior of captive Indian leopards. A study performed by Sellinger and Ha [1] showed that the jaguars at the Woodland Park Zoo (WPZ) in Seattle, Washington, were reacting to the intensity and density of visitors to the exhibit, with a trend toward increased amounts of pacing and aggression. The main focus of this research was to investigate the response of five Sumatran tigers (*Panthera tigris sumatrae*) in a zoo environment, during evening events (named Zoo lates). The zoo environment includes climatic, intraspecific and interspecific contact factors that can vary significantly from the in situ habitat where the species evolved. Animal responses are behavioral as well as physiological and monitoring these responses is essential for ensuring optimal animal welfare and offer insight to the species' behaviour and ex situ adaptability, producing valuable data relevant for their husbandry. For this project, a group of five Sumatran tigers was monitored comparing their behavior during evening social events (Zoo late Nights) and control evening during Summer 2014. Tiger behavior was monitored using focal animal sampling techniques, whilst crowd levels around the tiger enclosure was recorded every minute, flash photography as it occurred, and noise levels (maximum and minimum levels) were recorded every five minutes were recorded using a portable decibel reader. In order to evaluate the most significant (if any) visitor related disturbance factors on the tigers. The probability for each animal to move between enclosure zones in the 9-minute period of observation was analyzed by a logistic regression model. Direct observations indicate that the behaviour of the study animals was not significantly influenced on Zoo lates while the logistic model applied underlined the significance of several variables on the displacement of subjects. In particular, the total camera flashes and the maximum decibels were statistically significant ($P<.0001$), whereas the minimum decibels were borderline for significance. Qualitative variables (subject and crowd levels) did not influence the displacement, although a slight difference between subjects was observed. This study outlines the importance of monitoring animal behaviour during potentially stress inducing events and determining individual response to such environmental stimuli.

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A NEW TOOL TO EVALUATE CORTISOL CONCENTRATIONS IN ANIMAL HAIR

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The study of cortisol in hair requires the use of a highly sensitive assay. To date, hair cortisol concentrations (HCC) have been evaluated with different analytical methods as Radio Immune Assay (RIA), Enzyme Immunossay (EIA), Enzyme Linked Immunosorbent Assay (ELISA), ChemiLuminescent Immunoassay (CLIA) and High Performance Liquid Chromatography with Mass Spectrometry (HPLC/MS) or Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS). Amplified luminescent proximity homogeneous assay (AlphaLISA) is an ELISA-like, nonradioactive technology first reported in 1994 [1]. This technology allows the quantitative detection of molecules of interest in a light-induced chemiluminescence immunoassay using a microplate without wash step. The aim of the study was to compare RIA with AlphaLISA method and examine the sensitivity in evaluating cortisol concentrations in animal hair of the Cortisol AlphaLISA kit (PerkinElmer, USA), originally suggested for the analysis of buffer and serum samples. The study has been carried out on 4 samples of calf, 4 samples of foal and 8 samples of sheep hair. After washing with isopropanol, two extracts were obtained from each hair sample. One extract has been evaluated for HCC by RIA as previously described [2,3]. The second extract has been reconstituted with the buffer provided by the Cortisol AlphaLISA Kit and HCC evaluated by the use of this commercially available kit. The preliminary results indicate that the Cortisol AlphaLISA Kit is capable to detect cortisol also in hair samples with high sensitivity, with a detection limit of 17 pg/ml. Intra- and inter-assay coefficients of variation (CVs) were 4.0% and 9.0%, respectively. Cortisol concentrations ranged between 1.31 and 16.94 pg/mg, 6.30 and 57.65 pg/mg, and 2.16 and 45.08 in calf, foal and sheep hair, respectively. The HCC obtained by Cortisol AlphaLISA Kit and RIA showed a good correlation ($r=0.79$, $p<0.01$). Considering the possibility to use a low amount of extracted sample, its no-washing procedure and the performances showed, we can conclude that the Cortisol AlphaLISA Kit can be considered an excellent tool to evaluate cortisol concentrations also in hair derived from animal species.

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SILVAFEED® NUTRI P/ENC SUPPLEMENTATION TO FROZEN THAWED BOAR SPERM IMPROVES IN VITRO FERTILIZATION

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Boar spermatozoa are very susceptible to cryopreservation in that their membrane is rich in unsaturated phospholipids and poor in cholesterol. Cold shock leads to plasma membrane destabilization that, in turns, affects acrosome integrity and membrane lipid packaging and induces a loss of fertilizing potential [1]. Moreover, spermatozoa enter an "oxidative stress" characterized by a high production of ROS and a concomitant decrease of antioxidant defenses. For these reasons the pig remains one of the few species in which fresh semen is still preferred to the thawed one for insemination. Many recent studies evaluated the effects of supplementing frozen and/or thawing media with antioxidants such as catalase, ascorbic acid, resveratrol, EGCG and tannins, in order to enhance sperm quality and fertilizing ability [1-3]. Silvafeed®Nutri P/ ENC (SNP) (Silvateam S.p.a., Cuneo, Italy), is a mixture of additives rich in polyphenols (75% tannin content) commonly used in the daily ration of pigs for its nutritional properties and its ability to improve intestinal health and animal performance. The aim of the present work was to study the effect of supplementing boar sperm thawing medium with SNP on in vitro fertilization (IVF) and on the following sperm parameters: sperm motility (assessed by CASA), viability, acrosome integrity, mitochondrial function and lipid peroxidation (assessed by flow cytometry) and capacitation status (assessed by immunolocalization of tyrosine phosphorylated proteins). Thawed sperm cells were incubated 1 h at 37°C in BTS without (CTR) or with (5, 10, 20 µg/ml) SNP. After incubation sperm suspension was divided in three aliquots: one was used for IVF trials, one for sperm analysis, and the last one was capacitated for 1 h at 39°C 5%CO_2 in Brackett and Oliphant's medium. Oocytes inseminated with thawed spermatozoa pretreated with all the different SNP concentrations presented a significant ($P<0.001$) increase in penetration rate compared to CTR. In addition, 5 µg/ml SNP exerted a positive effect ($P<0.05$) on the total efficiency of fertilization, while different treatments did not affect tyrosine phosphorylated protein immunolocalization, used as capacitation parameter. Viability, total and progressive motility, acrosome integrity, mitochondrial functionality and lipid peroxidation were not influenced by SNP. Further studies are necessary to investigate the mechanism(s) by which SNP is effective in improving IVF outcome and to determine the possible positive effect of SNP addition to commercial thawing solutions during porcine AI with frozen-thawed boar semen.

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MAMMARY EPITHELIAL CELLS PROLIFERATION AND VIABILITY ARE MODULATED THROUGH A JUXTACRINE/PARACRINE EGFR-DEPENDENT ACTIVITY

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During puberty, the ductal system of mammary gland undergoes a tremendous proliferation, filling the fat pad in the form of Terminal End Buds (TEBs). According to studies in mice, ovarian estrogens drive ductal elongation via ERα expressed in a subset of epithelial cells: ERα-positive cells release Amphiregulin (AREG), which targets EGFR in the stromal compartment. Since only stromal EGFR has been proved essential for ductal elongation, ERα-negative cells are thought to proliferate through the intermediation of stroma [1,2]. In our model we hypothesized that locally produced EGFR-ligands can stimulate neighboring mammary epithelial cells, in a paracrine and/or juxtacrine fashion. For this purpose, we investigated the role of EGFR in mammary epithelial cells, regarding cell cycle, proliferation, gene transcription and translation. We also analyzed how EGFR ligands are modulated and which pathways take part in this regulation. Mammary epithelial cell lines from different species (immortalized murine and human cells, primary feline and bovine cells) were cultivated under various conditions and then analyzed: proliferation test, flow cytometry, rtPCR and Western Blot were aimed at showing the biological activity of EGFR. rtPCR and Western Blot were also used to unroll the pathways upstream of each ligand. All experiments were performed at least three times; data were analyzed using Student's *t*-tests, setting a significance at $p < 0,05$. Significative differences in G0/1 percentage and in proliferation were found between cells cultured in growth or starving mediums, added or not with AG1478 (a specific EGFR inhibitor), confirming the importance of EGFR for cell proliferation and survival. When cells were treated with EGF, EGFR activation alone was not sufficient to restore the basal growth levels, indicating that growth factors present in serum are needed to sustain proliferation. rtPCR analysis revealed that among the seven EGFR ligands, only AREG, EREG and HBEGF are modulated by a rapid, transient induction through an ERK1/2 dependent signaling pathway. The above results were consistent with Western Blot analysis that demonstrated a high p-ERK level under both growing and starving condition, knocked down only by EGFR inhibition.

The present study contributed to fill the gap in knowledge about the specific role of mammary epithelial EGFR, supporting the hypothesis of an epithelial paracrine activity. Moreover, a quite accurate description of the mechanisms regulating EGFR ligands was provided. Since little has been reported on EGFR-targeted therapy in veterinary medicine [3], a clear understanding of the epithelial EGFR-mediated signaling could result in a progress in mammary tumor diagnostics and therapies.

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NON-INVASIVE ASSESSMENT OF STRESS MARKERS IN HUNTING DOG

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The intense exercise causes to the organism oxidative stress and inflammation at gastrointestinal level. This is attributed to a change in blood flow that is shunted from the viscera to skeletal or heart muscle. The reduction in intestinal blood flow and the exercise-linked thermal damage to the intestinal mucosa can cause intestinal barrier disruption, followed by an inflammatory response [1]. Adaptations to exercise might be influenced by the gut microbiota [2]. Stool samples can be easily acquired without any discomfort to the subject and, together for the microbiota analysis, have been already used to quantify fecal cortisol metabolites (FCM) as a major indicator of altered physiological states that strongly correlates with stress, and IgA as indicator of intestinal immune protection. Moreover, as stool samples contain exfoliated cells, they have been utilized to quantify enterocytes gene expression profile in humans [3,4]. Considering these findings, the aim of the study was to investigate, in the stools of hunting dogs, the microbiota profile, the presence of some transcripts involved in inflammatory mechanisms, and to evaluate, in the same samples, FCM and IgA levels. The study included nine dogs of different age, weight, sex and breeds, clinically healthy, housed in individual boxes of the same kennel and fed with a commercial diet. The dogs were subjected to the same training regime for hunting wild boar. In order to counterbalance physiological variations, multiple day replicates were collected and pooled for each experimental point for each dog. The samples were collected at rest (180 days before training, T0), after 60 days of training (T1), after 60 days of hunting wild boar (T2) and finally after 60 days of rest (T3). From each sample total RNA extraction was performed. Then, the gene expression for occludin, calprotectin, proteinase activated-2 receptor (PAR-2) and heme oxygenase-1 (HO-1), was evaluated by qPCR. Moreover, FCM and IgA levels were quantified by RIA and ELISA assay, respectively. The fecal microbiota was profiled by paired-end sequencing of the V3-V4 regions of the 16S rRNA gene (Illumina MiSeq platform). No variation of FCM and IgA content was observed during the different phases, demonstrating that activity, in our model, did not exert strong stressor effects. On the contrary, alterations in microbiota structure in terms of beta diversity and relative abundance profiles, as well as gene expression were recorded, with a significant up-regulation of HO-1 and PAR-2 at T2 ($p<0.05$). These preliminary results confirm that physical activity impacts on the gut microbiota and modifies enterocytes permeability (PAR-2) increasing the expression of protective molecules (HO-1) in dogs, and support the idea that fecal mRNAs can contribute to improve the knowledge of gastrointestinal functionality.

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INCIDENCE OF CANINE BITES IN THE METROPOLITAN AREA OF NAPLES FROM 2010 TO 2016: PRELIMIMARY DATA

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Canine aggression is the most common complaint in veterinary behavior referral practice [1]. The family members are the most common targets of the aggression [2]. Statistical studies of dog bites to humans indicate that in most cases, people are victims of their own dog or of a dog they know [3]. Furthermore, dogs presenting aggression are at a higher risk of being abandoned [4] or even euthanized because of the aggression [5]. In South Italy, Naples' metropolitan area, the phenomenon of dogs biting people has caused much concern during the last few years. Thus, ASL Na1 Hospital started, on behalf of CRIUV's (Regional Centre of Veterinary Urban Hygiene) initiative, specific procedures to provide documentary evidence and to collect reports of dog bites. The epidemiological study is from 2010 through 2016. The research studied correlation between the dog sample's age, sex, breed and bite severity with sex of bitten. The collection of anamnestic data comes from the bites' registry officially used at C.R.I.U.V. 487 biting dogs were sampled, of which 377 males and 110 females. The total number of dogs was divided in 274 half-breed and 213 of breed. The small dogs (<10 kg) were 86, the medium dogs (10-20 kg) were 214 and the giant dogs were 187 (>20 kg). There was no real effect of breed type on bites shown. There were 498 bitten people in total, including 270 males and 228 females. Analysis of the results shows that male dogs are more involved in episodes of biting. Moreover, in relation to the size, medium and large dogs are responsible for the greatest number of bites. These data show a greater difficulty in the containment of medium and large dogs by the owner. This preliminary study will be completed with analysis of the following parameters: age of victim and severity of the bite, age, origin and training of the dog. Moreover, the study will investigate training method used by the owners and any correlation with the onset of potentially undesirable behaviors. Non the less, geolocation will be assessed to study correlations with urban areas at higher risk of delinquency.

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ARE DOGS STRESSED DURING READING SESSIONS WITH CHILDREN WITH PERVASIVE DEVELOPMENTAL DISORDERS?

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Animals give children the feeling of being supported and understood, without the thought of being judged for reading abilities [1]. A unique type of emotional support during reading sessions is widely recognised [1]: dogs are fully capable of being active and supportive listeners. However, we have little data on the behaviour and stress level of dogs during these activities, particularly with children with pervasive developmental disorders (PDDs). Although dogs could receive comfort during a reading session, they might also experience stress that might affect their willingness to work and overall performance [2]. Therefore, here we analysed dog's stress-related behaviours and physiological reactions (measured by saliva cortisol sampling) of dogs in a reading-to-dogs program with PDD children. Two healthy rescue dogs were involved after behavioural evaluation to determine their suitability for participating in five 40-minute reading sessions. During the sessions, no individuals other than the psychologist-experimenter, the veterinarian with expertise in animal behaviour and welfare and 4 PDD children were present. Dogs were free to choose their location and to act spontaneously in the room.

Saliva was collected at 3 time points: 15 minutes after arrival at the facility (T0), at the end of each reading session (T1), and 22 minutes after (T2). Stress-related behaviours (yawning, lip-licking, panting, withdrawal) [2] were measured via video-analysis (Observer XT, Noldus Information Technology, The Netherlands) as frequency and/or duration of occurrence during each reading session. One dog had higher salivary cortisol levels in T0 than in T1 and T2 compared to the other dog. However, no signs of behavioural or physiological stress, was detected in the dogs during and after the sessions. Thus, the presence of PDD children seemed not to be stressful for these animals. Further large-sample studies are needed for better understanding the benefits of the child-dog interaction to PDD children and the status of dogs during this particular activity, from a “One Health-One Welfare” perspective [4].

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BEHAVIOURAL AND PHYSIOLOGICAL SENSITIVITY TO BIOLOGICAL MOTION IN DOGS

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Biological motion perception refers to an organism's ability to identify biological stimuli from information about their motion. Experiments typically use moving light-point-displays, created by placing a small number of light-points in strategic locations on the human body to create the impression of a subject walking. So far, the majority of studies have been conducted on humans, revealing a preferential orientation towards the upright human displays compared to the same stimuli presented upside down, and this has been taken as evidence for identification of the upright stimulus as a human being. Only two studies investigated the perception of biological motion by dogs, producing inconsistent results [1,2]. Therefore, the aim of the current study was to determine what guides dogs' attention towards human light-point-figures, combining behavioral and physiological data. The experiment consisted of four presentations, where pairs of light-point-displays were projected on a screen for 30 s. In two of the presentations, the stimuli pair were an upright human vs. an inverted human; in the other two presentations, the stimuli pair were an upright human vs. an upright dog. The trial order was randomized and counter-balanced within the sample. The stimuli were the size of a real human, approximately 1.75 m in height, and a mid-sized dog, approximately 60 cm high at the shoulder. Dogs' orientation towards the stimuli or elsewhere was collected during the 30 s. Thermal images of the lacrimal caruncle were collected before the onset and after the offset of each presentation, to determine changes in eye temperature as a function of stimuli presentation. Findings revealed that dogs (n=24) looked longer towards the inverted human figures compared to the upright human figures, but only when these stimuli were presented first or second (P=.049). When dogs were presented with the upright dog and human light-point-figures, they directed significantly more attention towards the dog, regardless of the presentation order (P=.032). Thermal imaging data also coincided with behavioral data revealing that eye temperature significantly increased after the presentation of the upright and inverted human, but only when these were presented first or second (P=.012). It also increased when dogs were presented with the upright human and dog light-point-figures regardless of the order of presentation (P=.002). The combination of physiological and behavioral data indicates that dogs' preferential orientation is associated with sympathetic activation. In turn, this suggests that dogs' orienting behavior in this context is guided more by the stimuli being perceived as a novel, strange and/or potentially threatening (i.e. the inverted human and the dog). These results may justify the lack of clear orientation responses in previous studies on dogs' perception of biological motion and must be taken into account in further experiments on this topic. Finally, the results indicate that measurement of eye temperature is a useful complement to the interpretation of behavioral data in the study of dogs' visual perception.

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INVESTIGATION ON PAG-2 MRNA EXPRESSION IN WATER BUFFALO PBMC AND PMN FROM MATERNAL BLOOD AT THE PERI-IMPLANTATION PERIOD

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Pregnancy-associated glycoproteins (PAGs) constitute a large family of glycoproteins expressed in the outer epithelial layer of the placenta in eutherian species and are particularly numerous in ruminant ungulates. Phylogenetically PAGs can be defined as ancient (PAG-2) and modern (PAG-1). PAG-1 are produced in binucleate cells of both intercoltiledonary and cotyledonary chorion, while PAG-2 molecules are produced by both mononucleate and binucleate trophoblastic cells. The main objective of this study was to assess PAG-2 mRNA expression in maternal blood cells at the peri-implantation period in water buffalo; moreover, we wanted evaluate the earliest time in which PAG-2 could be detected in maternal blood. Thirty-two lactating buffaloes artificially inseminated were utilized. Blood was collected at Days 0, 14, 18, 28, 40 from AI (AI= Day 0). Pregnancy was diagnosed by ultrasound at days 28 and 40 from AI. Peripheral Blood Mononuclear Cells (PBMC) were recovered utilizing a density gradient separation (Lymphoprep™). Polymorphonuclear leukocytes (PMN) were isolated from the pellet at the bottom of the gradient tube after the lysis of erythrocytes. The purity of PBMC and PMN fractions was evaluated by flow cytometry. Expression of mRNA in blood cells fractions was measured by real-time PCR. Plasma PAG-2 concentrations were determined by RIA using bovine PAG as standard and tracer. PAG-2 values ≥1 ng/mL were considered indicative of conception. Out of the 32 buffaloes utilized for the study, 14 were confirmed pregnant (P group) and 18 were diagnosed not pregnant (NP group). Significant differences between P and NP buffaloes in plasma PAG-2 concentrations started from day 28 post AI ($P<0.01$) but the threshold of 1.0 ng/mL in P group was reached only a day 40, when sensitivity and specificity were 79% and 100%, respectively. Unlike PAG- 1 that is highly accurate for diagnosing pregnancy in buffalo starting from day 28 of gestation [1], PAG-2 resulted to be useful for pregnancy diagnosis only from 40 days. PAG-2 mRNA expression differed between P and NP groups, either evaluated in PMN or PBMC, starting from day 14 ($P<0.001$). At this period, the cut off values of 0.59 and 0.70 $2^{-\Delta Ct}$ in PMN and PBMC, respectively, showed a perfect discrimination capacity (sensitivity = 100%, specificity = 100%; AUC=1; CI 95%: 1-1; $P<0.05$). However, both estimated marginal means and multiple comparisons showed that PAG-2 mRNA expression was higher in PMN than PBMC ($P<0.001$). Differently from the appearance of PAG-2 in the blood, in the present study, an early expression of PAG-2 mRNA at Day 14 post-AI was observed. Despite further research is undoubtedly required, PAG-2 mRNA in peripheral blood leukocytes could be a promising candidate for the early detection of pregnancy and to better understand the role that the PAGs play during pregnancy in buffalo.

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THE INTERFERON GAMMA RESPONSE TO MYCOBACTERIUM AVIUM IN VITRO CAN BE CORRELATED WITH A HIGHER RISK OF CLINICAL KETOSIS IN DAIRY COWS

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Ketosis is a common metabolic disease often associated with reduction of immune competence and linked to other pathologies (mainly mastitis and metritis) of the transition dairy cow. These may be preceded by innate immune responses well before parturition [1,2]. The aim of this study was to evaluate the association between clinical ketosis after calving and adaptive immune responses during the transition period. Thirteen pluriparous Italian Friesian dairy cows were monitored from 21 days before till 28 days after calving. Cows were housed in a tie-stall barn with ad libitum feed and water. Diets were formulated to cover requirements according to the National Research Council Recommendations. During the trial, the animals' health status was checked every day and daily feed intake and rumination were recorded. After calving the cows were milked twice a day, and milk yield was recorded. Blood was collected from the jugular vein before the morning feeding, at different days from calving (DFC) from -21 till 28 DFC. Plasma samples were analyzed by a clinical analyzer (ILAB 650, Instrumentation Laboratory, USA) for energy parameters (glucose, NEFA and BHBA). Moreover, at -21 and 28 DFC a blood tube was collected and used in an IFN-gamma (IFNG) release assay for *Mycobacterium avium* subsp*. avium* on heparinized whole blood (internal method IZSLER, MP 13/011). Results were evaluated in terms of Delta OD (difference for IFNG between avian PPD-stimulated and control wells). Cows were retrospectively grouped according to their plasma BHBA concentrations after calving in Control (CTR, BHBA<1.4 mmol/L; 7 cows) and Ketosis (KET, BHBA>1.4 mmol/L; 6 cows). Data were analysed as a repeated measures study using the MIXED procedure of SAS with group (CTR or KET) as fixed effect. KET cows shower a lower feed intake during the 1st month of lactation with difference at 3 and 4 weeks after calving ($P<0.05$). Moreover, KET cows showed a lower milk production at 4th week (37.6 vs 40.3 kg/d of CTR, respectively; $P<0.1$) and, a lower rumination time (453 vs. 500 min/day of CTR; $P<0.1$). The markers of energetic metabolism confirmed the worse negative energy balance after calving in KET cows. In particular, BHBA concentrations were higher from -7 to 14 DFC ($P<0.05$), NEFA levels were higher from 0 to 28 DFC ($P<0.05$) and glucose concentrations were lower at 3 DFC ($P<0.05$). The IFNG response during challenge showed differences, both before (-21 DFC) and after calving (28 DFC), delta OD being higher in KET compared to CTR cows ($P<0.05$). In practice, cows mounting a higher IFNG response to *Mycobacterium avium* subsp*. avium* showed a higher risk to develop ketosis after calving. Considering that IFNG is related to metabolic pathways of energy use, a vigorous IFNG response to environmental microbial stressors may represent a risk after calving, when homeostatic control circuits are less effective. Our study confirms that peculiar features of the immune response of cows can affect the responses to metabolic changes after parturition.

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WATER BUFFALO SUBCLINICAL MASTITIS: CHANGES OF MILK MICROBIOTA AFTER TREATMENT WITH *Lactobacillus rhamnosus*

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Water buffalo mastitis represents a major issue in terms of animal health, cost of therapy, premature culling and decreased milk yield. The emergence of antibiotic resistance has led to investigating strategies in order to avoid or minimize the antibiotic use, especially during subclinical mastitis disease [1]. Use of bacteriophages, vaccines, nanoparticles, cytokines and also lactic acid bacteria may represent alternative strategies to antibiotics. *Lactobacillus rhamnosus* is part of the normal gut microflora, having meanwhile an immunostimulatory activity. The aim of this study was to investigate the change of milk microbiota after the therapeutic treatment with *Lactobacillus rhamnosus* of mammary gland quarters affected by sub-clinical mastitis. A number of 64 quarters were included in the study, of which 43 affected by sub-clinical mastitis (no signs of clinical mastitis and aerobic culture positive for udder pathogens) and 21 quarters were healthy (with no clinical signs of mastitis during the present lactation, with two consecutive Somatic Cell Counts (SCC) values lower than 500,000 cells/ml and aerobic culture negative for udder pathogens (H)). The experimental design was as follows: of subclinical mastitis quarters, a total of 11 were treated with antibiotics (SC-AB), 15 with *Lactobacillus rhamnosus* (SC-LB) and 17 with PBS as control (SC-PBS). Of healthy quarters, a total of 11 were treated with *Lactobacillus rhamnosus* (H-LB), and 10 with PBS as negative control (H-PBS). Samples were collected at two time points, T0 and T5 (days) and V4 region of 16S rRNA gene was amplified by PCR and sequenced using Ion Torrent Personal Genome Machine. Raw data were analyzed using Quantitative Insight Into Microbial Ecology 2 software. As determined at T0, the healthy core milk microbiota, defined as the asset of microorganisms shared by all samples, was represented by *Corynebacterium* and *Propionibacterium*. On the contrary, no genera were shared between subclinical mastitis milk quarters samples. Alpha and beta diversity, which highlights differences within and among samples, showed statistically significance differences between healthy and subclinical mastitis samples at T0 ($p=0.01$ for Shannon index and $p=0.007$ for Weighted Unifrac Distance Matrix, respectively). The microbiota structure of subclinical mastitis quarters changed after antibiotic treatment ($p=0.008$ for Weighted Unifrac distance matrix). The abundance of *Staphylococcus* decreased from 40.8% in SC-AB-T0 to 3.4% in SC-AB-T5 group ($p=0.008$), while *Methylobacterium* increased from 0.9% to 6.5% % in SC-AB-T5 group ($p=0.01$). On the contrary, the treatment with *Lactobacillus rhamnosus* did not alter the microbiota structure of subclinical mastitis quarters; interestingly, at taxonomic level, *Pseudomonas* increased from 1.3% to 4% in T5 ($p=0.02$). Microbiota structure of healthy quarters did not change after *Lactobacillus rhamnosus* treatment: at taxonomic level, *Lactobacillus* and *Sphyngomonas* increased their relative abundance from T0 to T5 ($p<0.05$). In conclusion, healthy quarters treated with *Lactobacillus rhamnosus* showed no alteration in milk microbiota; however, in subclinical mastitis quarters, these bacteria did not seem to improve the original quarter status. More studies are needed to investigate the probiotic role in mastitis treatment.

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ROLE OF IGA ANTIBODIES IN PIG ORAL FLUIDS FOR THE CONTROL OF PRRS VIRUS INFECTION

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The Porcine Reproductive and Respiratory Syndrome (PRRS) is a complex model of host/virus relationship, in which the role of adaptive immunity after infection is ill-defined [1]. More convincingly, clinical protection of pigs can be accounted for by (A) a reduced susceptibility of macrophages to PRRSV infection and replication; (B) an effective control over the inflammatory response caused by PRRSV and other environmental, infectious and non-infectious stressors. This kind of adaptation is likely to take place during the so-called "acclimatization" of gilts, i.e. the controlled exposure of PRRS-naïve gilts to PRRSV-infected pigs before the breeding period. In this respect, we had repeatedly observed on farm a clear correlation between a balanced IgA to IgG anti-PRRSV Ab ratio in oral fluids (OF) and block of environmental virus spread through OF samples of PRRSV-infected gilts. Vice versa, OF samples with peak IgG titers were correlated with peak PRRSV titers in OF, in agreement with major antibody-dependent enhancement (ADE) of virus replication (Amadori M., submitted).

Owing to the above, we decided to investigate the neutralization of PRRSV by OF samples with widely different IgA/IgG Ab titers to PRRSV in terms of s/p ratios in ELISA.

IgA and IgG Ab to PRRSV in OF were measured by ELISA (kit IDEXX P3) with anti-Ig isotype conjugates. After overnight antibiotic treatment, OF samples were tested for yield reduction of different PRRSV strains in swine pulmonary alveolar macrophages and monocyte-derived macrophages. Yield reduction of PRRSV was measured by Real-time RT-PCR . In agreement with our field studies, PRRSV yield reduction in pig macrophage cultures was shown to be critically dependent on the Ig antibody isotypes in OF samples, ADE being associated with IgG-rich samples. Most important, the extent of these effects varied as a function of the susceptibility to PRRSV replication of different macrophage batches, and also of the PRRSV strains under study. In particular, we could discriminate between ADE-positive and ADE-negative PRRSV strains. Next, we took to separating IgG and IgA in OF samples of PRRSV-infected pigs by means of protein A and size exclusion chromatography. The above results were confirmed by using separated Ig isotypes. In general, the combination of dimeric and monomeric IgA was correlated with the strongest reduction of PRRSV replication. Finally, we decided to check yield reduction in pig macrophages, pre-treated with separated Ig isotypes, washed, and then infected with different PRRSV strains. This treatment was also correlated with a substantial PRRSV yield reduction, which was Ab isotype and PRRSV strain-dependent, and went along with a down-regulation of CD163 and CD169 surface expression. On the whole, our data point at major role of IgA in the control of PRRSV excretion. This might be accomplished by extra or intracellular interaction of IgA Ab with PRRSV, as well as by signals leading to a reduced susceptibility of macrophages to PRRSV infection. Accordingly, major epigenetic regulations in pig macrophages exposed to PRRSV and/or OF antibodies should be investigated in the near future.

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CARACTERIZATION OF CF33 IN VITRO MODELS OF CANINE BREAST CANCER

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Mammary gland tumors (MGTs) are the most common neoplasms occurring in dogs; they are malignant in about 50% of the cases. Many studies on MGTs employed the CF33 cell line for in vitro tests; however, little is known about gene expression of parameters involved in the innate immune response, in DNA repair, in cell cycle regulation, in the ability to secrete cytokines, or in response to infectious or non-infectious stressor (1). The aim of our study was to evaluate the basal level of protein release and gene expression of pivotal molecules in the innate immune response and cell cycle regulation. We also investigated the ability of this cell line to respond to an infectious stressor. To this purpose, we performed two experiments; first, to evaluate the basal level of gene expression we selected a set of 31 immune-related and epithelial gene transcripts: the immune-related group were TNFA, IFNG, IFNb, IL1B, IL2, IL4, IL5, IL6, IL8, IL10, IL12, IL15, IL16, IL17, IL18, IL23, IL27, MYD88, NFKb/p65, TLR4, TLR5, MD2 and CD14, and the epithelial group were CD44, CXCR4, RAD51, p53, PTEN, Erb2, TGFB, BCRA. CF33 cells were grown until confluence at 37°C in DMEM enriched with 10% (v/v) fetal calf serum, a mixture of antibiotics and L-Glutamine 4 mM/L. Cells were tested at 37°, 39° and 42° passages; each experiment was repeated ten times. Next, we evaluated the ability of this cell line to respond to *S. typhimurium* and *S. monofasica*. Bacteria were sub-cultured for 2 h a 37°C and re-suspended at MOI 100 in DMEM and used to infect cells; untreated cells were employed as negative control. Bacterial penetration and innate immune response were evaluated as previously described (2). Differences between results were checked for significant differences by ANOVA. The significance threshold was set at $P<0.05$. Total RNA extraction, RT-PCR and set-up of Real Time qPCR reactions were done as previously described (3). Ribosomal protein S5 was used as housekeeping gene. All the genes under study were expressed with the exception of IL1B, IL-2, IL10, IL15, IL17, IL-18, IL27, IFNG, p53 and CXCR4. In particular, TGFB was expressed in 12 out of 30 samples, IL4 in 16 of 30 wells, IL6 in 14 of 30 samples, IL12 in 4 out of 30, IL-23 in 10 out of 30 while TNFA was unexpressed in 20 out of 30 wells. Regarding TLRs, TLR4 was expressed in 2 out of 30 samples and TLR5 in 4 out of 30 wells. NFKb/p65 was expressed in 12 out of 30 wells, CD14 in 8 out of 30 and ErbB2 in 6 out of 30. The other genes under study were expressed in all samples. Concerning *Salmonella* treatment both strains caused up-regulation of IL-8 ($P=0.0039$) and CD14 ($P=0.0060$) while we observed down-regulation of MYD88 ($P=0.0410$), NFKb/p65 ($P=0.0458$), p53 ($P=0.0120$), MD2 ($P=0.0310$) and TLR5 ($P=0.0022$). Our results outline the basal expression in CF33 of important genes involved in innate immune response and the ability of this cell line to respond to an infectious stressor. Moreover, we observed the ability of different strains of Salmonella to cause an inflammatory response in this cancer cell line.

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MODULATION OF INNATE IMMUNITY IN KIDNEY EPITHELIAL CELLS BY INFECTIVE AND NON-INFECTIVE STRESSOR

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Madin-Darby Canine Kidney (MDCK) are cell lines commonly used as models to study the characteristics of epithelial cells [1], they are involved to study viral infection of cells and in vaccine production [2]. Previous studies suggest the sensibility of MDCK to invasion and penetration of bacterial strain and the constitutive expression in MDCK of genes involved in the innate immunity response and cell cycle regulation [3]. However, there are no data about the ability of these cell line to respond to infective and non-infective stressor. The aim of this study was to evaluate the MDCK cells innate immunity response to non-infectious stressor (Cd2+) and infectious stressor (LPS). To this purpose we performed two experiments; in the first step we evaluated the ability of MDCK to respond to Cadmium (Cd2+) a non-infectious stressor, that at molecular level induces a cellular stress Cells were treated with 20 µM of Cd^{2+} dissolved in DMEM culture medium. After 3 h and 24 h of treatment at 37°C in 5% CO_2, mRNA was extracted to test gene expression. The second experiment provided the treatment of cells with 1 µg/mL of lipopolysaccharide (LPS) from *Escherichia coli* O111:B4 recognized an infectious stressor. After 3 h and 24 h of incubation t 37°C in 5% CO_2 mRNA was extracted to study gene expression. In both experiments we tested the modulation of followed parameters involved in innate immune response: IL-8, IL-6, IL-1β, TLR1, TLR3, TLR5, TLR9, INOS, CD14, MYD88, P65, TLR4, MD2, IL-18. Experiments were carried out in quintuplicate; cells treated with medium only were used as negative control. Each test was repeated twice. After 3 h of Cd^{2+} treatment we observed up regulation ($P<0.05$) of important pro-inflammatory cytokines (IL-8, IL-6, IL1β), INOS, TLRs (TLR1, 9, 3, 5) and down regulation of CD14. Cd^{2+} treatment at 24 h determined up regulation of MYD88, p65, IL-18 and down regulation of INOS, IL-1β, MD2 and TLRs. After 3 h of LPS treatment we detected up regulation ($P<0.05$) of MYD88 and down regulation of INOS, CD14, IL1β, TLRs (TLR 5, 4). No significant differences were reported after 24 h of treatment. Our results show the ability of MDCK to respond to infectious and non-infectious stressor; in particular, Cd^{2+} and LPS to modulate innate immune response in terms of gene expression as a function of time. These data suggest a possible alteration of host-pathogen interactions due to inflammatory response and modulation of TLRs expression.

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Yersinia enterocolitica INTERACTION WITH JEJUNAL EPITHELIAL CELLS

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Yersinia enterocolitica are zoonotic bacteria able to infect humans and animals, recognized as the third cause of foodborne disease in Europe (EU) in terms of prevalence (1). Important studies highlighted the molecular basis of pathogenesis of *Y. enterocolitica* infection, while scanty data are available about other environmental 1A biotypes, often isolated in cases of foodborne disease but not included in pathogenicity studies. Owing to the above, the aim of our work was to verify the modulation of intestinal innate immunity by different *Y. enterocolitica* strains. In our study, overnight cultures of 5 different *Y. enterocolitica* strains: 1B (O:8, ail+, ystA+, inv+, myfA+, ymoA+); 1A (O:9, ystB+, inv+, ymoA+); 1A (O:5, ail+, ystB+, inv+, myfA+, ymoA+); 1A (O:8, ystB+, inv+, myfA+, ymoA+); 1A (O:5, ystA+, ystB+, inv+, myfA+, ymoA+) isolated from wild boar livers were sub-cultured for 1 h at 37°C in BHI medium. Each bacterial strain was re-suspended at 100,000,000 CFU/ml in DMEM/F12 medium (2) and used to infect pig intestinal IPEC-J2 cells; untreated cells were employed as negative control. Innate immune responses were evaluated by real time PCR as previously described (2,3). Differences between data sets were checked for significant differences by ANOVA, and the significance threshold was set at $P<0.05$. Our results showed different abilities to modulate gene expression by the strains under study with respect to controls. In particular, *Y. enterocolitica* 1B determined a pro-inflammatory effect characterized by up-regulation of IL-8 ($P<0.0001$) and TNF-α ($P=0.0024$), and decrease of antimicrobial peptide gene expression: bD3 ($P=0.049$), bD4 ($P=0.049$). At the same time we observed down-regulation of CD14 ($P=0.011$), MD2 ($P=0.004$), TLR1 ($P=0.0415$), TLR4 ($P=0.0038$) and TLR5($P=0.0360$). *Y. enterocolitica* 1A strain 1 caused a pro-inflammatory response with increased expression of IL-8 ($P=0.0002$), TNF-α ($P= 0.0045$), bD1 ($P=0.049$), bD2 ($P<0.0001$) and down-regulation of NF-Kb1 ($P=0.0130$), bD4 ($P=0.0164$), MD2 ($P=0.0397$), and TLR4 ($P=0.0242$). *Y. enterocolitica* 1A strain 2 caused pro-inflammatory response with increased expression of IL-1β ($P=0.0168$), IL-8 (<0.0001), TNF-α ($P= 0.0076$), bD1 ($P=0.0010$), bD3 ($P=0.0086$) and down regulation of NF-Kb1 ($P=0.0392$), MYD88 ($P=0.0425$), MD2 ($P=0.0429$), TLR1 ($P=0.0256$) and TLR4 ($P=0.0212$). *Y. enterocolitica* 1A strain 3 determined a pro-inflammatory effect characterized by up-regulation of IL-1β ($P=0.0309$), IL-8 ($P=0.0493$), IL-18 ($P=0.0138$), and decrease of antimicrobial peptide gene expression: bD3 ($P=0.0010$), bD4 (P=0.08t). At the same time we observed down-regulation of CD14 ($P=0.011$), MD2 ($P=0.0132$), TLR1 ($P=0.0029$), TLR4 ($P=0.048$) and TLR5($P=0.0123$). *Y. enterocolitica* 1A strain 4 determined a pro-inflammatory effect characterized by up-regulation of IL-8 ($P<0.0001$), TNF-α ($P=0.0071$), and decrease of antimicrobial peptide gene expression: bD1 ($P=0.0078$), bD3 ($P=0.0163$). The adopted cell line had been shown to give valuable information about pathogenicity of bacteria (4). Our data suggest a potential pathogenic role of 2 out of 4 *Y. enterocolitica* 1A strains under study and different interactions with the host.

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MASTITIS RESISTENCE IN HOLSTEIN AND IN RENDENA CATTLE BREEDS

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The selective pressure for increased milk production in Holstein Friesian dairy cows has led to their higher propensity to develop diseases in the transition period, including mastitis, when compared to less selected and lesser producing dairy breeds which are typically characterized by a higher resistance to diseases. With the aim of investigating the factors associated to this phenomenon, this study applied a multidisciplinary approach to compare innate immune response patterns, metabolic parameters, milk protein profiles and the milk microbiota in 6 Holstein and 4 Rendena cows reared in the same farm and under the same management conditions. Quarter milk samples and blood plasma were collected from all cows at dry-off (T1), and 1 (T2), 7-10 (T3) and 30 days after calving (T4). Quarter milk samples were subjected to bacteriological culture, characterization of the milk microbiota by 16S metagenomics, milk protein profiling by electrophoresis and densitometry, somatic cell counting, measurement of the inflammation marker cathelicidin and assessment of different innate immune-related mediators such as lysozyme, CD45, IL-1β, TNF-α, PTX3, IL-1R8. In parallel, the main inflammometabolic parameters were measured in blood plasma samples. Holstein cows showed a more severe fat mobilization and systemic inflammatory response at T2 and T3 in comparison with Rendena cows. Rendena cows showed a greater muscle mass (i.e. higher creatinine) and an increased amino acid mobilization immediately after calving compared to Holstein. Upon bacteriological analysis, contagious bacteria such as *Staphylococcus aureus* and *Streptococcus agalactiae* were absent, but significant differences were seen in the general composition of the milk microbiota of the two breeds. The taxonomic profiles of both breeds were dominated by *Firmicutes* (mostly *Streptococcus* (average HF=27.5%, REN=68.6%)), followed by *Proteobacteria*, *Bacteriodetes*, and *Actinobacteria*. However, Rendena cows showed a lower microbial diversity and a more stable microbiota along the transition period. Concerning the milk protein abundance profile, pronounced differences were seen in colostrum (T2), with significantly higher amounts of immunoglobulins and other immune-related proteins in Rendena. Adding to this, the expression of innate immune related genes such as PTX-3, IL-1β, TNF-α, as well as the CD45/KRT5 expression ratio in milk cells, indicating the epithelial and leukocyte components, respectively, was lower in Holstein Friesian compared with Rendena at T2. In conclusion, several differences were observed among breeds, in spite of the same farming conditions. The observations reported in this work present numerous hints on the factors that may provide autochthonous, more rustic breeds with a higher resistance to metabolic diseases and mastitis.

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SCIENZE CLINICHE: SICLIM-VET

EFFECTS OF FOOT DISORDERS ON MEDITERRANEAN BUFFALO HEALTH AND WELFARE

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Lameness represents the third most important health-related cause of economic loss in the dairy industry after fertility and mastitis [1]. Although dairy Mediterranean Buffaloes (MB) and dairy cows share similar breeding systems predisposing to similar herd problems [2], published studies exploring its relevance and role in these ruminants are still rare and incomplete [3]. The aims of this study were to describe the clinical findings of foot disorders (FDs) in dairy MB and their influence on animal welfare, determined by assessment of locomotion score (LS), body condition score (BCS) and cleanliness score (CS). Of 1297 multiparous MB submitted to routine trimming procedures, 229 buffaloes showed at least one FD. The prevalence of buffaloes affected by FDs was 17.7%, while motility and lameness indexes were 84.1% (1091/1297) and 15.9% (206/1297), respectively. Overgrowth was present in 17.0% (220/1297), corkscrew claw in15.8% (205/1297), interdigital phlegmon in 0.9% (12/1297), white line abscess in 0.8% (11/1297), digital dermatitis in 0.1% (1/1297) and interdigital hyperplasia in 0.1% (1/1297). Simultaneous presence of FDs was recorded in 17.0% of MB (221/1297): overgrowth and corkscrew claw occurred together in 15.8% of cases (205/1297), overgrowth and interdigital phlegmon in 0.3% (4/1297), overgrowth and white line abscess in 0.8 (11/1297), digital dermatitis and interdigital hyperplasia in 0.1% (1/1297). The presence of FDs was always associated with lameness (LS>2), except from 23 MB with simultaneous overgrowth and interdigital phlegmon occurrence. The majority of MB within the under-conditioned group (95.5%, 43/45) and all those with CS>2 (122/122) had a locomotion score above the threshold of normality (LS>2). Furthermore, foot diseases such as interdigital hyperplasia, white line abscess and digital dermatitis or interdigital hyperplasia seemed to occur more frequently associated with decreased BCS and increased CS scores. This study describes for the first time the involvement of white line disease, interdigital phlegmona, digital dermatitis and interdigital hyperplasia in foot disorders of dairy Mediterranean buffalo and shows their association with an impairment of animal welfare.

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INFLUENCE OF *LACTOBACILLUS KEFIRI* ON GUT IGA SECRETION IN HEALTHY DOGS

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Gut microbiota plays an important role in absorption and metabolism of nutrients and has a trophic and protective function of the host. Commensal bacteria interact with cells of the intestinal immune system and regulate, among other things, the IgA secretion [1]. IgA provides intestinal immune protection, and it is supposed that lack of IgA in dog is correlated with chronic enteropathies [2]. *Lactobacillus kefiri* (Lk) is a probiotic isolated from kefir grains, fermented milks with a complex symbiotic microbiota, and approved for human use (Kefibios – Hulka srl - LKF01). It is able, in mice, to downregulate expression of proinflammatory mediators and to increase anti-inflammatory molecules in gut immune system, regulating intestinal homeostasis, incrementing IgA production and mucin induction [3]. No information about the influence of Lk administration on canine intestinal health has been already reported. The aim of this study was to verify the safety of Lk in dogs and its effect on the fecal IgA content. The study, authorized by the Animal Welfare Committee – UNIBO (Prot.3885 – July 21th 2017), included ten healthy dogs (6 pure breed and 4 mixed breed, 4 males and 6 females, median age 4 years and 1 month (min 2 years and 5 months – max 9 years and 8 months) without history of gastrointestinal diseases. The dogs received Lk at the dose of one billion of live microorganism, orally once daily for 30 days. Fecal samples were collected, and immediately frozen, from each dog in four periods (each time for three following days), before the beginning (T0), in the middle (T15), at the end (T30) of the Lk treatment and 30 days after Lk discontinuation (T60). Fecal IgA was measured by a dog specific IgA Elisa test. Results were statistically evaluated with D'Agostino & Pearson omnibus normality test, Friedman's test and Dunn's test. During treatment no dogs showed any side effect and fecal scores were constantly normal. Certain variability on IgA content (expressed as mg/g of dry feces) was present among the three following day samples and a high variability was observed among dogs at each experimental point (T0; T15; T30; T60). Mean value of fecal IgA reached the maximum value at the end of treatment (T30 - 15.48±17.34 mg/g), and then it was reduced at T60 (8.41±9.33 mg/g), without significant differences (p=0.62).

Our results suggest that Lk is a safe probiotic in dogs. The study confirms the needs of multiple day replicates to get a reliable information on fecal IgA content, but the variation of fecal IgA during treatment is not statistically significant; this could be due to the small number of patients included in the study or to their age and breed variability. Therefore, further studies are needed to clarify the role of this probiotic on dog's intestinal homeostasis.

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PACKED RED BLOOD CELLS AND STORED WHOLE BLOOD TRANSFUSIONS IN DOGS. A RETROSPECTIVE STUDY IN 86 RECIPIENTS TRANSFUSED AT A CANADIAN VETERINARY TEACHING HOSPITAL

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Veterinary transfusion medicine is an essential part of therapy for critical patients in small animals. Many updates have been made in recent years and blood transfusion (either packed red blood cell, PRBC or stored whole blood, SWB) has been used to treat different disorders inducing canine anemia to improve oxygen delivery to the peripheral tissues (1,2,3). The aims of this study were to classify anemia in transfused-recipients and to analyze the hematocrit (Hct) value increase post-transfusion in relation to: pre-transfusion Hct, type of anemia (acute or chronic), type of product used (PRBC or SWB), donor's Hct and storage age of the blood product. Medical records of 86 patients (different for breed, gender and age) who received about 10-20 ml/kg of PRBC or FWB were collected at the Veterinary Teaching Hospital of the University of Montreal, Canada. The anemia was classified according to Hct, MCV, MCHC, and regeneration degree. The pre-transfusion Hct (T0) was compared to post-transfusion Hct at T1 (range 0-4h), T2 (4-16h), T3 (16-34h), T4 (34-50h) and T5 (more than 50h after transfusion) (Spearman test). The pre-transfusion Hct was compared to post-transfusion Hct in dogs with acute or chronic anemia transfused with either PRBC (n. 100) or SWB (n. 19). Furthermore, the influence of the storage and Hct of blood donor (blood products) used on post-transfusion Hct was assessed (Mann-Whitney test).The main cause for transfusion was acute and severe, macrocytic and hypochromic anemia with a moderate-high grade of regeneration due to hemorrhage. Pre-transfusion Hct of the recipient influenced the degree of the increase of post-transfusion Hct only in chronic anemia (moderately inversely proportional at T1 and T4, rho >-0.500; and strongly inversely proportional at T5, rho <-0.600). The type of blood product did not affect post-transfusion Hct. There were no differences between the increases of Hct in recipient affected by acute or chronic anemia receiving blood products with different Hct values. The increase in Hct post-transfusion was greater when less than 10-days old stored blood product was used (P=0.0329). In chronic anemia, lower pre-transfusion Hct was observed which was related with greater increase of post-transfusion Hct. In both chronic and acute anemias, both PRBC and SWB showed to be useful for improving the post-transfusion Hct. The use of blood products less than 10 days old was associated with a greater increase in post-transfusion Hct, possibly related to a greater viability of the transfusion cells less affected by "storage lesion".

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EVALUATION OF CARDIAC ARRHYTHMIAS BEFORE, DURING AND AFTER TREADMILL EXERCISE TESTING IN POORLY PERFORMING STANDARDBRED RACEHORSES

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In sport horses, the exact influence of premature depolarizations (PDs) on athletic performance is still not well defined [1-5]. The aims of the present study are to report the prevalence of the most common cardiac arrhythmias detected during treadmill exercise in poorly performing Standardbreds, and to investigate possible differences, regarding some demographic, cardiac and performance indices, between horses with or without PDs. The study was conducted on 158 poorly performing Standardbred trotters, in which obvious causes of poor performance at rest were ruled out and plasma lactate curve threshold during incremental exercise test on a high-speed treadmill [6], and simultaneous Holter recording [7], were obtained. Statistical analyses (descriptive, exploratory, and multiple logistic regression) were performed to verify possible differences between horses with or without PDs regarding sex, age, minimum and maximum heart rate, difference between maximum heart rate and heart rate at 1 minute of recovery, maximum speed, maximum plasma lactate concentration, and time to fatigue. Fifty horses did not show any type of arrhythmia while 108 subjects had at least one type of arrhythmia. Supraventricular PDs (27/158, 17.1%) and ventricular PDs (66/158, 41.8%) were mainly observed during the first minute post exercise and in a lesser number of subjects during peak exercise. PDs were mainly observed as single or isolated premature beats, but also as pairs (24 horses) and as paroxysm of supraventricular and/or ventricular tachycardia (4 cases). Univariate statistical analysis (exploratory analyses) showed no differences regarding demographic, cardiac and performance indices, between horses with or without PDs; multiple regression analysis showed weak or borderline evidence of dependence of PDs occurrence from age (P=0.08), minimum heart rate (P=0.04), and maximum plasma lactate concentration (P=0.08). Our data suggest that PDs are frequently recorded in poorly performing Standardbred racehorses, but the results obtained in the present study did not clarify their role and clinical significance. Nevertheless, we would like to suggest that the presence of PDs in racehorses, recorded during and after strenuous exercise, should be further investigated.

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CLINICAL AND IMMUNOPHENOTYPICAL FINDINGS IN FOUR HORSES WITH DIFFERENT FORMS OF LYMPHOMA

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Lymphoma is the most common hematopoietic neoplasia in the horse. The anatomic site defines lymphoma as multicentric (generalized), alimentary (intestinal), mediastinal, cutaneous and solitary/atypical form [1]. No sex, breed or age predisposition have been found and there are no known etiological agents [2]. We describe the clinical, histological and immunophenotypical features of 4 cases found in the equine database of the Department of Veterinary Medicine of the University of Perugia (OVUD) to increases the knowledge about equine lymphoma. The patients were 3 geldings (Horse1; Horse2; Horse3) and 1 mare (Horse4), aged 7 to 26 years. The diagnosis was achieved through clinical findings, haematological results, cytological, histological and immunohistochemical evaluation for all cases while flow cytometric analysis (FC) was performed only for Horse2 and Horse4. DNA extraction from the neoplastic samples was also performed for all cases and a semi-nested PCR protocol targeting a conserved fragment of the glycoprotein B (gB) of Equid Herpesvirus type 5 (EHV-5) was performed. Anemia was identified in two horses (2 and 3), hyperfibrinogenemia and increased LDH activity were found in all patients. Based on the results, Horse1 had an alimentary form (small intestine) of lymphoma (epitheliotropic T-cell lymphoma), Horse2 a splenic form characterized by an isolated mass (diffuse large B-cell lymphoma) and Horse3 and 4 were affected by isolated extraocular (third eyelid) lymphoma (T-cells lymphoma and T-cell rich large B-cell lymphoma, respectively). All neoplastic samples were positive for EHV-5. Horse 1 and 2 were humanely euthanized, Horse3 and 4 were surgically treated; unfortunately Horse 3 died after two months, whereas after two years Horse4 is still alive. Alimentary lymphoma is the most common intestinal neoplasia in the horse, even if the diffuse infiltration of the mucosa is not frequently reported in old horses as in our case [1]. Isolated splenic lymphoma is rarely reported in horses and, to our knowledge, this is the first case investigated with FC [1]. Extraocular lymphoma is generally related to a systemic form of lymphoma, but in Horse3-4 no evidence of other lesions except the eye was found. To the author's knowledge, Horse4 is the first case of T-cell rich large B-cell lymphoma of the third eyelid diagnosed in the horse [3]. As in human and canine lymphoma, the combined use of cytological, histological and immunophenotypical technique are strongly suggested to achieve a correct ante-mortem diagnosis. In human and canine lymphoma increased concentration of LDH is due to the arise of specific LDH-isoenzymes; more studies should be performed in the horse [4]. On the basis of recent studies, the implication of EHV-5 in the pathogenesis of equine lymphoma should be investigated [2].

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TIME OF VICTIM'S DNA PERMANENCE IN JAWS OF ATTACKER DOGS

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Dogs attacks against humans represent a serious social, healthy and legal issue. During the last ten years more than ten lethal attacks occurred in Italy [1-3]. In order to fight this serious phenomenon, we need to use severe civil and criminal punishments towards anyone may omit, because of fraud or negligence, any measure to prevent or avoid aggressiveness events. Often these events remain unpunished as consequence of the impossibility to identify the animal makers of the tragedy and, consequently, the legal entities who are responsible of the management of the same. In the event of a legal dispute, presence of the victim's DNA, in the jaw of the dog attacker, may represent an indisputable date in order to give the relevant responsibilities. The objective of this work is to confirm the possibility of revealing the presence of the DNA of a hypothetical victim in the jaws of an aggressor dog and quantify the time in order to be able to give a precise indication on the useful technical time to prove the participation of one or more animals to an attack on people. Ten dogs were given the opportunity to belabor a simulacrum of a victim consists of a piece of beef. Subsequently they were performed on these dogs of the dento-gingival swabs at established times. Swabs was performed is the DNA extraction with subsequent search of STRs cattle. Our analysis shows that the bovine DNA traces persist in the dogs mouth up to an hour and a half after the attack simulation. Therefore, the method of investigation we propose to prove the dog or the dogs responsible of the attack was revealed efficient, provided that the swabs are made promptly.

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SUCCESSFUL TREATMENT OF 15 CASES OF CANINE TRAUMATIC AURAL HEMATOMA USING AUTOLOGOUS PLATELET RICH PLASMA (PRP)

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Platelet-rich plasma (PRP) derived from whole blood, is characterized by platelet (PLT) concentrations above baseline in a small volume of plasma that can accelerate the healing process [1] by providing elevate concentration of platelet-derived growth factors [2] which can stimulate cell proliferation and decrease of inflammatory reaction. Following the excellent results obtained on a small number of subjects previously reported in a preliminary study [3], in this prospective in vivo study we aimed to describe the clinical efficacy of autologous PRP in the treatment of canine traumatic aural hematoma secondary to otitis externa. After approval by the Ethics Committee of the University of Milan and with the owner informed consent, 20 ml of citrate whole blood were obtained from the cephalic vein of 15 dogs with traumatic aural hematoma of different breeds, 6 males and 9 females, with a age range of 1-15 years (mean±DS: 7,4±3,7 years). All subjects had a history of multiple centesis, sometimes associated with injections of cortisone in situ, with subsequent recurrences of hematoma. PRP was produced using a semi-automatic closed system (CPUNT 20, Eltek group, Casale Monferrato, Alessandria, Italy) for veterinary use [4]. The serum-hematic content of the auricular pinna was completely drained using one or more 20G needles (depending of hematoma organization) and, using the same hole of the drainage inlet, the PRP was then injected. The dogs were subjected to weekly follow up for a minimum of 45 days from the first treatment. In case of partial or total recurrence of the aural hematoma of the first follow-up, the treatment was repeated with the same procedure. No dog has been subjected to anaesthesia during the procedures. At D0 the aural hematoma was present for 17±13 days and the mean of the drained serum-hematic content was 23±30 ml. Four dogs had a partially organized aural hematoma. 1.3±0.6 ml of PRP were injected, with a mean concentration of 1185±908x103/µl PLT (minimum value: 308x103/µl PLT maximum value: 4141x103/µl PLT, 500% mean increase compared to whole blood). 12/15 subjects were treated with a single application (Group A1), 3/15 with two applications (Group A2). 2/15 subjects were lost after the first follow up. For the remaining 13/15 the mean healing time was 15.8±8.1 days (A1) and 24±5.2 days (A2). No subjects showed recurrences at 45 days follow up. No side effects have been registered. The in situ administration of PRP was effective in the treatment of traumatic aural hematoma secondary to otitis externa in dogs, leading to complete resolution of the disease in all treated subjects.

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EXPRESSION OF GUANYLIN, UROGUANYLIN AND GUANYLATE CYCLASE-C RECEPTORS ON DIFFERENT GASTROINTESTINAL TRACTS OF HORSE

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Guanylate cyclase-C (GC-C) is a type I transmembrane receptor encoded by the GUCY2C gene (1). Activation of GC-C leads to the triggering of a signaling cascade causing an uncontrolled release of electrolytes and water into the intestinal lumen, resulting at last in secretory diarrhea and dehydration (1). GC-C can be activated by endogenous ligands, the hormones guanylin and uroguanylin, and is also involved in mucosal barrier function, inflammation, modulation of intestinal cell proliferation and pain sensation [1,2]. In human medicine, the understanding of activities triggered by GC-C activation, guided the development of novel therapeutic approach to constipation, which resulted in clinical use of linaclotide, a synthetic 14-peptide, potent and selective agonist of GC-C and, more recently, of plecanatide and dolcanatide [3,4]. The aim of this paper was to investigate the gene expression of the guaniline, uroguaniline and GC-C receptors in different gastrointestinal tracts of horse. Tissue samples were collected from six female adult horses slaughtered at a public abattoir with no history of gastrointestinal disorders. Full-thickness samples were collected within one hour after the death from stomach, duodenum, jejunum, ileum, head and body of cecum, left and right dorsal colon, left and right ventral colon, pelvic flexure, transverse colon, descending colon and rectum. For each sample, total RNA was extracted from 100 mg of ground tissue. Primers on genes of interest (GUCA2a - GUCA2b – GUCY2c) were designed based on available sequences. For each primer pair, a preliminary qRT-PCR assay on the tissue bulks was performed. The amplification was performed in a CFX96 Touch instrument (BioRad, Hercules, CA). Data analysis was carried out with Bio-Rad CFX Manager software (ver. 3.2.2). To assess gene expression stability of reference genes, geNorm algorithm, included on CFX Manager software, was used and the expression ratio of the genes of interest was normalized relative to the abundance of the two reference genes (SDHA, HPRT) using the ΔΔCq method. A common pattern of expression throughout the gastrointestinal lumen for all three investigated transcripts was found. The expression was higher in jejunum, ileum, descending colon and rectum and the tissue where these transcripts appear least expressed is the stomach. For this reason, stomach has been chosen as reference tissue and its expression set to 1. This study demonstrated the expression of gene encoding for guanylin, uroguanylin and guanylate cyclase-C receptor in intestinal tract of horses. The highest expressions were found in the distal tract of the small intestine and in the large intestine. To the authors' knowledge, this is the first study on the gene expression of the guaniline, uroguaniline and GC-C receptors in different gastrointestinal tracts of horse. These findings open new scenarios for the therapeutic approach to enteric diseases of horse.

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OZONE EYEDROPS FOR BACTERIAL KERATITIS IN ANIMALS: A CASE SERIES

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Bacterial keratitis and in turn conjunctivitis is one of the commonest anterior segment disorders encountered in animals and humans, traditionally managed with topical instillation of antibacterial, non-steroidal anti-inflammatory and anti-collagenase agents [1]. A topical dosage of one or two drops of collyrium every 4 hours over three to seven days is recommended, even though resolution multiple and frequent instillation are often required to achieve clinical [1]. Due to the increasing loss of antimicrobic drug efficacy related to their overuse and the appearance of multi-drug resistant bacterial strains, the research has been driven towards the study of new antimicrobial agents, possibly also equipped with anti-inflammatory effect [2]. Ozonated liposomal dispersions have the same properties of gaseous ozone, are well tolerated by biological tissues and have biological properties as antimicrobial activity, wound healing promotion and oxidative stress protection [3]. The purpose of this case series is to describe the use of liposomal ozone dispersion in spontaneous bacterial keratitis in animals. A horse, a cat and a dog spontaneously affected by bacterial keratitis received topical application of liposomal ozone dispersion (Ozodrop®; FB Vision, Ascoli Piceno, Italy) three times a day until clinical remission of symptoms. All patients well tolerated the therapy and completely recovered from the conditions in a time comparable to the standard therapy. All ozone activities are related to oxygenated compounds that are able to eliminate pathogens by direct oxidation mediated by hydrogen peroxide and lipoperoxyde, and selective cytotoxicity on fast dividing cells, through bacterial lysis and cell death, negative regulation on mitochondrial activity in yeast and mould and disturbance on virus lytic enzymes activity [4]. Moreover, ozone promotes wound healing by releasing oxygen (O2), platelet-derived growth factor (PDGF) and transforming grow factor β (TGF-β) [5]. This preliminary study suggests that liposomal ozone dispersion may be effective for the treatment of bacterial keratitis in animals, having bactericidal and anti-inflammatory activity, in addition to promoting tissue repair effect. All of these therapeutic effects are contained in a unique ocular preparation stable over time reducing the risk of loss of patient and owner compliance. Topical treatment with ozone liposomal dispersion may avoid or reduce the need for topical antimicrobial drugs, preventing the emergence of bacterial resistance.

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MALASSEZIA OVERGROWTH IN 97 DOGS IN NORTHERN ITALY

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Malassezia spp. yeasts are normal inhabitants of the canine skin surface and are usually considered opportunistic pathogens. *Malassezia* dermatitis is a skin disease associated with *Malassezia* overgrowth (MO) and usually shows a good clinical and cytological response to appropriate antifungal treatment. *M. pachydermatis* is the main species associated to MO in the dog [1]. The aim of this study is to identify and quantify Malassezia yeasts from dogs with or without dermatitis and/or otitis and to evaluate the correlation with clinical signs and previous treatments. Ninety-seven dogs were enrolled: 13 clinically healthy (HD) and 84 dogs with MO. Clinical history and previous treatments were recorded. After a complete physical and dermatological examination, CADESI-3 scores were calculated. Skin samples for cytological and mycological culture were obtained in all dogs from axillae, interdigital webs, ear pinnas and ear canals and from lesions where MO had been evidenced by cytology. *Malassezia* overgrowth was diagnosed by cytology when more than 2 and 10 yeasts with typical morphology were counted in 5 random fields at 40x magnification from skin and ear canals, respectively [2]. After culture, colonies were identified as belonging to *Malassezia* genus according to microscopic and macroscopic morphology. If the yeasts grew on the Sabouraud Dextrose Agar other than on modified Dixon's Agar were suspected to belong to the non-lipid dependent *M. pachydermatis* species. Final identification was confirmed with molecular biology methods. All counts data were reported as a percentage and compared by the chi-square test. Normal distribution of the data was tested using the Shapiro-Wilk test. A Global Score (GS) was calculated taking into account numbers of yeasts counted in sampled regions. The association between the GS of *Malassezia* and the CADESI-03 score in dogs with MO was evaluated by the Spearman correlation index. The effect of therapy on GS was tested with Mann-Whitney Test. The quantitative data (GS and CADESI-03) were analysed using ANOVA. P-value <0.05 was considered significant. Of the 419 examined slides, a total of 124 (31 HD and 93 with MO) were positive on cytology to *Malassezia* yeasts. The greatest frequency ($P<0.001$) of isolation of *Malassezia* spp. was from skin lesions. GSs obtained from HD and dogs with MO were significantly different ($P=0.001$). In dogs with MO, GS was significantly higher ($P=0.015$) in the subgroup treated with antibiotics in the previous 3 months. GS was not significantly affected by treatment with steroids. Of 142 swabs obtained from 52 animals (39 with MO and 13 HD) 26 plates were considered positive (>70 UFC). All isolates were identified as *M. pachydermatis*. The frequency of yeast isolation from diseased dogs in the present study was significantly higher ($P=0.05$) when compared with HD and the highest frequency of yeasts isolation in dogs with MO was found in areas with skin lesions.
This study provides helpful insights into the occurrence of *Malassezia* in HD and in dogs with MO. Previous antibiotic treatments seem to be associated with an increased number of *Malassezia* yeasts on the MO affected areas.

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TRACHEAL WASH METABOLOME IN HORSES WITH RECURRENT AIRWAYS OBSTRUCTION (RAO)

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Metabolomics consists of the analysis of all metabolites that are present within an organism that provide unique insights into metabolic changes associated with disease [1]. Because of the high diagnostic potential of metabolomic studies, already established in humans with respiratory disease [2], we aimed to investigate the metabolomic profile of tracheal wash (TW) in horses affected by recurrent airway obstruction (RAO). Six horses with known history of RAO (1 male, 2 geldings, 3 females), median age 18 years, BCS 2.5-3.0, represented the study group (Group S). Six healthy horses with no history of RAO (1 male, 3 geldings, 2 females), median age 13 years, BCS 3.0, represented the control group (Group C). Tracheal wash was performed as described by others [3]; 5ml aliquot of TW, collected into sterile tubes with no anticoagulant, was used for cytological evaluation immediately after collection, another 1.5ml aliquot was stored at -80°C for metabolomic analysis. Endoscopic assessment of tracheal mucus (Grade 2-3) and cytologic examination by differential count of TW smears (neutrophils>30%), confirmed the acute phase of RAO in Group S [3]. The Mann-Whitney test was applied to find significant differences in metabolites concentrations between groups. P values <0.05 were considered statistically significant. Metabolomic analysis, determined by ^{1}H-NMR [4], showed a total of 38 molecules in TW samples and 13 out of 38 showed significant differences between groups. Mean values ± standard deviations of significant different metabolites between groups are expressed as mM/L $x10^{-2}$: Histamine (Group S=2.7±0.8; Group C=1.5±0.2), Valine (Group S=1.8±1.2; Group C=0.6±0.3), Leucine (Group S=4.3±3.1; Group C=1.2±0.8), Isoleucine (Group S=2.5±1.6; Group C=0.9±0.2), Glutamate (Group S=6.3±2.1; Group C=2.6±1.1), Aspartate (Group S=10.8±3.8; Group C=7.7±2.7), Taurine (Group S=20.2±9.9; Group C=10.7±8.8), Alanine (Group S=3.8±2.2; Group C=1.6±1.0), Lactate (Group S=18.9±18.4; Group C=102.5±114.9), Methylamine (Group S=0.2±0.1; Group C=0.5±0.5), Dimethylamine (Group S=0.05±0.03; Group C=0.11±0.02), Propylene glycol (Group S=0.2±0.1; Group C=0.5±0.4), and O-phosphocholine (Group S=0.2±0.1; Group C=0.5±0.3). Changes in given metabolites might result from the inflammatory process as well as from the oxidative stress associated with lower airway disorders occurring in horses suffering from RAO [5]. On the basis of our results, metabolomic analysis of TW could represent a valuable diagnostic tool in horses with RAO.

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EFFECTS OF INTERMITTENT HAEMODIALYSIS ON PLASMA VITAMIN E AND VITAMIN A IN NEPHROPATIC DOGS

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Renal failure is associated with disorders of vitamin E and vitamin A status [1,2]. Human patients on chronic haemodialysis tend to present deficiency of plasma vitamin E [3] and increase of vitamin A [4]. No data concerning the status of vitamin E and A are currently present in dogs submitted to haemodialysis. The aim of the present study was to evaluate the plasma concentration of vitamin E and vitamin A pre and post treatment in a group of dogs affected by acute and chronic kidney disease and submitted to intermittent haemodialysis (IHD). 30 dogs presented at the Service of Haemodialysis and Blood Purification of the University of Pisa, between September 2015 and April 2017, for acute kidney injury (AKI) and acute impairment of chronic kidney disease (AKI on CKD), and submitted to IHD were included. All patients underwent physical exam, abdominal ultrasound, renal panel and complete urinalysis. Plasma vitamin E and vitamin A were assessed through high performance liquid chromatography (HPLC) prior to haemodialysis and immediately after the treatment. Data were statistically analysed through GraphPad prism. Results were considered statistically significant for $p<0.05$. The study group was composed by 17 females and 13 males, of different breed, age and body weight. 7/30 dogs were diagnosed with AKI on CKD and 23/30 were diagnosed with AKI. The median pre-dialysis plasma concentration of vitamin E was 1.94 ppm (0.1-51.73 ppm); while the post-dialysis plasma concentration of vitamin E was 3.47 ppm (0.15-37.80 ppm). For vitamin A, the mean pre-dialysis plasma concentration was 0.58 ppm (±SD 0.30 ppm); while the post-dialysis plasma concentration was 0.55 ppm (±SD 0.30 ppm). No significant difference was found between pre- and post-dialysis plasma concentration of both vitamin E and vitamin A. Plasma concentration of vitamin E reduced significantly ($p=0.03$) from haemodialysis one to three. No significant difference in the number of dogs presenting reduction, increase or no variation in the plasma concentration of both vitamins, between pre- and post-dialysis treatment, was found. No significant correlation between plasma levels of vitamin E and vitamin A and plasma creatinine and urea was found. In the present study, the plasma concentrations of both vitamin E and vitamin A were lower than previously reported for azotemic dogs. Particularly, vitamin E deficiency seemed to worse with the number of dialysis. However, these plasma concentrations did not seem to be significantly affected by the hemodialysis treatment.

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PLASMA LEVELS OF HOMOCYSTEINE IN NEPHROPATIC DOGS SUBMITTED TO INTERMITTENT HAEMODIALYSIS

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In human medicine, hyperhomocysteinemia is a well-known cardiovascular risk factor, which has been associated with the progression of renal failure [1,2]. In human dialysis patients, hyperhomocysteinemia is considered a frequent condition, which can be corrected by standard haemodialysis only partially [3]. In veterinary medicine, blood homocysteine resulted increased in dogs with chronic kidney disease (CKD) of different etiologies [4]. The aim of the present study was to evaluate plasma homocysteine in a group of dogs with acute kidney injury (AKI) or acute on chronic kidney disease (AKI/CKD) treated with intermittent haemodialysis (IHD). Fifty dogs treated with IHD at the Service of Hemodialysis and Blood Purification (SEPEV) of the University of Pisa between September 2015 and July 2017 were included. Plasma concentrations of homocysteine pre and post dialysis were obtained, by using HPLC plasma method with the Homocysteine kit in plasma/serum (CHROMSYSTEMS ®, Diagnostics by HPLC & LC-MS/MS). Data were statistically analysed through GraphPad prism ®7, and a p-value <0.05 was considered significant. Twenty-three males and 27 females of different breed, age and body weight were included. 24/50 (48%) dogs were diagnosed as AKI, while 13/50 (26%) were diagnosed as AKI/CKD. There was no statistically significant difference between pre and post treatment homocysteine values in AKI and AKI/CKD dogs. Mean post-treatment homocysteine concentrations showed a non significant trend to reduce, compared to pre-treatment concentrations. No statistically significant difference in homocysteine concentration was found between AKI and AKI/CKD. No statistically significant correlation between plasma homocysteine and creatinine was found. No differences were also found in homocysteine concentration between survived and not survived dogs. In conclusion homocysteine seem not to change between AKI and AKI/CKD dogs, and not to be significantly affected by the haemodialysis treatment. Further studies with larger number of dogs and complete cardiological evaluation should be encouraged.

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SPECKLE TRACKING ECHOCARDIOGRAPHY OF THE CANINE LEFT ATRIUM: REPETEABILITY AND REFERENCE INTERVALS IN 80 CLINICALLY HEALTHY DOGS

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The left atrium (LA) plays a pivotal role in the overall cardiac activity and the accurate evaluation of its performance is a major clinical concern. Speckle tracking echocardiography (STE) is a new echocardiographic technique allowing to accurately evaluate the myocardial deformation [1-3]. The aim of the present study was to evaluate the repeatability of the canine LA STE evaluation and to establish specific reference intervals for different STE variables in healthy dogs. Eighty clinically healthy adult dogs were used. Each dog underwent physical examination, blood pressure (BP) measurement and complete transthoracic echocardiography. The STE examination was carried out on awake dogs as previously described [1]. The software of the ultrasound machine successively generated a curve of strain and strain rate (SR) for each segment of the LA wall. The following variables were measured: peak of atrial longitudinal strain (PALS) and peak of atrial contraction strain (PACS), and the contraction strain index (CSI) was successively calculated using the formula CSI = (PALS – PACS) x 100. Systolic SR (SR_s) and the early (SR_e) and late diastolic SR (SR_a) were measured accordingly. The intra- and inter-observer vaiability was evaluated by randomly selecting 10 dogs who were examined in two different days by the same echocardiographer (intra-observer variability), and in the same day by two different cardiologists (inter-observer variability). The coefficient of variation (CV) of measurements was calcutaed. An ANCOVA model was used to evaluate the effect of heart rate (HR), body weight (BW), age and sex on the variables of LA function. Furthermore, a linear regression analysis was carried out to evaluate the effect of BW, HR, and BP on the variables of LA function after log transformation. The 95% predictive intervals were successively calculated using the allometric equation and employed to establish the minimum and maximum reference intervals for each variable of LA function. The STE analysis was feasible in all dogs and measurements for each LA STE variable were obtained. The CV was low (<15%) for PALS, PACS and CSI, while higher CV was observed the SR variables. A negative and positive significant correlation with BW was found for PALS and PACS, and for SR_e and SR_a, respectively, and specific reference intervals were calculated for these variables. Heart rate and BP did not have any effect on the STE variables. In conclusion the STE is an useful and feasible technique for the evaluation of the LA function in the dog. Values of canine LA strain have a good repeatability while SR variables have lower repeatability. When interpreting values of PALS, PACS, SR_e and SR_a of an individual dog, the BW should be taken into account given the existing allometric correlation.

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EVIDENCE OF ATRIAL STUNNING AFTER TRANSTHORACIC ELECTRICAL CARDIOVERSION IN A DOG WITH LONE ATRIAL FIBRILLATION

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Atrial fibrillation (AF) is one of the most common arrhythmias described in animals. Transthoracic electrical cardioversion (TEC) is a valuable treatment option to restore normal sinus rhythm. In people, a typical complication of this procedure is the atrial stunning, a phenomenon characterized by reversible systolic impairment of the left atrium (LA), observed after TEC [1]. The evaluation of LA mechanical function after TEC provides useful information about the likelihood of remaining in sinus rhythm during the following months. Noninvasive ways to evaluate LA function in dogs are advanced echocardiographic techniques, such as Tissue Doppler Imaging and Speckle Tracking Echocardiography (STE) [2]. A 55kg, 5 years old, male castrated Dogue de Bordeaux has been referred to our cardiology to evaluate presence of a tachyarrhythmia. Complete clinical, electrocardiographic and echocardiographic examinations revealed presence of an AF with mean heart rate of 170 bpm, and mildly reduced left ventricular systolic function (LVIDDn: 1.5; LVIDSn: 1.23; EDVi: 70 ml/m^2; ESVi: 40 ml/m^2; FS: 18%; EF: 42%; E wave velocity: 99 cm/s), with minimal LA enlargement (diameter: 55 mm; LA/aorta ratio: 1.4; LA volume: 1.5 ml/kg). LA mechanical function assessed by STE revealed a severe impairment (Peak Atrial Longitudinal Strain [PALS]: 3% [normal based weight value>24%]). Complete blood work, including cardiac Troponin I, and thyroid function were unremarkable. Antiarrhythmic treatment with Amiodarone was started. After 2 weeks TEC was successfully performed and a normal sinus rhythm was restored. Echocardiographic follow up revealed a restoration of LA systolic function, which was still impaired (maximal LA volume: 1.1 ml/kg; minimal LA volume: 0.7 ml/kg; A wave velocity: 20 cm/s; A wave VTI: 3.4 cm; TDI a' velocity: 6 cm/s; PALS: 7%). Two weeks later, left ventricular parameters changed remarkably (LVIDDn: 1.6; LVIDSn: 1.27; FS: 21%; EDVi: 67 ml/m^2; ESVi: 34 ml/m^2; EF: 49%; E wave velocity: 72 cm/s). LA dimensions had reduced, with amelioration of LA systolic function (LA diameter: 48 mm; LA/aorta ratio: 1.3; LA maximal volume: 0.9 ml/kg; LA minimal volume: 0.6 ml/kg; A wave velocity: 46 cm/s; A wave VTI: 4.4 cm; TDI a' velocity: 8 cm/s; PALS: 19%). At 1 month after TEC, a complete normalization of LA function was observed (LA diameter: 45 mm; LA/aorta ratio: 1.1; La maximal volume: 0.8 ml/kg; LA minimal volume: 0.8 ml/kg; A wave velocity: 57 cm/s; A wave VTI: 5 cm; TDI a' velocity: 10 cm/s; PALS: 27%).

This case shows clinical evidence LA reversed remodeling after TEC. The significantly depressed LA function observed after TEC was secondary to the atrial stunning, that progressively resolved over time. The systematic echocardiographic evaluation of LA function in dog that undergo TEC, might be helpful in predicting which subjects are more prone to maintain normal sinus rhythm after the procedure, and those who will instead show AF recurrence.

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CLINICAL AND ECHOCARDIOGRAPHIC FEATURES AND LONG TERM SURVIVAL IN DOGS WITH LEFT ATRIAL TEAR

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Left atrial tear (LAT) is an acute and life threatening condition, rarely observed in dogs with myxomatous mitral valve disease (MMVD). LAT is accompanied by acute pericardial effusion, and variable signs of cardiogenic shock, congestive heart failure (CHF) or sudden death [1]. Echocardiography is a valuable tool to diagnose LAT, by detecting pericardial effusion, MMVD, and an echogenic intrapericardial structure consistent with a clot. Although it is considered a severe condition, with variable prognosis, little is known about long term outcome in dogs presented with LAT. This is a case control matched study. Electronic database of the veterinary cardiology services of the Universities of Bologna and Zurich have been retrospectively interrogated searching for dogs with clinical and echocardiographic diagnosis of LAT. Fifteen dogs of different breeds have been selected. Clinical, echocardiographic, treatment, and survival data have been annotated. The same number of dogs with the same severity of MMVD as assessed on the basis of the ACVIM consensus classification [2], without LAT were selected, and acted as control dogs for direct comparison. Kaplan-Meier curves have been generated with survival data deriving from dogs having or not experienced LAT. Regarding the group of dogs with LAT, the most represented breeds were Chihuahua and Dachshund. Nine dogs were in ACVIM class C and the remaining 6 in class B2. The most commonly observed signs were dyspnea, syncope, and weakness, while only 2 dogs showed signs of shock. Ten dogs had CHF. Mean left atrial diameter/aortic diameter ratio (LA/Ao) was 2.2±0.4 and 2.3±0.5 at admission and at the resolution of PE (T1), respectively, while left ventricular internal diameter at end diastole indexed on the body weight (LVIDDi), was 1.2±0.3 and 1.3±0.2 at admission and at T1, respectively. There was no statistical difference between echo variables at the two time points. Only 3/15 dogs received a pericardiocentesis, while the others were medically treated. Drugs variably used were furosemide, pimobendan, benazepril, amlodipine, and hydrocodone. Regarding the comparison between the two groups, there was no difference in body weight, age, blood nitrogen urea, male/female prevalence, LA/Ao, and LVIDDi. At the end of the study, 13/15 dogs with LAT died for cardiac related causes, 5 of them during the first week after admission. In the control group, 10/15 dogs died, 1 of which during the first week after admission. The median survival time for dogs with and without LAT was 52 and 406 days, respectively, with no statistical difference (P=0.223). When excluding the animals that died during the first week, the median survival time was 427 and 406 for dogs with and without LAT, respectively (P=0.982).In this study a detailed analysis of clinical, echocardiographic and survival information about dogs with spontaneous LAT has been carried out. Although a relatively high percentage of dogs with LAT died during the first week after hospitalization, there is no apparent impact of this event on long term survival.

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CORRELATION BETWEEN SONOGRAPHIC HONEYCOMB APPEARANCE OF THE SPLEEN AND CYTOLOGIC OR HISTOLOGIC DIAGNOSES IN A FELINE POPULATION

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Honeycomb pattern (HCP) of the spleen is a sonographic feature characterized by a general diffuse inhomogeneity with multiple hypoechoic well-defined nodules. While the association between HCP and splenic lymphoma in dogs is acknowledged [1], studies aimed at establishing the clinical relevance of HCP in cats are lacking. A correlation between HCP and lymphoma has been anecdotally reported [2], while a recent study, investigating in cats the association between splenic sonographic appearance and cytological diagnosis, demonstrated that a moth-eaten or marbled appearance of spleen is not necessarily related to malignancies [3]. The aim of this study was to assess the correlation between feline splenic HCP and the final cytological or histological diagnosis, and to determine the positive predictive value of HCP for the diagnosis of splenic lymphoma. In addition, we aimed to determine whether the use of a high-frequency linear transducer allowing a high-resolution imaging of the spleen may increase HCP conspicuity on sonographic images and thus positively affect its identification. The medical records of cats with a sonographic splenic HCP were retrospectively searched for those having an abdominal ultrasound examination and the availability of cytological or histological samples of the spleen. All images and videos were evaluated by an experienced radiologist in order to record spleen size, shape and margin appearance, other parenchymal alterations and splenic hilar lymphadenopathy. A retrospective review of cyto-histopatologic samples was also performed by a pathologist and a clinical pathologist, and the final diagnosis was made by consensus. In the presence of a cytological suspect of lymphoma, diagnosis was confirmed with clonality testing by polymerase chain reaction (PARR). A total of 33 cats with HCP fulfilled the inclusion criteria. Five cases were diagnosed by histology and 28 by cytology, confirmed by PARR in 19 uncertain cases. There were 15 lymphoid hyperplasia, 8 lymphomas (4 B-cell, 3 T-cell and 1 large granular lymphocytes), 6 splenitis, 3 extramedullary hematopoiesis and 1 histiocytic sarcoma. The positive predictive value of HCP for splenic lymphoma was 24%. Splenomegaly was the most frequent sonographic feature associated with HCP and was observed in all lymphoma cases. Images of the spleen obtained by both linear and microconvex transducers were available in 24 cases, while the spleen was imaged only by a microconvex and a linear array transducer in 4 and 5 cases, respectively. The high-resolution linear array transducer enabled the visualization of HCP in all cases, while the microconvex array only in 15/24 (62%). According to our findings, HCP is not predictive of splenic lymphoma in cats. Therefore, when HCP is detected, further investigations are needed to obtain a final diagnosis. The use of high-frequency transducers is recommended to properly recognize HCP or subtle changes in feline splenic parenchyma.

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TREATMENT OUTCOME OF STRONGYLOIDIASIS IN SHELTERED DOGS

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Canine strongyloidiasis has been documented in many European countries, including Italy [1]. In dogs, information on the treatments against *Strongyloides stercoralis* infection is scant and anedoctal, mostly based on single cases [2] or small case series [1,3,4]. To evaluate the treatment outcome of natural infection in sheltered dogs, 17 dogs positive to *S. stercoralis* at Baermann test have been weekly monitored over a period of 8 weeks after treatment. Dogs lived in a municipal shelter housing about 800 dogs in the province of Bari where clinical cases of *S. stercoralis* infection were previously reported [1]. Dogs have been treated with ivermectin 200 µg/kg/sid/os for two consecutive days (the same protocol used for human strongyloidiasis) [5] and remained in the same environment. From each dog, single faecal samples were weekly collected directly from the ampulla for direct smear, faecal floatation, Baermann test and real-time PCR [6]. At the enrolment, of the 17 selected positive dogs (i.e. 5 males and 12 females) with an age ranging from 3 to 10 years and a weight ranging from 16 to 28 kg, only two animals showed clinical signs suggestive of strongyloidiasis (diarrhea, weigh loss). In one of them diarrhea disappeared one week after treatment, and he remained negative from the first treatment till the end of the study, whereas the other, who was cachectic at the presentation, never recovered and died after the treatment, despite the negative fecal results. In the remaining dogs (n=15) all fecal samples tested after treatment resulted negative for *S. stercoralis* at direct microscopy and Baermann test. qPCR confirmed negative results in all samples throughout the study except for 4 dogs resulting positive only at the first monitoring after treatment, then constantly negative as the others. Data of this study show that ivermectin was highly effective in the treatment of *S. stercoralis* infection of sheltered dogs as demonstrated by the persistent fecal negative results both at Baermann tests and rt-PCR throughout the post-treatment follow up. The application of correct deworming protocols is necessary to reduce the environmental infective larval burden and, therefore, protect dogs and workers from the risk of infection.

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POSTOPERATIVE ULTRASONOGRAPHIC APPEARANCE OF INTESTINAL SURGERY IN SMALL ANIMALS: RETROSPECTIVE STUDY (JANUARY 2009 - JANUARY 2018)

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Small intestinal surgery (SIS) is commonly performed in small animals but postoperative (PO) complications, such as dehiscence, can also develop [1]. Abdominal ultrasonography (US) is a useful technique to evaluate the PO intestinal course after surgery, but few studies have addressed this issue in the veterinary literature [2,3]. The aim of this retrospective study was to describe the US findings during standardized PO intestinal monitoring in order to characterize the normal/abnormal PO course. The study included all canine and feline patients undergoing SIS between January 2009 and January 2018, with US monitoring performed at an interval of 24 (T1), 48 (T2), and 72-96 (T3) hours after surgery. For each patient the following US parameters were evaluated: visualisation of the surgical site, wall layering and thickness, extension and motility of intestinal surgical site and also echogenicity of the surrounding omental/mesenteric fat. Abnormalities in the remaining portions of the gastrointestinal tract, presence and amount of abdominal effusion and pneumoperitoneum, pockets of fluid collection, as well as lymphadenopathy were recorded. Two groups of animals were included: Group 1 - normal PO course (G1) composed by twenty animals (15 dogs and 5 cats) and twenty-six surgical sites (8 enterotomies, 7 enterectomies, 8 intestinal biopsies, 2 perforation closures and one serosal patching) and Group 2 - complicated PO course (G2) composed by six dogs and nine dehiscences. All of 26 surgical sites of G1 were sonographically visualized and were characterized by irregular, hypoechoic serosal margins, focal thickened wall bowel with altered (7.7% of cases) or absent (92.3% of cases) wall layering, presence of hyperechoic double-walled foci (suture material), normal (80% of cases) to reduced intestinal motility and hyperechoic surrounding fat. Furthermore, mild (33% of cases) to moderate (77% of cases) abdominal effusion, mild (65% of cases) to moderate (35% of cases) pneumoperitoneum were noted in all patients, while abdominal pockets of fluid collection were found in 8 cases. All intestinal anastomosis leakages of G2 were sonographically visualized and were characterized by the same US features of normal PO course associated with hyperechoic spots or lines accompanied by reverberation artefacts crossing the wall. These features were compatible with intraparietal gas. Based on our results, normal PO intestinal surgical sites can be identified thanks to characteristic US features. Using a standardized US monitoring, the presence of surgical complications as dehiscence could be identified. Visualisation of intraparietal gas was a strong US sign of dehiscence.

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EVALUATION OF PLASMA PROCALCITONIN CONCENTRATIONS IN HEALTHY AND SICK COWS

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Since years, there has been an increased interest in measuring the plasmatic concentrations of acute phase proteins in cattle to provide a method for the early detection of inflammation or bacterial infection [1]. The most commonly occurring bacterial diseases in cattle, sometimes resulting from or leading to bacteremia and endotoxemia, are toxic mastitis, toxic metritis, pneumonia, endocarditis, septic arthritis and gastro-intestinal conditions [1]. Procalcitonin (PCT) has been investigated as a biomarker of sepsis in veterinary species [2-8]. The aim of this study was to evaluate plasma PCT concentrations in healthy and sick cows. Twenty healthy control cows and 20 sick cows were included. Sick cows included presented a positive Systemic Inflammatory Response Syndrome (SIRS) score, plus a given diagnosis of the previously mentioned bacterial diseases. Plasma PCT concentrations were measured with a commercial ELISA assay for cattle (MyBiosource.com). Differences in PCT concentration between healthy and sick cows were evaluated with a Student t-test. Healthy cows included in the study presented no clinical signs or laboratory changes related to any disease. Sick cows enrolled in the study showed clinical signs and laboratory data related to peritonitis (n=6), septic arthritis (n=5), toxic mastitis (n=3), toxic metritis (n=2), pneumonia (n=2), endocarditis (n=1), enteritis (n=1). The average plasma PCT concentrations in healthy cows and sick cows were 719.4 pg/mL (19-1669 pg/ mL) and 1130 pg/mL (208.3–2058 pg/mL), respectively ($P<0.03$). Plasma PCT concentrations were higher in both healthy and sick cows compared to values found in other species [2-6] and in calves [7-8]. Plasma PCT concentrations were statistically higher in sick than in healthy cows. These results confirmed an increase in plasma PCT concentrations in cows with bacterial diseases, as previously reported in horses [2-6] and calves [7-8].

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URINALYSIS IN DOGS WITH ACUTE PANCREATITIS

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In acute pancreatitis (AP), kidney injury (KI) could occur via hypovolemia, cytokine-induced ischemia, inflammation and oxidative stress [1]. In people, urinalysis and some urinary biomarkers have been proposed as useful prognostic tools in AP [2]. At the best of our knowledge, there are no studies evaluating urinalysis in canine AP. The aim of the study was to evaluate urinalysis parameters and urinary GGT-to-urinary creatinine (uGGT/uCr) in canine AP and their association with the outcome. AP diagnosis was made if there were compatible clinical (≥2 of the following: abdominal pain, diarrhea, vomiting or hyporexia) and laboratory parameters (acute inflammation), abnormal SNAP® cPL test and abdominal ultrasound abnormalities supporting a diagnosis of AP. Dogs with preexisting diagnosis of chronic kidney disease and/or managed by hemodialysis were excluded. Urine samples were collected and analyzed within 12h from AP diagnosis. For uGGT/uCr, a cut off value of 105 U/g was used [3]. KI was defined if dogs had urinary casts and/or proteinuria. Sediments were classified as active if there were one or more of the following findings: bacteriuria, moderate number of casts and >5 RBCs, WBCs, or epithelial cells/HPF. Dogs were divided into 2 groups (survivors and non-survivors) according to outcome at 15 days from their admission. Normal distribution was assessed using D'Agostino-Pearson test. Specific gravity (SG), urinary protein-to-creatinine ratio (UP/UC) and uGGT/uCr were evaluated in association to the outcome using Mann-Whitney U-test. pH was compared between outcome groups using t-test. Chi square test was used to evaluate dipstick parameters in association with the outcome. Fisher's exact test was used to compare the severity of UP/UC (≥2) and the presence of kidney injury to the outcome. Odds ratio (OR) was calculated. Seventy client-owned dogs with owners' consent were retrospectively included. Twenty-four dogs (34%) died. Seven out of 24 dogs were euthanized due to poor clinical condition or to progressive disease. Urine samples were collected by free catch (n=43), cystocentesis (n=19) or catheterization (n=8). Forty dogs showed active sediment (57%). KI was detected in thirty-six dogs (37%) and was associated with mortality (p=0.01 OR 3.9 95% CI 1.3-11.56). Non-survivor dogs showed higher dipstick bilirubin levels compared to surviving dogs (p=0.005). By excluding dogs with active sediment, UP/UC ratio ≥2 was associated with mortality (p=0.001 OR 47.5 95% CI 4-571.9). SG, pH and the other dipstick parameters were similar between groups. uGGT/uCr was available in 40 dogs (57%). Twenty-one dogs (53%) had uGGT/uCr over the cut off level and it was not associated with the outcome. No statistical differences were found in uGGT/uCr values between survivor and non-survivor dogs. In our study, UP/UC ≥2 seems to be a negative prognostic factor in dogs with AP. Further studies on uGGT/uCr during canine AP are warranted.

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EFFECTS OF SYNTHETIC COLLOID AND HYPERTONIC SOLUTION ADMINISTRATION ON PLASMA ONCOTIC PRESSURE IN DOGS

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The power exerted by macromolecules on semipermeable membrane, proportionally to their number, is the oncotic pressure (COP). In whole blood, COP is due to plasma proteins, in particular to albumin [1]. The aim of this study was to evaluate in dog, the impact of hydroxyethyl starch 130/0.4 (HES) or hypertonic saline 7.5% (HS) infusion on plasmatic COP, during fluid resuscitation to treat shock. Dogs admitted to the Veterinary Hospital were included if they met following inclusion criteria: diagnosis of GDV and presence of shock. Exclusion criteria were: colloid or blood components administration one month before enrollment or positive history for cardiac, pulmonary, renal and hepatic failure. At baseline (T0), CBC, biochemical panel [with albumin (ALB)], packed cell volume (PCV), total solid (TS) and plasmatic COP (Osmomat 050, Gonotec) were determined. Dogs were randomized to receive 10 ml/kg of HES or 4 ml/kg of HS over 15 minutes. After the bolus (T1), blood was collected to repeat the analyses. The statistical analysis was conducted with t-Student test or Wilcoxon rank-sum test to compare variables at T0; ANOVA or Wilcoxon matched-pairs signed-ranks test to compare results obtained at T0 and T1. It was considered significant a value of $P<0.05$. Twenty-one dogs were included: 11 in HES group [6 females and 5 males, median age of 10 years (min 1-max 13) and median weight of 35 kg (min 17-max 55)] and 10 in HS group [4 females and 6 males, median age of 10 years (min 2-max 14) and median weight of 37 kg (min 20-max 61)]. At inclusion, there was no statistically significant difference regarding age, weight, PCV, TS, ALB and COP between the two groups. Results in the group HES were [median (min-max)]: ALB of 0.3 g/L (0.2-0.4), TS of 6.5 g/dl (5.8- 9.2), PCV of 50% (30-55) and COP of 20 mmHg (17-25). Results in the group HS were: ALB of 0.3 g/L (0.2-0.3), TS of 7.4 g/dl (5.2- 8.9), PCV of 44 % (39-51) and COP of 21 mmHg (16-24). At T1, in both groups were observed a statistically significant reduction of PCV [40% (28-48), HES, P=0.003; 37% (28-42), HS, P=0.0001), TS (5 g/dl (4-8), HES, P=0.0005; 6 g/dl (4-8), HS, P=0.0028) and ALB (2 g/dl (1-3) HES, P=0.0002; 2 g/dl (2-3) HS, P=0.0044); whereas COP decrease only in HS group (15 mmHg (12-21), P=0.0001). These results indicate that, in dogs affected by shock, a bolus of HS can decrease plasmatic COP, whereas a bolus of HES can contribute to preserve it. Previous studies have obtained contrasting results, reporting increase or decrease of COP after HES administration, but they were conducted using different type of HES (600/0.75) or higher dose (40 ml/kg). [2,3]

A similar decrease of PCV, TS and ALB was detected at T1 in both groups, indicating a same degree of hemodilution induced by HES and HS. During fluid resuscitation of hypoalbuminemic dogs, clinicians should take into account the effect of HS solution on COP.

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DRY-MOUNT FECAL CYTOLOGY IN DOGS WITH ACUTE AND CHRONIC ENTEROPATHY

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Dry-mount faecal cytology (DFC) is a technique for faecal examination in dogs with gastrointestinal diseases. [1,2] Recently, three different sampling methods were evaluated in healthy dogs (HDs) suggesting some differences in results due to the selected method. Moreover, clusters of epithelial cells and neutrophils were observed more frequently in dogs with diarrhoea compared to healthy dogs. [3] The aim of this study is to describe swab faecal cytology in dogs with acute and chronic enteropathy compared to healthy dogs. Client-owned HDs and dogs with a diagnosis of acute (AE) and chronic enteropathy (CE) were enrolled. AE group included dogs with a history of diarrhoea for less than five days. CE group included dogs with diarrhoea for at least 3 weeks. HDs were recruited from dogs judged healthy during routinely admission for vaccination. In all dogs a DFC via a rectal swab was performed. A plastic swab was moistened with sterile saline solution, inserted in the rectal ampulla with an inclination of 45° for 1 to 4 cm (according to the size of the patient), rotated on the mucosal surface for 4-5 times, extracted and rolled on slides for cytology. All smears were stained with a Romanovsky staining (Diff Quik® Bio Optica Milano SpA - Milan, Italy) and examined by the same cytopathologist. Ten healthy dogs were initially used to determine the normal reference range for seventeen selected cytological features. Then, cytological smears from the three groups were evaluated and each cytological feature was scored as normal or abnormal. For each cytological feature, exact tests were used to evaluate associations between the three groups. A p-value <0.05 was considered significant. One hundred and twenty dogs were enrolled and divided equally into the three groups. No differences in age and gender were observed between groups. Presence of erythrocytes, cocci bacterial monomorphism and sporulating bacteria were significantly different between HD and AE groups ($p=0.0004$, $p=0.0002$ and $p=0.0476$, respectively). Neutrophils, lymphocytes and macrophages were significantly different between HD group and both AE and CE groups ($p<0.0001$, $p=0.0117$, $p=0.0143$ and $p<0.0001$, $p<0.0001$ and $p=0.0009$, respectively). Plasma cells were significantly different between CE and both HD and AE groups ($p<0.0001$ and $p=0.0052$, respectively). Bacterial phagocytosis was significantly different between the three groups ($p<0.0001$). In this study presence of erythrocytes, inflammatory cells, bacterial monomorphism, sporulating bacteria and bacterial phagocytosis were significantly different between healthy and sick dogs. Moreover, plasma cells were observed more frequently in CE group and bacterial phagocytosis in AE group. Since faecal cytology is a cheap, fast and non-invasive procedure, this study showed as DFC could be useful in clinical practice.

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MONITORING OF HEMOSTASIS BY THROMBOELASTOMETRY AND ACTIVATED CLOTTING TIME DURING HEMODIALYSIS: A CASE SERIES OF DOGS TREATED WITH HEPARIN

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Treatment with anticoagulant during hemodialysis (HD) is frequently applied to avoid coagulation of the extracorporeal circuit. Response to anticoagulant depends on the animal, disease and session of treatment. Monitoring of the coagulation status is essential to avoid complications. [1] The aim of this case series was to describe which method, between activated clotting time (ACT) and thromboelastometry (TEM), best identifies coagulation status during treatment with heparin in dogs undergoing hemodialysis. Three dogs admitted to the Veterinary Hospital of Pisa, for acute renal failure, were included. Before the dialysis session (T0), after 2 hours (T1) and at conclusion of HD (T2-4 hours) blood was collected for determination of ACT (Hemocron, Accriva Diagnostics) and TEM (ROTEM, Tem International GmbH). Heparin dose [bolus (25-50 UI/kg) followed by 50-100 UI/kg/h] was modified during treatment based on ACT values, to reach a target of 150-180 s (200-250 s whether blood coagulates in the circuit). Abnormal TEM analysis was defined as more than one TEM parameter outside of institutional reference interval, in a single profile. Changes that characterize a hypercoagulable trend are a decrease of CT or CFT and increase MCF or α angle, whereas an increase in CT or CFT, and a decrease in MCF or α angle indicate a trend toward hypocoagulable. Dog A performed 3 hemodialysis (HD) sessions and dogs B and C one. Below, all results were indicated for ACT, whereas only abnormal profiles were reported for TEM. Dog A: HD1-ACT (s): T0,155 – T1,146 - T2,147, TEM at T0: decrease CT (19s), increase in MCF (77mm) and α angle (86°) in ex-TEM profile; HD2-ACT (s): T0, 202- T1, 181-T2,180, normal TEM profiles in all times evaluated; HD3-ACT (s): T0, 153- T1,179-T2,172s; TEM at T2: increase in CFT (427s), decrease in MCF (32mm) and α angle (37°) in in-TEM profile. In dog A, hypercoagulable TEM was recorded at T0 in HD1 and hypocoagulable TEM at T2 in HD3, wheres ACT was in the therapeutic range.
Dog B: ACT (s): T0, 150- T1, 148- T2,165; TEM at T0 and T1: increase in MCF (T0, 77- T1, 76mm ex-TEM, and T0, 35- T1, 38mm fib-TEM) and α angle (T0, 82- T1, 83° ex-TEM and T0, 86-T1, 85° fib-TEM), normal TEM profiles at T2. In dog B, TEM was hypercoagulable at T0 and T1, and it was normal at T2; ACT was always in the therapeutic range. Dog C: ACT (s): T0, 164- T1, 221- T2, 169; TEM profile: increase in CFT (T0, 41- T1,27- T2, 30 mm ex-TEM, and T0, 28 mm in-TEM), MCF (T0, 85- T1,82- T2,81mm ex-TEM, T0, 82mm in-TEM and T0,49-T1,41-T2,47 mm fib-TEM) and α angle (T1,83- T2,85- T3,84° ex-TEM, T0,84° in-TEM and T0,84- T1,86- T2,85° fib-TEM), normal in-TEM profile at T2. In this dog, several episodes of clots formation have occurred. In dog C, TEM was hypercoagulable in all times evaluated, whereas ACT resulted in therapeutic range at T0 and T1, and at T2 it reached the range recommended in presence of clot. In these 3 dogs, a discrepancy between TEM and ACT was recorded. These observations seem to indicate that TEM monitoring could be more accurate than ACT during heparin therapy for hemodialysis, and it was more correlated to hemostatic status of dogs [2].

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FUNCTIONAL ENDOSCOPIC SINUS SURGERY (FESS) IN A CAT WITH NASAL TUMOR: CASE REPORT

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Primary nasal tumors are rare, but they represent 40% of chronic nasal diseases in cats. Lymphoma is the most common tumor affecting the nasal cavities of cats. Adenocarcinoma and squamous cell carcinoma are the most frequent epithelial tumors. Early diagnosis facilitates therapy and improves prognosis (1). Endoscopy and computed tomography (CT) of the nasal cavity provide useful information for therapeutic plan (1), even if the definitive diagnosis requires histopathological or cytopathological analyses (1). Pharmacological therapy, chemotherapy, radiation therapy and/or surgical approach represent the treatments of nasal neoplasia, considering the histologically nature of the tumor (1). This report describes a functional endoscopic surgery (2) of nasal tumor in a cat. An 11 year-old, 5.8-kg of weight, neutered European male cat was referred to the Veterinary Teaching Hospital of the University of Perugia for four month history of sneezing, nasal discharge and respiratory distress unresponsive to symptomatic therapy. In the previous 5-6 days the cat also presented disorexia and successively anorexia. The physical examination revealed lethargy, bilateral haemorragic mucopurulent nasal discharge with air flow reduction and stertorous respiratory sounds. Latero-lateral, oblique and a ventro-dorsal open mouth Xray projections of the skull showed a regular hyperdense area in the middle part of right nasal cavity. No evidence of bone involvement was visible. The right frontal sinus appeared filled with hyperdense material. CT scan was performed evidencing an irregular shaped mass that involve the aboral part of the right nasal cavity and the sphenoidal sinus. A mild periferical contrast enhancement of the mass was observed. Ostheolisis was present in small area of the ventral and right lateral sphenoidal wall close to the mass. A mild atrophy of the right turbinate was observed too. The right frontal sinus was filled with semi-fluid hyperdense material. An endoscopic guided biopsy was planned in order to obtain an adequate tissue sample for histopatologic examination. The cat in general anesthesia was positioned in dorsal recumbency with the head flexed in order to place palatine bone perpendicularly to the table. A 0° degree 3 mm KARL STORZ endoscope (7220AA) was used for the endoscopy. The mass was localized endoscopically in the medium meatus, near the rostral part of the ethmoidal bone. Using RHINOFORCE® (miniaturized cutting tool) and a gripper, the mass was completely removed. All these operations were made under endoscopic guidance. After this procedure a nasal hemostatic tampon was applied for one day. The patient was able to eat again in short time and was asymptomatic for 6 months after procedure. The samples obtained were analyzed; the histopathological and immunochemistry exams revealed a type B lymphoma. In conclusion, according with human literature (2) about functional endoscopic surgery, even though the purpose of this procedure was to obtain appropriate biopsy samples, this procedure also allowed to restore a normal nasal flow and sinus drainage.

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IMPLEMENTATION OF ULTRASOUND-BASED TECHNIQUE FOR THE ASSESSMENT OF MUSCLE GLYCOGEN DEPLETION AFTER EXERCISE IN HORSES: PRELIMINARY RESULTS

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Muscle glycogen is a form of carbohydrate storage, and a major source of energy during exercise. Glycogen concentration in muscle tissue is an important determinant for endurance capacity during high intensity exercise [1], so that its measurement provides a meaningful marker of athletes' conditioning and diet/training appropriateness suitability [2]. The aim of the present study is to verify the feasibility on equines of a recently developed non-invasive approach for muscle glycogen assessment in man using ultrasound (US) imaging [3].

Intra-muscular glycogen depletion following exercise was assessed after preliminary sessions for the definition of target muscles, probe position/orientation and exercise protocol. The measurements were performed by means of US system Esaote ClassC using a linear probe in a long axis orientation, set at 12.5 MHz frequency, on left middle gluteus (GL) and semitendinosus (ST) muscles. Images were obtained on 3 horses at rest and after exercise on a high speed treadmill (Säto I, SATO, Sweden). The horses performed 5 minutes warm-up and 15 minutes steady-state exercise (average speed: 6.5 m/s ± 1.8 SD; heart rate: 171 bpm ± 21 SD). US images were obtained both before and within 20 minutes after exercise. Image intensity in the grey scale was considered as the outcome measure inversely related to tissue water content (an indirect measure for muscle glycogen concentration).

The variation coefficient of consecutive measurements with probe re-positioning on the same muscle was 5.07%±2.98%. Preliminary data show that overall muscle image intensity significantly increased after exercise (+ 30% p 0.017, paired t-test), being significant in ST (mean +22% ±11%, p=0.039) and not in GL muscle (mean +55% ± 60%, p=0.298).

The use of US technique appears to be sensitive in detecting changes in tissue water content related to glycogen depletion following exercise. It is therefore a technique of potential impact for the evaluation of equine athletes, even though present results on a small sample need to be further implemented and validated through glycogen direct measurement by biopsy technique.

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PLASMA LEVELS OF ENDOCANNABINOIDS IN DOGS WITH PRIMARY CHRONIC ENTEROPATHIES

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Diagnosis of canine chronic enteropathies (CE) is challenging and requires a thorough clinical, laboratory and histological evaluation □1□.Nevertheless, disorders related to CE are further retrospectively classified by their responsiveness to sequential therapeutic trials □2□.
Convincing evidence suggests that the endocannabinoid (eCB) system is expressed in the gut □3□ and that eCBs are involved in the control of gut homeostasis suggesting an important pathophysiological role in the gastrointestinal tract □4□.
The purposes of the study were to estimate plasma levels of 2-arachidonoylglycerol (2-AG), arachidonoylethanolamide (anandamide, AEA), palmitoylethanolamide (PEA), and oleoylethanolamide (OEA) in healthy dogs, and to assess their potential usefulness as diagnostic tool for canine CEs. Dogs with CE were divided into 4 groups: Food Responsive (FRE), Antibiotic Responsive Enteropathy (ARE), Idiopathic Inflammatory Bowel Disease (IBD) and Protein Losing Enteropathy (PLE). Plasmatic 2-AG, AEA, PEA and OEA levels were determined by liquid chromatography/mass spectrometry (LC-MS). All data were expressed in pmol/ml as medians (interquartile range), and were compared using non-parametric tests. The diagnostic accuracy was assessed by a ROC-curve. *P*-values <0.05 were considered significant.
In healthy dogs (CONTROL, n=30) plasma levels of 2-AG, AEA, PEA and OEA were: 4.1 (2.80-6.40), 1.7 (1.50-2.20), 24.5 (17.50-31.60) and 52.1 (39.50-74.60), respectively. Dogs with CE (n=33) showed significantly higher (P<0.01) 2-AG [10.7 (3.87-29.77)] and PEA [40.5 (30.05-49.27)], compared to the CONTROL, while no statistical differences were found for AEA and OEA. In particular, in the comparison among the CONTROL, FRE (n=10), ARE (n=9), IBD (n=9) and PLE (n=5) groups, FRE showed higher levels of PEA [48.9 (40.5-57.3), P<0.05] and OEA [93.0 (65.3-122.1), P<0.05], ARE had higher levels of 2-AG [11.2 (6.4-24.9), P<0.01], the IBD group showed increased 2-AG [15.4 (7.5-130.3), P<0.01] and PEA [41.2 (38.2-50.2), P<0.05], while PLE dogs had increased concentrations of 2-AG [38.4 (11.2-92.1), P<0.01] and decreased amounts of OEA [26.2 (18.2-38.0), P<0.05] and PEA [11.9 (9.9-23.7), P<0.05]. The overall accuracy of 2-AG to exclude (-LR 0.18, Sensitivity 94.4%, Specificity 70% at □3.8) or predict (+LR 7.78, Sensitivity 77.78%, Specificity 90% at >6.4) ARE or IBD was very high (AUC 0.90). Additionally, plasmatic OEA showed a good accuracy (AUC 0.77) to exclude (-LR 0.18, Sensitivity 90.0%, Specificity 55.56% at □53.3) or predict (+LR 4.5, Sensitivity 50.0%, Specificity 88.89% at >90.7) FRE. Potential limitations were the sample size and the lack of eCBs analysis in intestinal biopsies. It can be concluded that 2-AG and OEA may be a promising diagnostic tool for differentiating FRE from ARE and IBD in dogs with chronic gastrointestinal signs.

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HORSE RECOVERY AFTER COLIC SURGERY: ASSOCIATED FEEDING PATTERNS - PRELIMINARY RESULTS

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Colic is a severe and quite frequent disease for horses [1]. When surgery is necessary, the nutritional management in the post-operative period is crucial. The aim of this retrospective study was to analyze different factors and understand if they may influence recovery duration after colic surgery. Data were collected from records of 37 surgical colic cases from the hospitals of the Department of Veterinary Science of Turin (Italy), and of the Department of Veterinary Medicine of Lisbon (Portugal). Multiple correspondence analysis (MCA) was used to investigate the correlation between the different parameters and the recovery length. A ranking class dataset was clustered according to the length of recovery classes and it was used to calculate the Kendall's tau correlation coefficient. The correlation significance was then assessed according to Bonferroni corrected multiple comparison p-values. The results showed that a short recovery length was associated with: shorter surgery time and endovenous fluid therapy; higher body condition score (BCS) at admission; early intake of adequate amount both of dry matter (DM) as forage (hay and fresh grass) and total DM intake. The association of short recovery with shorter surgery times (<1.5 h) may imply the effect of other factors, like the anesthesia, that could influence the intestinal motility [2]. As well, brief postsurgical IV fluid therapy (<12 h) is probably associated with short recovery because continuous administration of fluids has a relevant impact on the normal desire to drink water and, consequently, to eat [3]. A higher BCS (6-7.5 on 9-point scale) resulted to be associated with a reduced recovery time; on the contrary, the majority of the horses with a longer postsurgical recovery time had a low BCS (3-4). This is probably due to the fact that thinner horses have less reserves to mobilize during recovery compared with fleshy ones. From the present study it was showed that horses with a short recovery received at least 0.1% DM/Body weight as forages during the first 24 h, increasing to at least 0.3% DM/BW on the second day after surgery. Total DM intake was at least 0.55% on the second day after surgery and 0.85% on the fourth day after surgery. Despite the fact that often a diet with short fiber or complete pelleted feed is suggested [4], the present analysis did not yield to any significant association between shorter recovery times and the use of fibrous mix, with fibers shorter than 10 cm. On the other hand, the collected data showed that the time to first defecation after surgery did not covariate with the length of the post-surgery period. This could be explained by the fact that arguably the fecal ball production depended on the type of surgery rather than on patient recovery. In conclusion, this work was intended as a preliminary retrospective study in order to unravel the effects of postsurgical management on horse patients. It determined that early feeding has a main role on the length recovery of horses undergone a colic surgery. However, how different types of diets can affect the duration of hospitalization still remains to be investigated.

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INTRACORONARY CYTOPROTECTIVE GENE THERAPY IN CANINE PATIENTS WITH DILATED CARDIOMYOPATHY

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Dilated cardiomyopathy (DCM) is a myocardial disease of dogs and humans characterized by progressive ventricular dilation and depressed contractility and it is a frequent cause of heart failure. Conventional pharmacological therapy cannot reverse the progression of the disease and, in humans, cardiac transplantation remains the only option during the final stages of cardiac failure. Cytoprotective gene therapy with the Vascular Endothelial Growth Factor-B167 (VEGF-B167) has proved an effective alternative therapy, halting the progression of the disease in experimental studies on dogs [1,2]. The aim of this work was to test the tolerability and feasibility of intracoronary inoculation under fluoroscopic guidance of VEGF-B167 carried by adeno-associated viral vectors in canine DCM patients. Ten patients underwent the gene delivery procedure. The intraoperative phase was well tolerated by all dogs. Clinical and echocardiographic assessment at 7 days post-procedure in all dogs showed stable clinical conditions that could be superimposed to those pre-procedure. The results of this work indicate that intracoronary gene delivery is feasible and tolerated in dogs with DCM. Further monitoring/investigations are ongoing to evaluate the effects of this procedure on disease progression.

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ZOONOTIC VISCERAL LEISHMANIASIS EPIDEMIOLOGYCAL SURVEY IN MOLISE REGION, SOUTHERN ITALY

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Dogs play an important role as hosts and reservoirs of several vector-borne infections, including zoonotic visceral leishmaniasis [1]. Mediterranean basin is historically considered endemic for ZVL; however strong differences may exist in terms of prevalence among different areas and data about seroprevalence in inner areas of southern Italy are very few. Therefore, the aim of this study was to investigate the seroprevalence of *Leishmania infantum* in dogs living in the Molise region, southern Italy. A grid-based approach within a Geographical Information System (GIS) was used in order to uniformly sample the dogs throughout the entire region [2]. For this purpose, a grid representing quadrants of 10x10 km was overlaid on the regional map within the GIS. Thus the Molise region was divided into 55 quadrants and the study was designed to sample 6 hunting, 6 stray and 6 sheep dogs in each quadrant. The study of leishmaniasis seroprevalence was performed through an immunofluorescence antibody test (IFAT; provided by the National Reference Center for Leishmaniosis, Palermo, Italy) to detect anti-*Leishmania* antibodies in serum samples. When possible, dogs were submitted to physical examination and a clinical form was filled out. A total of 752 samples were collected (347 from hunting, 180 from stray and 225 from sheep dogs) and 77 samples (10.2%; 95%C.I.= 8.2-12.7) showed a titer ≥1:160 for *L. infantum*, with a higher prevalence in hunting dogs (9.5%; 95%C.I.= 6.7-13.2). Of 77 positive dogs, 30 were submitted to physical examination: 12 dogs showed clinical signs (40%). The present study showed that leishmaniosis is present in dogs in the Molise region. The detection of the infection in dogs with or without clinical signs reinforces the importance of increasing the veterinary community, owners and public health authorities' awareness.

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SHORT-TERM EFFECTS OF EQUINE ASTHMA TREATMENTS ON AIRWAY SMOOTH MUSCLE PLASTICITY

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Increased airway smooth muscle (ASM) mass is a hallmark of equine asthma [1]. ASM cells are characterized by phenotype plasticity, that is, the ability to modulate their functions in response to physical and chemical factors. ASM phenotype switching from contractile (differentiated) to proliferative (dedifferentiated) could enhance airway remodeling [2]. In vitro studies suggest that inflammatory mediators can modulate ASM phenotype switching and, in turn, foster airway remodeling. In vivo data supporting these observations, however, are scarce and they often disregard the effect of asthma treatments. The objectives of our study are 1) to relate the expression of selected markers of ASM cell phenotypes in the central airways of asthmatic horses to lung function, airway remodeling and tissue cytokine expression, and 2) to assess how fluticasone and salmeterol regulate these parameters. We studied 12 asthmatic horses in exacerbation of the disease. Lung function (impulse oscillometry system) and endobronchial biopsies (EBB) were performed before and after 4 weeks of inhalation therapy with fluticasone (2.5 mg BID, n=6 horses) or salmeterol (0.25 mg TID, n=6). EBB were processed for histology and for qRT-PCR. ASM and extracellular matrix remodeling were evaluated on Movat penthachrome-stained tissue sections. We assessed the mRNA expression of myocardin and of a 7-amino acid insert in the motor domain of smooth muscle myosin heavy chain (sm-MHC) called SM-B, two markers of ASM contractile phenotype. ASM proliferation was studied by immunohistochemistry (PCNA+ myocytes/ASM area). The mRNA expression of IL-13, IL-4, IL-5, IL-6, IL-8 and IL-17 was quantified to eludicate possible regulators of ASM phenotype switching. The effect of time and treatments were evaluated with 2-way ANOVA with Sidak post tests. Correlations were performed with Pearson or Spearman tests, depending on data distribution. Asthmatic horses had severe obstruction at baseline as indicated by their respiratory resistance and reactance values. In absence of treatment (baseline), the expression of myocardin/sm-MHC positively correlated with airway resistance ($r=0.57$, $p=0.05$). SM-B expression negatively correlated with ASM proliferation ($r=-0.69$, $p=0.01$) and tended to correlate with ASM cell size ($r=0.57$, $p=0.06$). However, this was not observed when the expression of SM-B was corrected by total sm-MHC. The expression of SM-B/total sm-MHC was also strongly correlated to the expression of IL-13 ($r=0.87$, $p=0.0005$) and IL-6 ($r=75$, $p=0.005$). Fluticasone and salmeterol similarly improved respiratory resistance and reactance after 4 weeks ($p<0.05$). Both treatments decreased ASM cell size ($p<0.05$). Fluticasone reduced ASM proliferation ($p=0.002$). Salmeterol reduced myocardin/sm-MHC expression ($p=0.001$). In conclusion, we provide in vivo evidence supporting the implication of IL-13 in ASM cell differentiation. Our data suggest that, during equine asthma exacerbations, differentiated contractile ASM cells could contribute to airway remodeling and disease presentation. Lastly, the expression of markers of ASM cell contractile vs. proliferative phenotype appears to be regulated by different mechanisms, only partly interconnected.

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A CASE REPORT OF PLEURO-PNEUMONIA, BACTERIAL ENDOCARDITIS AND DISSEMINATED INTRAVASCULAR COAGULATION IN A 18-MONTHS TROTTER GELDING.

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The article reports a case of pleuropneumonia, endocarditis and disseminated intravascular coagulation (DIC) in a horse. The patient was an 18 months old gelding Trotter horse presented for dyspnea and fever. The horse has been already treated with antibiotics and showed diarrhea 15 days before admission. At admission, the horse showed lethargy, anorexia, distal edema and poor body condition (BCS 2.5/6). The rectal temperature was 37.3°C, mucous membranes were congestive with petechial hemorrhages, the capillary refill time (CRT) was prolonged, and the sub-mandibular lymph nodes were enlarged. The examination of the circulatory system revealed sinus tachycardia and the presence of a grade IV/VI, holodiastolic decrescendo murmur with point of maximal intensity in correspondence of the aortic valve. Bounding and hyperkinetic arterial pulse was detected. The examination of the respiratory system revealed tachypnea, attenuated lung sounds, especially ventrally. The examination of the digestive system was normal. Laboratory findings showed increased HCT, TP and creatinine, leukocytosis, neutrophilia and left shift. The PT and aPTT were prolonged, the FDP were increased, while fibrinogen and PLT were low. Lung ultrasound (US) showed diffuse comet tails, hypoechoic areas located in the cranio-ventral pulmonary fields, localized pleural effusion on the left and diffuse on the right hemithorax. The US of the abdomen was normal. A BAL was performed and BALF was analyzed for cytology and culture. The TNCC and differential cell count were compatible with bacterial pneumonia. A pleural tap was obtained from both the hemithorax aseptically and was analyzed for cytology and bacteriology. The TNCC and differential cell count were compatible with bacterial pleuritis. Pleural and BALF cultures were negative. Echocardiography revealed an irregularly thickened aortic valve, with hyperechoic vegetative lesions on the aortic valve leaflets. Color Doppler echocardiography showed severe aortic valve regurgitation. The left atrium and the left ventricle were moderately enlarged. According to clinical, diagnostic imaging and laboratory findings, a diagnosis of severe pleuropneumonia, aortic endocarditis and DIC was done. The horse was treated with fluid therapy, broad-spectrum antibiotics (ceftiofur 4 mg/kg SID IM), FANS, omeprazole (4 mg/kg SID PO). The DIC was treated with intravenous equine plasma and sodium heparin. After 24h of fluid therapy, the creatininemia was within normal ranges, thus gentamicin was added. Two days after admission the horse was mildly improved (CRT <2 sec; reduction of the WBC count and improvement of the coagulative profile) and was stationary for 6 days. Seven days after admission the horse became profoundly depressed, anorexic, and the clinical exam revealed pyrexia and severe dyspnea. On day 8 the subject showed abdominal pain not controlled with drugs. The GI exam revealed no gut sounds and rectal exploration was compatible with colic. Due to severe impairment of the cardiovascular system and colic syndrome, the horse was humanely euthanized.

Necropsy revealed hydrothorax and hydropericardium with moderate left ventricular hypertrophy. A large based and irregular thrombus was present at aortic valve. Multifocal atelectasis and emphysematous areas were evident in the lung associated with thickening of the arteriolar muscular layer.

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PRESENCE OF *ESCHERICHIA FERGUSONII* IN THE STOMACH OF HORSES WITH OR WITHOUT GASTRIC ULCERS

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Literature concerning the gastric mucosal microbiota in healthy horses is scarce and the role of the bacterial community of the equine GI tract in diseases such as gastric ulcers is not fully elucidated [1,2]. In the last few years, *Escherichia fergusonii* has been described as an emerging pathogen in both human and veterinary medicine, and it has been isolated by specimens of glandular lesions from the stomach of horses [3]. The aim of this study was to investigate the presence of *E. fergusonii* in the stomach of asymptomatic horses and correlate it with the presence of gastric lesions. Twenty-five pleasure horses, subjected to little or no physical exercise, were enrolled in the study. Gastroscopy was performed in each animal and gastric biopsies were sampled from normal squamous mucosa and from squamous gastric lesions graded at least 2/4 [4] and located along the margo plicatus (2 samples for each horse). All the biopsies were submitted to genomic DNA extraction and analyzed using a PCR protocol amplifying a fragment of yliE gene to detect *E. fergusonii* DNA [5]. In our study, *E. fergusonii* was detected only in 1/50 (2%) sample obtained from normal squamous mucosa. This result differs from what was reported by Husted and colleagues regarding the etiological role of this bacterium on gastric glandular ulcers [3]. The difference could be explained by the different location of sampling (glandular vs squamous). Furthermore, none of the horses showed clinical signs or presented the risk factors reported in the literature for Equine Gastric Ulcer Syndrome (EGUS) [4], but nonetheless lesions ranged from 2 to 4/4. Further studies are needed to confirm this finding and to evaluate the role of other bacteria in the development of lesions in the stomach of asymptomatic horses

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A RETROSPECTIVE EVALUATION OF *VIPERA ASPIS* ENVENOMATION IN DOGS TREATED WITH AN EQUINE-DERIVED F(AB')2 VIPERID ANTIVENOM

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Viper snakebite is an important health problem in dogs in Italy, where four poisonous species of the *Vipera* genus are commonly found: *Vipera aspis, Vipera berus, Vipera ammodytes* and *Vipera ursini* [1]. The clinical features of viper envenomation in dogs are characterized by local tissue injury and systemic signs, including increased vascular permeability, hypotension, hemolysis, anemia, thrombocytopenia, coagulopathy, respiratory depression, myonecrosis, nervous system dysfunction, and acute renal failure [2]. This study describes the clinicopathologic signs and the outcome in dogs envenomed by *V. aspis* treated with an equine-derived F(ab')2 viperid antivenom (Sclavo Diagnostics International Srl, Siena, Italy) [3]. The study protocol was approved by the Ministry of Health, Department of Veterinary Public Health, Food Security and Bodies for Health Protection (DGSAF 001453-P-01/08/2012). The medical records of 80 dogs presented to 13 veterinary facilities in Tuscany and diagnosed with *V. aspis* envenomation were retrospectively reviewed. Data included the signalment, date, history, physical examination and laboratory findings, disease progression, treatment, hospitalization time period and outcome. Data were statistically analyzed with Fisher's exact test and Student's t-test. A value of $P<0.05$ was considered significant. Before treatment, envenomed dogs mostly showed decreased sensory response (58/80), hematuria (38/80), tachypnea and/or tachycardia (34/80). The most common clinical pathology abnormalities were increased creatine kinase, alkaline phosphatase and aspartate transaminase (33/80), prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT) (27/80), proteinuria and increased urine protein-creatinine ratio (27/80). All dogs received fluid therapy, glucocorticoids and the viperid antivenom, which was administered by intravenous infusion or subcutaneous injection at the dosage of 1 ml/kg (100 mg/kg) body weight. Five dogs died during the study (6% mortality rate), after an average time of 4 days following the bite (range 1-15 days). The large majority of the dogs incuded in this study (75/80) survived following the administration of the specific equine-derived F(ab')2 viperid antivenom. The antivenom resulted effective in stabilizing or reversing the effects of progressive envenomation syndrome and in improving the clinical conditions within 8 hours. On the contrary, no significant change was observed in the hematological and coagulative parameters and a significant worsening was observed for WBC and RBC count ($P<0.05$). In conclusion, the specific equine-derived F(ab')2 viperid antivenom was associated with an improvement of neurologic and other systemic effects and the resolution of most of the clinicopathologic signs in the envenomed dogs.

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ULTRASONOGRAPHIC EVALUATION OF DIGITAL LESIONS IN HOLSTEIN DAIRY COWS

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Dairy cattle lameness is one of the major problems in current dairy farms [1]. Better knowledge of clinical lesions insurgency and consequences could help early recognition and treatment. Ultrasonography has been proven useful in evaluation of the solar horn tissue and of the soft tissue layer (especially digital cushion). The aim of the present study was to correlate digital lesions with ultrasonographic evaluations of the foot. Fifty-six Holstein dairy cows were enrolled in the study. Two months after calving functional trimming was performed on rear limbs and lesions were classified and scored for severity [2]. Ultrasonography was then performed using a portable unit and a linear probe [3]. Digital cushion thickness was measured in three points [4] and four grades of increasing echogenicity were established. Ultrasonographic visible alterations were measured long their vertical axis (L1) and their horizontal axis (L2). Claws were divided in two groups: healthy and affected. One-way analysis of variance (ANOVA) was applied to assess the effect of lesions presence on ultrasound imaging and Pearson's correlation coefficient was determined. Of the 224 ultrasonographed claws, 84 were healthy (37.5%) and 140 affected (62.5%). The most common pathology was solar hemorrhage (48.5% of all pathologies), followed by solar ulcer, (16.4%). Other pathologies were white line disease, horizontal fissure and axial fissure. Statistically significant ($P<0.001$) differences were found in the echogenicity appearance between healthy claws and all the affected ones, with the healthy ones being mainly anechoic. No statistically significant differences were found for the 3 digital cushion thickness measures between healthy and affected group. Vertical axis (L1) measures showed a statistically significant ($P<0.001$) difference between white line disease, solar ulcer and axial fissure against solar hemorrhage and horizontal fissure. Horizontal axis (L2) measures showed a statistically significant ($P<0.001$) difference between all pathologies against horizontal fissure. These results confirm ultrasonography as a reliable tool for detecting an increase in digital cushion echogenicity that occurs with pathologies insurgency. Our findings suggest that the cushion’s composition, rather than its thickness, has an important role in the incidence of foot pathologies. Ultrasonography has proven useful also in determining lesion extension by measuring the L1 and L2 parameters. Ultrasound could be also used after trimming practice to classify animals depending on their digital cushion echogenicity, therefore identifying animals keen on developing foot diseases.

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TREATMENT OF CANINE ORAL SQUAMOUS CELL CARCINOMA USING ELECTROCHEMOTHERAPY. A CASE SERIES REPORT

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Non-tonsillar squamous cell carcinoma (SCC) is the second most common oral tumor in dogs [1]. It is locally aggressive with a low-moderate rate of metastasis. Surgery and radiation therapy can both be used for local control. However, these two treatments are not always feasible due to owner's willing or financial concerns. Electrochemotherapy (ECT) is a local ablative technique that uses electric pulses to enhance the intracellular diffusion of cytotoxic drugs and can be used to treat several types of solid tumors [2].

The aim of the study was to evaluate the feasibility of ECT in the treatment of oral canine SCC. Twelve dogs with SCC were retrospectively enrolled (March 2005 – October 2017). ECT was combined with IV bleomycin (15000 UI/m2): alone in 11 cases and post-surgery in one. Two pulse generators were used: Cytopulse Oncovet® (6 cases) and Cytopulse PA4000® (6). The following parameters were considered: tumor size, clinical staging (TNM), electroporation parameters, response rate (RR), (as complete remission [CR] and partial remission [PR]; RECIST guideline) [3], median survival time (MST, time from diagnosis to death or to the last follow-up), recurrence rate, disease-free interval (DFI, median time from ECT treatment to recurrence) and treatment toxicity (6-point scale) [4]. The median size of the tumors was 1.65 cm (range 0.3–8 cm). TNM was as follows: 6/12 T1, 3/12 T2 and 3/12 T3. The pulse frequency used was 5 kHz in 6 dogs and 1 Hz in 6 dogs. The voltage used was 1,200 V for 8 cases and 1,000 V for 4. The RR was 92% (11/12; 9 CR and 2 PR). Two dogs underwent a second ECT treatment. Seven dogs died during the study period and 3 experienced recurrences. MST for dogs dead with the tumor (n=2) was 110 days and for dogs dead without the tumor (n=5) was 923 days. Among the remaining 5 surviving dogs, one experienced tumor recurrence and 4 were considered in CR at the last follow up. Overall tumor recurrence rate was 33.3% (4/12 cases). Median DFI and MST for dogs with recurrence were 48.5 days (range 9-83) and 118 days (range 99-1891), respectively. All dogs with T1 stage SCC obtained CR and showed no recurrence (median follow-up 1112 days). Treatment toxicity was ≤2 in 11/12 dogs and only one experienced 3 points of toxicity. No associations were noticed between tumor size, T stage, voltage or pulse frequency and treatment efficacy or toxicity. ECT for canine oral SCC could be seen as an alternative treatment to excisional surgery or radiation therapy. However, more cases should be collected and investigated in a prospective trial in order to compare ECT with the other standard treatment.

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CLINICAL, ULTRASONOGRAPHIC AND THERAPEUTIC FEATURES OCCURRED IN A DOG AFFECTED BY MASSIVE PLEURAL LARVAL MESOCESTOIDIASIS

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Pleural cestodiasis represents an uncommon condition caused by larval forms of tapeworms from the genus *Mesocestoides*. Adult parasites are non-pathogenic and live in the small intestine of carnivores. In contrast, larval mesocestoidiasis is a potentially lifethreatening disease that occurs occasionally when larval stages of *Mesocestoides* colonize mesothelial cavities of dogs and cats, increasing abnormally their numbers through asexual division [1]. Despite several Authors described clinical cases of peritonitis associated to mesocestoidiasis [1], data about the effect of pleural invasion by larval stages of *Mesocestoides* are scant and most of the times occurs as occasional post-mortem finding. The present work aimed to describe medical and surgical approaches occurred in a dog affected by a massive pleural larval mesocestoidiasis. A 4 year-old, female, mixed breed dog, 20 kg, was referred to the Veterinary Teaching Hospital of Perugia for severe respiratory distress. Thoracic ultrasound revealed severe pleural effusion and multiple rounded anechoic fluid-filled cystic structures fluctuating within the fluid and disseminated all over the visceral and parietal pleura. Abdominal cavity presented hyperechoic reactive fat and few small and irregular cystic structures. An ecoguided thoracentesis was performed and almost 2 liters of turbid milkish fluid, with many free fluctuating whitish structures (ranged from few mm to 2 cm), were aspirated. Samples of fluid were microscopically examined and revealed the presence of acephalic larvae (i.e., no suckers at the anterior end) of cestodes. Diagnosis of infection by larval stages of *Mesocestoides* spp. was performed on the basis of a PCR approach targeting the cox1 gene [3]. A therapy with oral fenbendazole 50 mg/kg twice a day associated with cephazoline 30 mg/kg IV twice a day and oxigenotherapy was started. Fenbendazole was administered for a period of 28 days. Due to an incomplete resolution of the clinical features, in agreement with the owners, the dog underwent to thoracic exploratory surgery to perform a wide surgical debridement and to remove metacestodes specimens. The histopathological analysis of pleuric tissue samples revealed a severe chronic multifocal lymphomonocytic and granulomatous mediastinitis associated to parasitic larvae. After surgery the patient continued therapy with oral fenbendazole 50 mg/kg once a day for a period of 3 months to prevent reoccurrence of pleural mesocestoidiasis and to treat the abdominal one. A follow-up ultrasonografic examination was performed monthly and, although some parasitic structures were always evident in abdomen, no clinical signs were reported after 12 months from the first visit. Pleural mesocestoidiasis in the dog showed characteristic ultrasound features. Ultrasound was very useful in evaluating the relative number and size of parasitic specimens during pharmacological therapy. Fenbendazole appears to be only partially effective against larval mesocestoidiasis as reported in the literature. [1] A surgical therapeutic approach was considered necessary to improve the patient quality of life and reduce the recovery time [1].

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CELL BLOCKS IN CANINE EFFUSION CYTOLOGY: METHODS COMPARISON AND TECHNICAL ISSUES

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Cell morphology assessment and cell type identification represent a crucial issue in cavitary effusions analysis, particularly in non-inflammatory disorders. Fluid-to-solid specimen conversion could complete the standard analysis providing blocks of cells that can be stored and used for ancillary tests. Two techniques have been described: the agar cell block (ACB) and the cell tube block (CTB). [1,2] Aim of this study was to compare ACB and CTB applied to canine cavitary effusions and define whether the presence of RBC may affect ACB quality. Canine spontaneous pleural, pericardial, and peritoneal effusions were processed as routine including refractometric total protein, automated cell count (ADVIA120), direct smear and cytospin preparation for cytological evaluation. Based on these findings, hemorrhagic, neoplastic, and doubtful effusions were included if at least 2 ml of fresh fluid in EDTA tube were available. ACB and CTB were prepared as previously described, formalin fixed paraffin embedded blocks were processed as histological samples and 4 µm sections were stained with hematoxylin and eosin. Cell blocks yield was defined as proportion of samples resulting in evaluable sections. Sections were scored (0 to 2) for background, diagnostic material, degeneration, and architecture; the overall quality (0 to 8) was determined by the sum of the scores of the single parameters. [3] To investigate the effect of RBC on ACB sections' quality, samples were divided in hemodiluted (≥10^6RBC/µl) and non-hemodiluted and compared by independent-samples Mann-Whitney U test. ACB and CTB quality and the effect of RBC lysis on hemodiluted ACB were evaluated by related-samples Wilcoxon signed-rank test. Thirty-three effusions (16 pleural, 14 pericardial, 3 peritoneal) from 33 different dogs were enrolled. ACB and CTB yield was 100% (33/33) and 58% (11/19) respectively. Overall quality of ACB and CTB sections showed no significant difference; mean total score was 6.3 (95% CI 5.81-6.91) and 5.9 (95% CI 5.05-6.76), respectively. Similarly, no significant differences were detected for any single parameter. ACB sections from hemodiluted samples (n=7) showed significantly lower scores for background (p=.008), diagnostic material (p=.021) and overall quality (p=.011) compared to sections of non-hemodiluted samples (n=10). RBC lysis pretreatment (n=6) improved the background (p=.003) and overall quality (p=.001) without affecting cell morphology. In conclusion, hemodiluted samples provided lower ACB quality and a previous RBC lysis treatment is recommended in these cases. Both ACB and CTB provided sections of good quality but CTB procedure was unsuccessful in a significantly higher proportion of cases. Unless technical improvement or evidence of case-specific advantages of CTB, our results indicate ACB as first choice technique for fluid-to-solid conversion of canine effusions. Diagnostic usefulness needs to be prospectively investigated.

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ANIMAL EXPERIMENTATION: RESEARCH PROJECTS AND EVALUATION PROCEDURES. PERSONAL OBSERVATIONS

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Italian legislation on animal experimentation (D.Lgs.26/2014), in compliance with Directive 2010/63/EU on the protection of animals used for scientific purposes, states that "no project can be carried out without a favorable evaluation by part of the Competent Authority".
In Italy, two Competent Authority have the purpose of authorizing procedures: 1) Ministry of Health and 2) Animal welfare body (Organismo preposto al Benessere Animale-OpBA). Ministry of Health acquires the opinion of the experts of the Istituto Superiore di Sanità or of the Consiglio Superiore di Sanità to authorize or reject the project. OpBA carries out the tasks related to the application of the requirement by legislation [1]. Although it is recommended the training of personnel who participate in various capacities in animal testing, to date no decree has been issued defining the procedures for recognition of the level of qualification of such personnel for research staff [2,3]. Four years after the entry into force of D.Lgs. 26/2014, we wanted to gather the main critical points identified in the Ministry's opinions in research projects in the Animal Facility of the University of Study "Magna Græcia" in Germaneto, Catanzaro.
Since October 2015, 15 projects were presented (13 on mice and 2 on rats), of which 60% (8 on mice and 1 on rats) received a negative opinion, 33.3% (5 on mice) were approved and 6.6% (1 on rats) is awaiting of evaluation. Of the 15 projects submitted, 73.3% (11 projects) received an application for integration (6 received a negative opinion and 4 were approved) by the Competent Authorities. For 10 projects (66.6% of total), the Ministry's assessment times exceeded the time limits indicated by law and in some cases it was necessary to wait even a further month before the opinion was issued.
The reported data show that the main problems encountered concern the following points in Annex VI of the project sheet: 20.1) the use of methods to replace, reduce and refine the use of animals in the procedures; 20.2) the Risk/Benefit analysis; 21) experimental methodology and statistics; 24) adverse effects and measures to reduce, avoid and alleviate any form of suffering; 26) severity classification of scientific procedure. Our survey shows that researchers often use summary information, sometimes repetitive and not specific, for the various proposed protocols, associated with a lack of appropriate knowledge for the experimental models indicated. Researchers not trained in the experimental model to be used for their research project often see the animal as an object and not as a sentient being, underestimating the damage and the degree of suffering inflicted on the animals used [4].
Appropriate training for researchers and technical staff could lead to improved animal welfare and the scientific quality of the results. Furthermore, in order to reduce the time needed for evaluation by the Competent Authority, it could be useful to involve a greater number external scientific experts, with specific competences regarding the research on animals and the different experimental models, thus avoiding also possible conflicts of interest.

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[2] *http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32010L0063:IT:NOT*
[3] *http://ec.europa.eu/environment/chemicals/lab_animals/pdf/guidance/education_training/it.pdf*
[4] *http://ec.europa.eu/environment/chemicals/lab_animals/pdf/guidance/severity/en.pdf*

AGREEMENT BETWEEN DIFFERENT METHODS TO CALCULATE LACTATE THRESHOLD IN STANDARDBRED RACEHORSES

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In equine sports medicine, the anaerobic threshold is traditionally defined as the Onset of Blood Lactate Accumulation (OBLA), namely the speed at which blood lactate concentration reaches 4 mmol/L [1]. Nevertheless, in human medicine it has been recognized that a fixed value does not consider the inter-individual differences in lactate metabolism. Therefore, several methods have been developed to define the individual Lactate Threshold (LT) [2]. Aim of the present study was to calculate the individual LT in racehorses by four different techniques applied in human medicine and to evaluate the agreement between the methods. From a large cohort of horses investigated for performance profiling, fifteen healthy and well performing Standardbred racehorses (aged 3.1±0.8 years old) were selected. Each horse underwent an incremental treadmill test as previously reported [3]. Blood samples were collected for each speed step of the test by means of a jugular catheter. Plasma lactate was measured with an enzymatic colorimetric method. Lactate values were analyzed with a dedicated software [4] and LT was determined by the following methods: a) Inflection Point (IP), i.e. the estimated speed corresponding to the location of the point of intersection between two linear splines on lactate curve; b) Lactate Threshold by logaritmic transformation (Log-Log LT), tracing the log lines, lactate and load, followed by a linear regression analysis to determine the transition between the two log lines; c) OBLA at 4 mmol/L; d) Initial Rise of 1 mmol/L, as the speed at which lactate increased 1 mmol above baseline. LT values obtained by the four methods were statistically compared by Friedman test and Dunn's Multiple Comparison test. Correlation between the values calculated by different methods was evaluated by Spearman test. Statistical significance was set at $p<0.05$. Statistical analysis showed a highly significant difference ($p<0.0001$) between the different threshold values. In particular, differences were found between IP and Log-log LT ($p<0.0001$) and between IP and Initial Rise of 1 mmol/L ($p<0.0001$). Correlation was significant only between OBLA and Initial Rise of 1mmol/L ($p<0.05$). Results showed lack of concordance between the different methods, therefore they cannot be used interchangeably, as reported in human sport medicine [5]. Further studies are needed to determine the gold standard technique to calculate LT in horses and the method that better associates with performance.

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RITUAL SLAUGHTERING AS A JUSTIFICATION FOR ALLOWING OFFICIAL VETERINARIANS TO EXPRESS CONSCIENTIOUS OBJECTION: SITUATION AND FUTURE PROSPECTS

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Current laws on the treatment of animals in Western countries state that animals be stunned before being slaughtered in order to avoid causing unnecessary suffering to the animals concerned [1]. Nonetheless, in many countries, for the respect of religious freedom, exemptions from this legal requirement have been granted to Jewish and Muslim communities, which perform ritual slaughter without stunning. As much concern has been raised regarding the welfare of animals slaughtered without prior stunning, these derogations are widely considered to be inconsistent with the objectives for animal welfare during the slaughter process contained in the law in force [2]. The slaughterhouse veterinarians shoulder primary responsibility in upholding the safety of meat, but they are also responsible for ensuring animal welfare. When playing their official role, performing inspections at the time of ritual killing, a number of them experiences a dilemma and would like to refuse to be a party to any of these practices. The aim of this study was to take into consideration the struggle that wages within the slaughterhouse veterinarians who are concerned about humane treatment of all animals at the slaughterhouses, and regard the suffering caused by ritual slaughtering as “unnecessary”, but despite this are required to witness ritual slaughters in order to perform official controls. Actually, even if the practice of ritual slaughter is evaluated as an expression of religious freedom, every democratic society should wonder how to reconcile the different interests. The existence of individual rights should be met either when one person claims an individual right to ritual slaughter and when another one claims individual protection of his or her sensitivity and ethic. A solution would be to express conscientious objection to the practice on ethical ground, because ensuring animal welfare is an inherent part of the veterinarian’s professional ethics. Unfortunately, conscientious objectors are recognized only if there is a law allowing them to express their position. At present, Italy has a law on conscientious objection that recognizes such a right in a few cases only (e.g. animal experimentation). Instead, until now, the legislator has not considered extending the possibility of granting the right to conscientious objection in the case of veterinary supervision on ritual slaughters. Furthermore, a recent answer given on behalf of the European Commission to a Parliamentary question concerning the provision to negotiate a stunning method for sheep and cattle slaughtered in the EU, whose meat is destined for halal export markets, confirmed that the EU can’t impose any particular slaughtering method. After collecting the views of the veterinarians working in three large slaughterhouses and after performing an analysis of the current legislation on the protection of animals at the time of killing, the Authors have explored the feasibility of introducing legislation recognizing a right to conscientious objection for slaughterhouse veterinarians. The results of the survey encourage to move in this direction, but the law in force may constitute an obstacle.

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CLINICAL INSIGHTS INTO FELINE AELUROSTRONGYLOSIS: A LARGE CASE SERIES

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The cat lungworm *Aelurostrongylus abstrusus* causes a lower respiratory tract disease in cats worldwide, whose clinical manifestations are non-specific and overlap with those of other conditions [1]. Bronchoscopy is one of the most useful techniques for providing a diagnosis in small animals with airway or lung diseases [2], though its role in feline aelurostrongylosis has not been fully investigated [3]. This study aimed at describing history, clinical and radiographic findings of 60 cats with *A. abstrusus* infection. Furthermore, the usefulness of bronchoscopy and bronchoalveolar lavage (BAL) in the diagnosis and management of 25 cats with feline aelurostrongylosis was also evaluated. Affected cats included 32 males (6 neutered) and 28 females (9 sterilized) with a median age of 18 months (3-132). All cats lived outdoors or had free access to outdoors. The most frequent complaints reported by the owners were coughing (n=27), ocular and/or nasal discharge (n=14), respiratory difficulty (n=8), anorexia or hyporexia (n=8), reduction of the activity (n=7), weight loss (n=6) and sneezing (n=5). On physical examination tachypnea, was observed in 21 cats and pyrexia in one; 5 cats had pale mucous membranes, while one was cyanotic. Thorax auscultation revealed adventitious breath sounds as follows: increased vesicular breath sounds in 27 cats, crackles in 12 and wheezes in 8. In 12 cats, no relevant clinical complaints were recorded and physical examinations were unremarkable. Thoracic radiography revealed abnormalities in 59 out of 60 cats. The most common radiographic patterns were bronchial (48/59) and interstitial (46/59) cats. An alveolar pattern was recorded in 12 patients, while 5 showed nodular lesions. In 2 cats, enlargement of caudal pulmonary arteries was recorded. Bronchoscopy was performed in 25 cats and revealed excessive bronchial mucus (n=21), airway hyperemia (n=9), bronchiectasis (n=7), epithelial irregularities (n=4), stenosis (n=3), airway collapse (n=2) and nodular regions (n=1). Eosinophilic (n=8), neutrophilic (n=8), lymphocytic (n=2) and mixed neutrophilic/eosinophilic (n=2) inflammations were observed at BAL fluid cytology, while 5 cats had BAL cytology within normal limits. Bacteria were isolated from BAL in 7 cats, with cytological evidence of sepsis in only one. Larvae of *A. abstrusus* were cytologically detected in 7/24 cats. Clinical history and physical findings of feline aelurostrongylosis are non-specific and all cats with outdoor access that have respiratory signs should be tested for *A. abstrusus*. In this cohort, several cats were subclinically infected, but radiographic changes were present in all of them but one, confirming that radiographic abnormalities may be evident before the onset of clinical signs. While bronchoscopy did not enhance the diagnosis of aelurostrongylosis, it represents a useful tool to detect bronchial abnormalities that might affect management or prognosis.

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EVALUATION OF PROTEINURIA IN HEALTHY RABBITS BY DIPSTICK AND URINARY PROTEIN: CREATININE RATIO IN SAMPLES COLLECTED BY CYSTOCENTESIS

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The diffusion of rabbits like a pet has led this species live for up to nine or eleven years. This extension of longevity is often accompanied by an increase in geriatric disorders, such as kidney diseases. Both acute kidney injury and chronic kidney diseases are considered relatively common disorders in older domestic rabbits [1]. Proteinuria in urine with an inactive sediment is considered to be one of the earliest signs of diseases of renal tubules or glomeruli [2]. The aim of this report was to evaluate the diagnostic reliability of a semiquantitative (dipstick) and a quantitative (urinary protein:creatinine ratio – UP/Cr) method for the detection of proteinuria in rabbits. Twenty-one clinically healthy rabbits, admitted for routinary wellness visit, based on the physical examination and haematology, biochemistry and urinary routine analysis were enrolled. For all of the animals included in the study an owner consent was obtained. Ten/21 were breed miniature lop (47.6%) and 11/21 Netherland dwarf breed (52.4%). Eight out of 21 of these were whole males (38.1%), 5/21 castrated males (23.8%), 7/21 neutered females (33.3%), and 1/21 whole female (4.8%) . Urine collection was performed by US-guided cystocentesis. Urine samples of 3 ml were refrigerated and examined within two hours of collection. After centrifugation of samples at 1000 rpm for 10 minutes, the supernatant was used for the detection of specific gravity (SG) by a refractometer and then analyzed by use of patch test strips (Combur-test, Roche Diagnostics) to have a semiquantitative estimation of proteinuria, as well as urinary pH, glucose, ketones, bilirubin, hemoglobin/myoglobin. The quantitative proteinuria evaluation was performed on the same supernatant by means of UP/Cr, using an automatic liquid chemistry analyzer (Pictus 400, Diatron). The values of proteinuria detected with the dipstick were 1+ in 15/21 subjects (71,4%), corresponding to about 30 mg/dl with SG ranging from 1.010 to 1.042 (median 1.033), 2+ in 5/21 rabbits (23.8%), corresponding to about 100 mg/dl with SG ranging from 1.037 to 1.060 (median 1.053) and negative in 1/21 animal (4.8%) with SG of 1.012. The UP/Cr values were 0.36 ± 0.24 (mean ± SD). A small amount of proteinuria has already been observed in rabbits [2] in urine samples collected from a spontaneous micturition. However, the determination of proteinuria by this collection method, does not allow to differentiating plasma proteins from proteins of different origins. The results of the present study shown that a small amount of protein can be normally present in the urine of healthy pet rabbits. This positivity was detected both with the dipstick and with the UP/Cr methods, but the semiquantitative proteinuria must be related to the urinary SG to achieve a proteinuria estimation which is thus subjective and not reliable. Therefore, in our opinion, the determination of UP/Cr is the most correct and recommended method to quantify proteinuria also in pet rabbits, as reported in other species.

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OBSERVATIONAL STUDY ON THE PALATABILITY OF FOUR COMPOUNDS IN THREE DIFFERENT FORMULATIONS IN NON-HUMAN PRIMATES

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Palatability is an important factor influencing the administration of oral medications in humans and animals. In wildlife and zoo animals, because ingestion of the correct amount is usually of critical importance and because some substances can be unpalatable, parenterally administration or gavage is sometimes necessary. These procedures require the capture and physical restraint of the animal as well as, highly skilled staff they also carry a risk of injury to both the animal and the operator [1]. To minimize stress on primates, the oral route would usually be the most desirable one, however the oral delivery of many drugs has been limited by their palatability. The aim of this prospective observational study is to describe palatability of four different flavors (chicken, apple, bacon, mixed fruits]) in three different formulations (capsules [EcciVetTM,Fagron], pasta and syrup [SysSvetTM, Fagron) to facilitate drug administration in non-human primates. The study was conducted at the Bioparco (Rome), between 1/07/17 and 30/10/2017, and included a total of 31 non-human primates, belonging to 3 different families: *Cercopithecidae* (*Cercocebus, Mandrillus sphinx*) 6 females and 4 males, ageing from 3 to 30 years, *Lemuridae* (*Lemur catta, Lemur macaco*, Red ruffed lemur) 7 females and 8 males, ageing from 3 months to 17 years and *Homiminae* (*Pongo orangutans, Pan troglodytes*) 12 females and 4 males, ageing from 3 to 42 years. Each subject received a single flavor which was presented each time in all three of the different formulations once a day for three consecutive days. The consumption of each portion was verified by one out of 7 different caretakers and direct observation and palatability ratings after each dose were obtained. Based on one of the most common scales used for evaluating the acceptability of medicines in children [2], to assess palatability a modified 3-point scale (0=not eaten: dislike; 1=partly tasted: not sure; 2=completely eaten: like; 3=like very much: request for an additional dose) was used. Data were analyzed using non-parametric tests and a p-value <0.05 was considered significative. In all of the groups, capsules were less readily accepted as compared with pasta and syrup ($p<0.01$). In particular, among the *Cercopithecidae* and *Homiminae* families, no differences were observed between pasta or syrup formulation, whereas *Lemuridae* seemed to prefer syrups. Regarding the different flavors, all the non-human primates belonging to the three families found the four flavors palatable, however the highest scores were observed in *Lemuridae* for mixed fruits vs bacon taste ($p<0.05$), and in *Homiminae* for bacon vs apple taste ($p<0.05$). Pasta and syrup were well accepted by all the primates while capsules were less palatable, particularly for subjects belonging to the *Lemuridae* family. In conclusion the four flavors used in this study, considering the responsiveness of non-human primates, could be utilized to successfully mask objectionable tastes such as that of some medicines, especially antibiotics.

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ASSESSMENT OF THE MICROVASCULAR PERFUSION USING ORTHOGONAL POLARIZATION SPECTRAL IMAGING IN HEALTHY NEWBORN FOALS

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Although different methods to measure tissue perfusion are available in equine neonatology, they are not representative of microvascular derangements. Noninvasive microcirculatory visualization by Orthogonal Polarization Spectral (OPS) capillaroscopy has been used in anesthetized animals and in human neonates and septic patients [1-3]. This technique has never been described in foals. Purposes of this study were to evaluate the feasibility of the OPS imaging to visualize the capillary microvasculature in conscious newborn foals, the differences between two sites and times of measurements, and the measurements reproducibility. Nine healthy newborn foals were selected. Three different sites at the upper and lower lip mucosa were assessed by OPS, using a hand-held capiscope, at 24 hours and at 4-5 days after birth. All procedures were carried out with the approval of the Ethical Committee of the University of Bologna. An informed consent was given by the owners. Video sequences were recorded at each site and timepoint by a single operator. Each video was assessed independently by two observers for quality and for semiquantitative calculation of microvascular parameters, including Vascular Density (VD), Microvascular Flow Index (MFI), Proportion of Perfused Vessels (PPV), and Functional Capillary Density (FCD), as previously described [1]. Data were analyzed using unpaired and paired t-test to assess differences between sites and time-points, respectively. Bland-Altman plots and intra-class correlation coefficient (ICC) were used to assess measurements reproducibility. Significant differences were found only for VD between 24 hours and 4-5 days in the upper lip (observer 1: p=0.04; observer 2: p=0.009), and between the upper (40.7±6.9l/mm) and lower lip (31.2±9.1l/mm) at 24 hours (observer 1: p=0.031; observer 2: p=0.0081); no significant differences were found for all the variables. The bias and 95% limits of agreement between observers were: VD 1.6 (-1.7–4.8), MFI 0.2 (0–0.4), PPV 6.3 (0.1–12.5), FCD 289 (-81–659) for the lower lip and VD 0.2 (-3.0–3.4), MFI 0.4 (-0.1–0.6), PPV 11.9 (1.7–22.1), FCD 482 (74–890) for the upper lip. ICC for measurement reproducibility was good for all parameters (0.64-0.79) for the lower lip, and was good for VD and FCD (0.76-0.79) and fair to moderate for MFI and PPV (0.1-0.41) for the upper lip. The microcirculation is feasible in the conscious newborn foal. The lower lip has the greatest success with reproducibility, and both sites could be used in order to generate comparable results. The preliminary PPV and MFI normal reference ranges, outlined by this study, are comparable to those outlined in the literature. The applicability of the OPS in foals and in particular the methods of measurements require further in vivo evaluations. This technique could allow to detect microvascular changes in both normal and cardiovascularly compromised states, particularly in septic foals.

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EVALUATION OF THE ERYTHROCYTE MEMBRANE LIPIDOME PROFILE IN HEALTHY DOGS AND ASSESSMENT OF THE POTENTIAL ROLE AS DIAGNOSTIC TOOLS IN CANINE DIABETES MELLITUS AND CHRONIC ENTEROPATHY

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Analysis of red blood cells (RBC) membrane lipidome represents a powerful diagnostic tool in humans for assessing the quantity and quality of fatty acids and for the follow-up of the membrane remodeling under physiological and pathological conditions [1], however a systematic study to evaluate membrane homeostasis in dogs has not yet been established. The aim of this study was to compare RBC membrane lipidome profiles of healthy dogs (HD, n=17) with dogs newly diagnosed with diabetes mellitus (DM, n=6) and dogs with chronic signs (i.e. >3 weeks) of enteropathy (CE, n=6). All dogs receiving dietary ω3 supplementation were excluded from the study. The RBC membranes were isolated from EDTA-treated blood and a cluster made of 10 saturated [SFA (palmitic; stearic)], monounsaturated [MUFA (palmitoleic; oleic; vaccenic)] and polyunsaturated [PUFA (linoleic; dihomo-gamma-linolenic; arachidonic; EPA; DHA)] fatty acids was determined by Gas-Chromatography. The HD were 5 males (1 neutered) and 12 females (3 sterilized), with a median age of 38 months (2-98); DM dogs were 2 males and 4 females (2 sterilized), with a median age of 120 months (96-158), and all CE dogs were male (2 neutered) with a median age of 81 months (12-126). In HD SFA, MUFA and ω6 levels were close to each other, while the ω3 values showed a wider variability (mean 1.67%; SD 0.91%). The RBC fatty acid-based membrane lipidome profiles in DM and CE dogs compared to HD showed different trends connected to metabolic transformations along the fatty acid pathways. The CE dogs had decreased levels of palmitic acid ($p<0.01$) and higher stearic acid ($p<0.01$) whereas DM did not show significant changes in these values, compared to HD. The MUFA levels were interestingly diverse in the two health conditions: higher in DM ($p<0.01$) and lower in CE ($p<0.05$) compared to HD. In particular, CE dogs had lower levels of palmitoleic ($p<0.05$) and vaccenic acids ($p<0.01$), while DM dogs showed an increased content of palmitoleic ($p<0.01$) and oleic acids ($p<0.01$). As regards of ω6-PUFA, only in DM arachidonic acid levels differed if compared to the HD, in particular lower levels were observed ($p<0.01$). ω3-PUFA levels were increased only in DM dogs in comparison to HD, both for EPA ($p<0.05$) and DHA ($p<0.05$) values. These preliminary data have clear limitations as for the sample size, the lack of data in geriatric healthy dogs and the lack of retrospective diagnosis of disorders associated with the chronic enteropathy. The variability of ω3 values found in erythrocyte membranes of healthy dogs, can be probably due to the individual dietary variations. However, it can be preliminarily observed that the SFA-MUFA pathway shows significant involvement in canine diabetes mellitus, with a higher palmitic-palmitoleic and palmitic-oleic transformations due to an accelerated delta-9 desaturase enzymatic activity. On the other hand, CE dogs showed increased levels of stearic, and decreased palmitoleic and vaccenic acids suggesting an activation of elongation pathway, leading to profound changes of membrane fluidity and permeability properties. In conclusion, erythrocyte membrane lipidome of dogs may be successfully applied in veterinary medicine, providing important information of different profiles under normal and pathological conditions.

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SEVERE PARANEOPLASTIC HYPERCALCEMIA SECONDARY TO AN ORAL MELANOMA AND PALLIATIVE TREATMENT WITH ZOLEDRONIC ACID IN A DOG

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Paraneoplastic syndromes (PNSs) are neoplasm-associated disorders arising from tumor secretion of hormones, peptides, or cytokines or from immune cross-reactivity between malignant and normal tissues. Sometimes, PNSs are the first evidence of a neoplastic disease they cause more significant morbidity than the tumour itself. The most common cause of hypercalcaemia in dogs is an underlying malignancy, and neoplasia is diagnosed in approximatively two-thirds of dogs with hypercalcemia [1]. Melanoma-associated PNSs are anecdotally in dogs and hypercalcemia occurs in 1-2% of humans with malignant melanoma, usually due to osteolysis and resulting release calcium into the bloodstream [2,3]. A 10-year-old male Boxer was referred to the Veterinary Teaching Hospital (VTH) of Teramo after a 3-days history of weakness, anorexia, vomiting, polyuria and polydipsia (PUPD). A non-movable mass on the lingual aspect of the right mandible was biopsied by the referring veterinarian. Histopathology was consistent with malignant melanoma. On physical examination at VTH, a weak femoral pulse, prolonged capillary refill time and abdominal pain with palpation were present. The melanoma on the lingual surface of the right mandible was approximately 3 cm in diameter. Abnormalities detected via CBS, serum biochemistry and urinalysis revealed neutrophilic leucocytosis [13,360/µL; Reference Interval (RI) 3000-12000], hypercalcemia (17.8 mg/dl; RI 8-12), and hysosthenuria (USG 1010). A large area of bone resorption of cranial part of right mandibular body was observed at radiographic examination visible on both laterolateral and dorsoventral projections. Abdominal US and a 3-view radiography of the thorax were within normal limits. Increased ionized calcium (Ca++ 2.45 mmol/L; RI 1.25-1.50), low parathyroid hormone (PTH <3 pg/ml; RI 20-130) and normal vitamin D3 (51.60 µg/L; RI 18-58) and PTH related protein levels (0.0 pmol/l; RI 0.0-1.0) ruled out hyperparathyroidism and humoral malignancy, suggesting hypercalcemia as PNS secondary to bone resorption. The dog received saline solution, gastroprotective and antiemetic drugs (ranitidine 3 mg/kg EV BID, maropitant 1 mg/kg SC SID, metoclopramide 1.1 mg/kg/die CRI) and restart eating during the second day of hospitalization despite high Ca++ levels (2.16 mmol/L; RI 1.25-1.50). One administration of 4 mg of zoledronic acid, diluted in 100 ml of saline solution in a slow intravenous infusion over 30 minutes, was able to normalize Ca++ in three days (1.45 mmol/L; RI 1.25-1.50); the owners refuse further diagnostic and therapeutic procedures and the dogs was discharged after 4 days of hospitalization. After 8 weeks of follow-up the dog remains normocalcemic and it was active, with a good appetite and without PUPD. In conclusion, hypercalcaemia is a rare complication of melanoma and, in both humans and dogs, is essentially due to aggressive bone lysis with an uncoupling in bone turnover. As observed in the present case, bisphosphonates such as zoledronic acid can offer short-term palliation.

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SURVEY ON MANAGEMENT AND WELFARE OF DOGS IN THIRTY SHEEP AND GOATS FARMS IN THE TUSCAN EMILIAN APENNINE AREA

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The dog was the first domesticated species [1]. With the domestication of other species and the dawn of pastoralism dogs started to be used to herd and protect livestock [2]. Pastoral context is peculiar since humans share the same environment with dogs and other domestic species. The aim of this study was to acquire information on dogs' management and welfare in pastoral environment, in order to identify any critical point for public health and to enlighten any difference in management between the following dog categories: livestock guarding dogs (LGDs), herding dogs (HDs), house dogs (PDs), truffle dogs (TDs). Data were collected by visiting 30 sheep and goat farms in the Tuscan Emilian Apennine area and interviewing the farmers. 184 dogs were included: 91 LGDs, 45 HDs, 41 PDs and 7 TDs. Only 7% (13/184) were pedigree dogs. As regards LGDs, the most represented breed was the Maremma Sheepdog, while most herding dogs' breed was Lupino del Gigante, a local Italian breed not officially recognized by the Italian Kennel Club. 81% (149/184) of dogs were regularly registered, with a higher percentage for sheepdogs (89%, 121/136) than PDs (61%, 25/41) and TDs (42%, 3/7). 65% (119/184) of dogs were vaccinated, with a higher percentage for HDs (87%, 39/45) than LGDs (59%, 65/91) and PDs (63%, 26/41); 65% (119/184) were wormed and 88% (162/184) treated for ectoparasites. Neutered dogs were only the 12% of sheepdogs (17/136) and 22% of PDs (9/41); all TDs were intact. None of the dogs were subjected to mutilations such as tail docking and ear cropping. 60% of farmers (18/30) used to chain up their dogs for short periods, 2 farmers kept some dogs restrained all the time. Most farmers fed their dogs only once a day, with petfood and dry bread, but in 17/30 farms (57%) both sheepdogs and house dogs had access to afterbirths and livestock carcasses. Given the dogs' temperament, faeces were collected from the ground whenever possible. Coprological examinations were positive for Tricocephali in 12/20 (60%) farms, Cestodes in 7/20 (35%) and Ascarids in 5/20 (25%) farms. Data concerning the official registration of dogs confirm that, at present, the Italian law no. 281/1991 to prevent the stray problem is not being implemented properly. Estrus control appears to be scarcely felt by farmers and it becomes an issue only if it compromises the sheepdogs' work. Another alarming aspect is the farmers' habit to let the dogs having free access to afterbirths and carcasses. In fact, the high prevalence of Cestodes observed in our sample, given the coexistence of all the host implicated in its biological cycle, throws up the red flag for the possible presence of *Echinococcus granulosus*, an important zoonotic agent. Strikingly, the prevalence of Cestodes we found in dogs is very similar to the prevalence of hydatidosis observed in adult sheep from Italian farms, which therefore has to be considered an endemic disease [3].

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IRREVERSIBLE PULMONARY HYPERTENSION IN A DOG NATURALLY INFECTED WITH *ANGIOSTRONGYLUS VASORUM*

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Angiostrongylus vasorum is a metastrongyloid nematode affecting the pulmonary arteries and the right heart of dogs and other animals. The most common clinical signs are cough, dyspnea and tachypnea due to bronchopneumonia but bleeding disorders, haemorrhagic diathesis, ocular and neurological signs are also been reported. Furthermore, the adult worms trigger lung vascular inflammation and pulmonary thrombosis, that are important mechanisms of secondary pulmonary hypertension (PH) [1]. At thoracic auscultation increased or harsh lung sounds, crackles and a systolic tricuspid heart murmur can be heard. Clinical diagnosis is often challenging and definitive diagnosis is obtained by detecting parasite L1s at the Baermann test or the circulating antigens with a rapid kit (AngioDetect®, IDEXX). The present report describes a case of irreversible pulmonary hypertension in a dog naturally infected with *Angiostrongylus vasorum*. An 8-year-old-female shorthaired Italian Hound was referred with a history of respiratory distress and exercise intolerance over the previous month. The clinical examination showed cough and dyspnea, while the thoracic auscultation revealed increased bilateral respiratory sounds and a right grade 2/6 systolic heart murmur. Radiographic imaging of the thorax showed peripheral alveolar pattern, nodular pattern and lung consolidation affecting areas through lung fields. A lungworm infection was suspected and was further confirmed by a positive antigenic test (AngioDetect®, IDEXX). The presence of *A. vasorum* L1s was recorded at Baerman test whilst no microfilariae were found at the Knott's examination. Complete blood count, biochemical and coagulation profile were performed. The electrocardiogram was within normal range while echocardiography showed a systolic tricuspid regurgitant jet with a TR maximal peak velocity of 3.2 m/s recorded at continuous-wave Doppler, with an estimated pressure gradient of 46 mmHg using the modified Bernoulli equation. The dog was treated with a spot-on solution containing imidacloprid 10%/moxidectin 2,5% (Advocate®, Bayer Animal Health). Four weeks after treatment the dog scored negative for *A. vasorum* larvae, cough and dyspnea were absent and findings at the thoracic radiographs were improved. Nonetheless, the echocardiography showed a tricuspid regurgitation flow similar to that found at the pre-treatment examination. After other four weeks, the animal was still negative for *A. vasorum* with no respiratory clinical signs but still had echocardiographic evidence of mild PH. Therefore, serial echocardiographic assessment to estimate the pulmonary artery pressure should be included in the clinical follow up of dogs treated for angiostrongylosis considering that severe cases of PH could present right heart failure, collapse and sudden death [2]. This exam is important to support clinicians to provide a more accurate prognosis to owners of *A. vasorum*-infected dogs.

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EVALUATION OF OXIDATIVE STATUS IN BALF OF HEALTHY AND SICK EQUIDS

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The oxidative stress has been associated with the pulmonary inflammatory disorders in horses suffering from recurrent airway obstruction (RAO) [1], summer pasture-associated obstructive disease [2] and inflammatory airway disease (IAD) [3]. The aim of this study was to evaluate the oxidative status of BALF in healthy and clinically sick equids. Nineteen horses and 4 donkeys were included. The protocol was approved by the Ethical Commette, University of Pisa (no. 14875/12). Inclusion criteria: not performing equids; no treatments during the last 30 days; same management. BAL was performed [4] under sedation (alfa$_2$-agonist). BALF was collected in EDTA, heparin and with no anticoagulant. BALF in EDTA was used to evaluate TNCC and differential cell count (DCC), heparinized BALF and no anticoagulant aliquots were centrifuged at 1,200 rpm for 10 minutes, frozen at 20°C in 4 eppendorf vials and analyzed in a single batch. D-ROMs (Reactive Oxygen Metabolites-derivatives) were assessed by a spectrophotometric method (Slim, SEAC, Italy) (505 nm) using commercial kits (d-ROMs test, Diacron Labs Srl, Italy), while NPBI (Non-Protein Bound Iron) and AOPP (Advanced Oxidation Protein Products) were evaluated by HPLC as proposed by others [5,6]. Based on the TNCC and DCC, the equids were divided retrospectively into three groups [3]: 1) healthy (n=5), 2) mixed IAD (n=11), 3) RAO (n=7). Data's distribution was verified through Shapiro-Wilk normality test. Mann-Whitney test for unpaired data were used to evaluate differences between groups. Pearson's test was used between pro-oxidant (NBPI or d-ROMs) vs end-products (AOPP) in the IAD and RAO groups. Statistical significance was set at $p<0.05$ (GraphPad Prism 6.0 USA). Statistical differences were obtained for d-ROMS healthy vs IAD ($p<0.02$) and healthy vs RAO ($p<0.002$), NPBI healthy vs IAD ($p<0.05$) and healthy vs RAO ($p<0.04$), AOPP healthy vs IAD ($p<0.0005$) and healthy vs RAO ($p<0.04$). A positive correlation was found between d-ROMs and AOPP in IAD ($p<0.011$; $r=0.726$).

This study showed an increased oxidative potential both in the mixed IAD and RAO group vs healthy horses. d-ROMs, NPBI and AOPP were higher in the pathological horses. These results support the hypothesis of an increament in oxidation status in equids affected by low airway inflammatory diseases.

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SCIENZE CLINICHE: SICV

ASSESSMENT OF UV-A/RIBOFLAVIN CORNEAL CROSS-LINKING EFFICACY FOR THE TREATMENT OF EXPERIMENTALLY INDUCED CORNEAL LESIONS IN AN EX VIVO ANIMAL MODEL

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The aim of this pilot study was to evaluate the histological changes induced by UV-A/riboflavin corneal cross-linking in experimentally induced corneal lesions in an ex vivo animal model.

Methods – Three groups of long-term (7 days) cultured porcine corneas were used, such as the control group (healthy), the injured group (alkali-induced corneal stromal melting) and the treated group (injured and treated with Vetuvir, 30 mW/cm2 for 3 min). All samples were kept in an organ culture medium for a week and then processed for histological analysis characterization. By using automated- image analysis of HE-stained sections, we collected quantitative data on histological features across the 3 groups. Differences were evaluated using both parametric and nonparametric ANOVA-type inferential methods.

Results – Statistically significant differences ($P<0.001$) were found among the tree groups in the mean of “brightness” (the proxy feature we used as measure of the relative extension of the injured areas in the corneal stroma). Specifically, the treated group showed a significant effect on the repair process after cross-linking treatment in comparison with the injured group (Tukey’s tests, $P<0.001$) and an equal level of brightness as in healthy (Tukey’s tests, $P=0.359$). Multi-aspect-type nonparametric analysis confirmed the effects in the mean of brightness showing also a significant difference in scatter between healthy and treated groups ($P<0.001$). These findings suggest that the tissue was recovered ‘in mean’ while keeping a higher heterogeneity.

Conclusions – The obtained results prove the effectiveness of cross-linking on the repair process from a histological point of view. The results support the rationale of the study and encourage further investigation in terms of extending both the sample size and the evaluating the repair process from a cellular and molecular point of view. One of the future aspects of this project could be the implementation of clinical trials of cross-linking on small domestic animals in order to evaluate whether technique may provide a valid alternative and /or complement conventional therapy also in the every-day clinical practice.

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PRELIMINARY EVALUATION OF ALFAXALONE FOR IMMERSION ANESTHESIA IN FISH

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Goldfish (*Carassius auratus*) is the most commonly fish kept as pet. Immersion anesthesia is more often performed than parenteral anesthesia in fish (1). Alfaxalone induces sedation and anesthesia because it selectively modulates gamma aminobutyric receptors. The purposes of this study is to assess the anesthetic effects of 6 mg, 7 mg and 9 mg alfaxalone/L on induction and recovery times in fifteen goldfish obtained from a fish private pond, that underwent skin scraping, gill exam and stool collection (ethical committee approval: 252 University of Parma, 2017, 6th November). They were considered healthy following a visual exam based on the evaluation of equilibrium, swimming, operculum movements and physical appearance. They were randomly divided into three groups (n=5). Each fish was transferred to an individual induction tank. Anesthesia was induced with the following alfaxalone doses: 6 mg/L (group G6), 7 mg/L (group G7) and 9 mg/L (group G9). The anesthetic stages (sedation, light anesthesia, surgical anesthesia and recovery) were evaluated assessing approach reaction, equilibrium, operculum movement and reaction to tactile stimulus (1). Sedation induction time, light anesthesia induction time, surgical anesthesia induction time, and recovery time (from the fish transfer to the recovery tank to the time of recovery of normal approach reaction, swimming, equilibrium and opercula movements) were recorded. The data were analyzed with ANOVA. The times in minutes (min) were reported as the least-squares means (LSM) ± standard error of the mean (SEM). P values <0.05 were considered significant. Sedation time of 6 mg/L dose (6.00±0.40 min) was significantly longer compared to 7 mg/L (3.80±0.40 min) and 9 mg/L doses (4.00±0.40 min). Light anesthesia and surgical anesthesia times of 9 mg/L dose (8.00±1.04 min; 10.20±1.84 min) were significantly faster compared to 6 mg/L dose (14.40±1.04; 20.80±1.84) and 7 mg/L dose (12.60±1.04; 19.60±1.84). One fish belonged to the group G7 and one belonged to the group G9 showed the cessation of opercular movement when surgical anesthesia stage was achieved. A steady flow of oxygenated water delivered through the oral cavity and across the gills was required to stimulate the buccal flow/heart rate reflex (1). No significant differences were recorded in recovery time. Alfaxalone is a reliable anesthetic induction agent in goldfish. Immersion in water concentration of 6 mg alfaxalone/L provided a smooth induction of surgical anesthesia stage in approximatively 20 minutes. As expected, higher doses shorten induction times but increase the incidence of respiratory depression (2).

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SURGICAL TREATMENT AND OUTCOME OF MENINGOCELE OR MENINGOMYELOCELE IN DOGS

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The purpose of this study is to retrospectively review the outcome of dogs surgically treated for meningocele (MC) or meningomyelocele (MMC).

All the dogs included in the study had a MRI diagnosis of MC or MMC and were surgically treated with a minimum post-operative follow-up of two months. Surgery consisted of isolation of the meningeal protrusion, nerve root adhesion resolution and reconstruction of the meninges.

Seven dogs were included in the study with a median age of 4 months (range 2 to 24 months), five French and two English Bulldogs. The main clinical signs were fecal (7/7 dogs) and urinary incontinence (6/7 dogs) with a decreased anal tone and sensation of the perineum, and paraparesis (3/7 dogs). MRI revealed the presence of four MMC and three MC in the lumbo-sacral region. Adjunctive spinal cord anomalies (arachnoid diverticulum, syringohydromielia and tethered cord syndrome) were reported in three dogs.

Surgery was carried out without complication and during the immediate post-operative period the neurological deficits temporarily worsened in three dogs. In the long-term period 4/7 dogs presented a stable condition, while improvement of the paraparesis and urinary and fecal continence was observe in 2/7 dogs and complete urinary and fecal continence was present in 1/7 dog.

MC and MMC are rare, probably underestimated, neural tube anomalies [1]. Our study suggests that the incidence of the disease can be similar between French [2] and English Bulldogs [3,4]. The main clinical sign is incontinence and, less frequently, abnormal gait. Our results suggest that surgery at young age can improve or impede the progression of the disease, as an improvement of the paraparesis and variable results for incontinence were observed. Notably no worsening of the neurological signs was noticed in the long-term follow-up.

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EVALUATION OF ABNORMALITIES OF THE NAVICULAR SPONGIOSA: CORRELATION BETWEEN RADIOGRAPHIC AND MAGNETIC RESONANCE FINDINGS

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The aim of the study was to investigate the correlation between radiological and magnetic resonance (MRI) findings observed in the navicular bone (NB) spongiosa. Radiographic examination is the first step in the diagnosis of navicular disease in horse even if it had low sensitivity in identifying medullar abnormalities [1] while MRI is considered the gold standard in the evaluation of the navicular bone [2].

Horses were selected for inclusion in the study that had lameness localized to the foot, undergone both radiographic and low-field MRI examinations of the foot in the period between February 2007 and May 2017. Navicular spongiosa was graded in both radiographic and MR images with a grading system composed by a number that indicated the gravity of lesion and by a letter to state the localization of the abnormalities.

Sensitivity, specificity, positive and negative predictive value of radiography for identification of spongiosa abnormalities were calculated considering MRI as gold standard. A K-Cohen test was used to calculate the association between NB spongiosa grades obtained using radiological and MRI examination. The distribution between categories was evaluated using a Chi-square test. Intra-observer agreement was calculated using a K-Cohen test.

Ninety-four horses were included in the study and a total amount of 129 navicular bones were evaluated.

The sensitivity of radiology in identification of spongiosa abnormalities was 80%, the specificity was 100%, the positive and the negative predictive value were respectively of 100% and 52%. Intra-observer agreement was substantial (0.88). There was a statistically significant difference between the distribution of the overall grades of the NB while the association between radiographic and MRI examinations was moderate (0.473).

Despite the moderate correlation between radiographic and MRI grades, x-rays allowed to recognize the presence of cystic-like lesions in the 85.7% of cases observed on MRI images; cyst-like lesions have been not detected when localized in the more distal aspect of NB. A good correlation (95.8%) was observed in absence of lesions. X-rays were less sensitive than MRI in detecting mild alterations of NB spongiosa and unable to detect those localized in the proximal border. Using radiography, abnormalities of the distal border were overestimated when of low grade and underestimated when of high grade. Radiography had a rather low sensitivity for identifying NB spongiosa abnormalities especially when localized in the proximal and distal border or when of low grade and MRI had to be considered the gold standard in the evaluation of NB [3].

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MINIMAL INVASIVE PIEZOELECTRIC OSTEOTOMY IN VENTRAL AND DORSAL DECOMPRESSIVE SURGERY FOR CERVICAL SPONDYLOMYELOPATHY: CLINICAL AND HISTOLOGICAL EVALUATIONS

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Piezoelectric osteotomy is based upon the principle of the indirect piezo effect which consists in the property the piezoelectric crystals have to be affected by a spatial deformation if subjected to an electric field with a given ultrasonic frequency. When transferred onto the instrument tip, the linear and micrometric oscillations produce a selective mechanical cut of the mineralized tissue with no significant damages to the underlying soft tissues. The employment of piezosurgery bone scalpel in both ventral slot and dorsal laminectomy offers some putative not negligible advantages in terms of minimal invasiveness, but few data still have been reported about its real efficacy and safety. To test the efficacy, safety and feasibility of piezosurgery on a consecutive institutional series of ventral and dorsal decompressive procedures aimed to cure cervical spondylomyelopathy. In forty-two dogs all the procedures were entirely performed by means of Mectron Piezosurgery® bone scalpel (Mectron Medical Technology, Genoa, Italy). Efficacy, precision, safety and blood loss were evaluated intraoperatively and postoperatively case-by-case by the senior author (A.C.). Histology on bony specimens allowed to evaluate the effects of piezosurgery on osteocytes and osteoblasts. Osteotomies were sharp and precise in all cases, leading to the sparing of soft and neurovascular tissues. Operative field was blood-free and heat-free. The wide range of inserts, different in shape and length, allowed to treat elegantly and effectively the deep-seated field of ventral slot with a near to zero complications. The same results were obtained with dorsal laminectomy. A prompt and fast recovery of functions was observed in the most of the treated cases. Histology on bone flaps showed the presence of live osteocytes and osteoblasts at the osteotomized surface. In Conclusions Piezoelectric bone surgery has proved to be effective, selective, simple to perform and very safe in ventral slot and dorsal decompression for wobbler syndrome. Equally effective to drills and saws, piezoelectric bone scalpel results minimal invasive on the neighboring soft tissues and neurovascular structures.

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ULTRASOUND ELASTOGRAPHY FOR EVALUATION OF TENDONS AND JOINT CAPSULES IN HEALTHY DOGS AND HORSES: A PRELIMINARY STUDY

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Ultrasound strain elastography (USE) is a non-invasive technique that uses ultrasounds to provide information about tissue stiffness and elasticity [1], based on the principle that tissue compression by an ultrasound probe produces a displacement within the tissues [2]. This technique has been proven useful to evaluate human and horse muscoloskeletal system, in healthy and pathological tendons, which show an altered elasticity [3-5]. The aim of this study was to investigate the feasibility of the USE on various tendons and joint capsules in healthy dogs and horses, as well as to describe their elastographic appearances. We included in the study 5 dogs and 5 horses. Ten normal biceps brachii (BT), calcaneal (CT) and supraspinatus tendons (SST) and ten normal fetlock joint capsules (JC) were examined using the USE. Each structure was examined transversely and longitudinally except for the calcaneal tendon due the unfeasibility of a transverse study in that region [6]. Semi-quantitative and qualitative analysis of each structures was performed using the strain ratio (SR). The elastographic studies were performed at the area considered the most vulnerable to injury: in the BT the SR of the tendon was calculated in the bicipital groove; in the SST the SR was measured at his insertion on the greater tubercle of the humerus and in the CT, the SR was calculated at its insertion on the calcaneus. For those tendons, different structures located within the region of interest have been chosen as control structures, respectively the SST, the supraspinatus muscle and the standoff pad above the CT. Regarding the JC, the SR was measured at its insertion on the dorsal proximal aspect of the first phalanx, using the dorsal digital extensor tendon as reference. All examined tendons appeared hard and the capsules soft on both qualitative and semi-quantitative analysis. In conclusion, the findings of the present study indicate that the USE is easily realizable in those areas and is a feasible method to determine elasticity values of muscoloskeletal structures. Further studies comparing the elasticity values of normal and pathologic tissues are necessary to determine the diagnostic role of this technique.

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COMPARISON OF THREE CELL FREE NOVEL BIPHASIC SCAFFOLDS FOR ONE-STAGE TREATMENT OF OSTEOCHONDRAL DEFECTS IN A SHEEP MODEL

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Osteochondral defects are a common problem in both medical and veterinary practice with a difficult resolution since the currently used techniques have important limits especially concerning the cartilagineous tissue regeneration. In recent years an interest in the subchondral bone has growth considering it as an important element in the osteochondral defect healing. Considering this, researchers have focused their attention on creating instruments that can be suitable to repair both bone and cartilagineous tissues simultaneously to enhance the possibility of osteochondral lesions repair (1). The aim of this study was to evaluate the regenerative performances of three cell free scaffold in collagen and hydroxyapatite and collagen hydroxyapatite wallostonite in a sheep animal model. In our study, a novel three-dimensional collagen/HA osteochondral substitute is proposed, surrounded in circumference by a thin layer of collagen (2), and characterized by a stable preintegration between the osteo- and chondral components, easy surgical handling, and compliant at the implant site. A novel configuration also evaluated is the HONEY scaffold in which hydroxyapatite columns are inserted inside a collagen slur (3). The use of different kinds of scaffolds were used to permitted to identify which one had the better regenerative characteristics, since they had different constructions methods, materials and shape, and to evaluate finally which one could represent the best choice. The study started after the planning and realization of the devices and then implanting them in an in vivo model represented by sheep. The animals were divided in four groups, three were treated using different kind of scaffold while one represented the control group. Evaluations were made at 3 and 6 months through TC, MRI and histological examinations. The in vivo study on the sheep model to evaluate the efficacy of the proposed scaffolds for repairing osteochondral defects demonstrated the biocompatibility of three cell-free, biphasic scaffolds in an osteochondral defect. The novel “HONEY” configuration of the scaffold with Hydroxyapatite allowed for a better reparative process, although we are still far from obtaining a complete restoration of the defect.

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INTERTROCHANTERIC VARUS OSTEOTOMY FOR THE TREATMENT OF THE CANINE HIP JOINT INCONGRUENCE

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Intertrochanteric Varus Osteotomy (ITO) is a surgical procedure performed on immature dogs with coxa valga (>146°) [1]. The aim of ITO is to reduce the coxa valga by means of varisation of the femoral inclination angle to achieve an angle of 135°, namely 10° less than normal. This technique is not common in veterinary medicine, probably because canine hip dysplasia (HD) is not usually associated with coxa valga. Moreover, the beneficial role of varus osteotomy for the treatment of hip osteoarthritis (OA) has been recognised in human medicine since the 1920s, and it has been reported that osteotomy delays the need for hip replacement in young patients with OA by more than ten years [2]. The purpose of the present study was to describe a novel rationale of ITO, enrolling dogs with an early diagnosis of HD even if the angle of inclination of the femoral neck is not increased. Moving the femoral head closer to the center of the acetabulum, as is suggested in the human literature, the force acting on the cartilage surface of the acetabulum can be reduced by redistributing the weight-bearing load. The results can be reduction in mechanical stress and improvement in joint congruence. Inclusion criteria, novel preoperative planning, pre- and postoperative radiographic study and long-term outcome are described. Dogs under 12 months of age with hip laxity and a positive Ortolani sign, with radiographic signs of hip incongruence, coxofemoral subluxation, the center of the femoral head placed lateral to the dorsal acetabular rim, a Norberg angle (NA)<105°, percentage of acetabular coverage (PC)<50%, normal or increased femur inclination angle, and absence of or mild signs of OA were enrolled. Dogs which presented with deformity of the head and acetabulum were excluded. The inclination angle was measured using the Symax method and the amount of varisation osteotomy was calculated to move the axis of the femoral head and neck through the center of the acetabulum[3]. Clinical evaluation (lameness, pain, range of motion) and radiographic examination (hip congruence, NA, PC, and OA) were carried out pre- and postoperatively at 1, 2 and 6 months, at 4 and 8 years. Eleven dogs (15 hip joints) underwent ITO (mean weight 32.2±11.5 kg, 9 males and 2 females, mean age 10.2±2.2, 6 right and 9 left limbs). No dogs showed lameness and pain 3 months after treatment, and the range of motion decreased in hip extension. The radiographic measurements after surgery increased significantly, according to the Wilcoxon test, as compared to the preoperative values. Joint congruence was preserved at each time point, and the OA increased slowly; however, a relationship between the radiographic and the clinical evidence was not found. Seven hip joints were evaluated over a very long period of time (4-8 yrs), and no acetabulum was flattened, no femoral head was subluxated and joint congruence was preserved. These findings could encourage the use of ITO, known since 30 years, on greater number of selected subjects for the treatment of early stage HD in dogs with radiological signs of joint incongruence.

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FOURTY-ONE CASES WITH CRANIAL CRUCIATE LIGAMENT RUPTURE TREATED WITH POROUS TTA: THREE YEARS OF FOLLOW UP

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Tibial Tuberosity Advancement (TTA) is an extracapsular surgical technique based on a linear osteotomy that determines a cranial advancement of the tibial tuberosity in patients suffering from anterior cruciate ligament rupture. The aim is to neutralize the cranial tibial thrust (CTT) and to reach a 90 ° angle between the patellar tendon and the tibial plateau when the knee has an extension of 135 ° (physiological angle), so to obtain an independent stabilization from the cranial cruciate ligament [1]. In our study, the use of a Ti6AI4V ELI titanium scaffolds (porous wedge, developed by Instituto Tecnològico de Canaria, Las Palmas) has been adopted for the Porous TTA, with excellent properties of osteointegration, osteoconduction and osteoinduction when subjected to cyclic loading. Based on a previous scientific work on an ovine model, the use of this type of porous scaffolds has subverted the previous models, proposing itself as a valid alternative. Scaffold production technology is based on the direct mechanical manufacturing called Electron BeamMelting (EBM) [2]. For this study, 41 cases of patients belonging to canine species, mixed breed, medium-large size, of live weight between 10 and 80 kg, aged between 1 and 13 years, were enrolled. The inclusion criteria in the study and subsequent follow-up investigations were based on clinical evaluation of patients (different gaits), functional tests of the joint (drawer test and tibial compression), score obtained by LOAD questionnaire (Liverpool Osteoarthritis in dogs), radiographic diagnosis in sedation with a 135 ° positioning of the joint and baropodometric investigations (stance analyzer). The use of porous scaffolds has allowed to obtain encouraging results in patients subjected to Porous TTA, in terms of LOAD score, radiographic and clinical evaluations. In conclusion, Porous TTA is an excellent method for functional recovery of the knee joint following partial and total rupture of the cranial cruciate ligament.

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TRAMADOL IN SLOW INFUSION IN THE IN THE FUNCTIONAL BALANCE OF THE FOOT IN BOVINE

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The study was approved by the University of Messina Review Board for Animals Care. Experiments were performed following Italian law (D.M. 116192), Europe law (O.J. of E.C. L 358/1 12/18/1986), and USA laws (Animal Welfare Assurance No A5594-01, Department of Health and Human Services, USA). Moreover, the owners of each bovine was informed about the study and signed a consent.

The study conducted in brazil on the identification and subsequent treatment of pain, of veterinarians, in cattle and horses shows that: the tramadol it is used by 39% of brazilian veterinarians [1-3]

The objective of the study, is to evaluate the analgesic and sedative effects of slow intravenous injection of tramadol in bovine.

Twenty bovine (3±1 years and 600±20 kg of weight) divided into two groups receive, during the functional balance of the foot, tramadol bolus IV B group (1 $mgkg^{-1}$) or tramadol (1$mgkg^{-1}$) slow intravenous administered over 10 minutes BL group. Heart rate (HR), respiratory rate (RR), sedation and analgesia were assessed before and up to 20 minutes following drugs administration. The sedation has been evaluated, considering ataxia, assessed through a score scale 0/4 and measuring head height above aground. Analgesia were evaluated through Numeric Rating score scale (0/10).

Pre, post-treatment and comparison difference between the three groups, physiological and behavioural variables were compared using the Friedman's test and 2-way ANOVA with $p<0.05$ considered significant.

Heart rate and RR, they increase, during the functional balance, respect to baseline in both groups. However, in increase of HR and RR is greater than the BL group. Ataxia scores is low in both groups: group B 0(1-1) and group BL 0(1-2). The head height above aground it does not change following both treatments .The analgesia score, they are lower in BL group than B group :1(3-3) /4(7-7) vs 10(9-9) p=0.000.

Tramadol slow intravenous administered over 10 minutes, it provides in cattle, better analgesia compared to fast bolus administration.

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POROUS TITANIUM SCAFFOLDS AND THEIR USE IN ORTHOPEDIC SURGERY

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Scaffolds are used for three-dimensional tissues regeneration to allow the organization of cells in complex structures. In bone tissue engineering they must promote cell adhesion and deposition of new tissue, which can colonize or replace the artificial implant over time.

Scaffolds must possess a geometry that is highly interconnected trough poruses with a large area/volume ratio that allows cell growth, distribution and neovascularization of the structure from the surrounding tissue.

Porosity and pore's architecture influence physical and mechanical properties of the scaffold, indeed their control allows to obtain strength and elasticity similar to those of cortical bone [1].

Titanium and its alloys are widely used in bone tissue engineering due to highly biocompatibility, corrosion resistance and osteointegration which make the perfect for their use in orthopedic surgery [2,3].

In our paper we describe some possible application in orthopedic surgery of titanium alloy Ti6Al4V-ELI scaffold, developed in Istituto Tecnològico de Canaria, made through electron bean melting technology (EBM). With this technology can be obtained three-dimensional implants with a " Trabecular Titanium " microstructure, multiplanar, regular, with hexagonal cells, characterized by a highly porosity that mimics the trabecular bone [4].

Our applications refer to the use of custom made porous titanium scaffold in experimental and cinical application.

In all this cases we performed in post-operative clinical and radiological evaluations.

Clinical evaluation didn’t show any adverse reaction to the biomaterial.

Radiographic follow-ups showed a progressive integration of the scaffold in bone tissue, and the latest one (12 months follow-up) showed how the scaffold was perfectly integrated into the surrounding bone tissue.

The porous titanium scaffolds are important tools for orthopedic surgery because their ability to create an ideal environment for new bone tissue formation while allowing a early functional recovery.

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TOTAL LAPAROSCOPIC GASTROPEXY WITH PDS STAPLES IN THE DOG

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The aim of this study is to propose a totally laparoscopic gastropexy technique to be performed with completely resorbable PDS staples. 6 giant dogs (4 Great Danes and 2 Neapolitan Mastiffs) were selected with an average weight of 67 kg, 4 females and two males. The dogs were placed in dorsal decubitus and the abdomen prepared for aseptic surgery. A Verres needle was inserted through the eleventh right intercostal space in the caudal back to insufflate the abdomen with CO_2 (maximum intra-abdominal pressure, 10 mmHg) using a laparo-insufflator. An incision was made through the umbilicus and a 5 mm trocar inserted into the abdomen for the introduction of a 5mm 30° laparoscope. Two portals for 5 mm instruments were placed under laparoscopic guidance, in the same frontal plane respectively 6-7 cm to the left and right of the laparoscope. An atraumatic 5 mm bowel grasper was introduced through the instrument portal. The ventral gastric wall, proximal to the pylorus and cranial at the attachment of the large omentum, was gripped and raised towards the right and ventral abdominal wall. The appropriate peritoneal area was located 3 cm caudal to the 13th rib and 3-4 cm lateral to the right rectus abdominis muscle. The peritoneum area and the relative gastric serous corresponding to the area identified for the anchorage were debrided with a radio-system, in four or five points. The distance between each point was about 0.5-1 cm. Using a specific applicator (Ethicon securstrap) the PDS staples were applied through the serum stomach layer and the abdominal wall positioned along the coagulation line. The hold of the pexy was checked and the pneumoperitoneum was stopped, the trocars were removed and the surgical wounds sutured. The dogs underwent ultrasound examination of the right flank region to monitor the integrity of the pexy in follow-ups at a distance of 7 15 and 30 days after the operation. The technique proved to be quick and simple with surgical times below 15 min. No subject showed intra- and post-operative complications and recovery was excellent. The mini-invasiveness of the procedure allowed minimal post-operative care and a resumption of normal activities as early as the day after the operation. The ultrasound checks showed the presence of the pexy and the lack of mobility of the stomach in all the checks carried out. The morbidity of GDV in treated subjects is absent until the last follow-up (4 months).

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PAIN ASSESSMENT IN CALVES UNDERGOING RING CASTRATION

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The rubber ring method for the castration of calves is quick, easy, cheap and reliable, but there are several studies indicating that it causes significant pain during and after the procedure [1]. The aim of this study was to evaluate the impact of the ring castration of 2-month-old calves on potential indicators of pain or stress. These indicators included serum cortisol concentration, leukogram, behavioural posture and activity evaluated using the UNESP-Botucatu unidimensional composite pain scale [2], local temperatures and scrotal clinical evaluation. Moreover we used the in-field measurement of Leukocyte Coping Capacity (LCC) on whole blood as a different method to evaluate suffering condition [3]. Twenty healthy calves (90±4 kg of body weight, 2 months of age) were selected for the study and randomly assigned to either sham (n=10) or ring castration (n=10). Calves were handled in a similar manner for analogous amount of time and castrated with rubber rings using an elastrator or not castrated. Behavioural assessment, blood sampling, temperature recording and scrotal evaluation were repeated 1 hour before castration, 30 minutes after ring application and at day 3, 7 and 14 after ring application. The same timepoints were used in the sham group. Chronological changes of cortisol, leukogram, pain scale score, local temperature and LCC were analysed within each group and between groups using the SAS statistical software. $P<0.05$ was considered significant. After the ring application, the scrotal sac appeared swollen at day 3, mummificated and partially detached at day 7 and at day 10 it was surgically removed. Pain scale score showed very little pain in castrated animals at any timepoints (mean score range 0.3-0.6 out of 10) and no pain in sham calves (0 out of 10). No significant differences were recorded between sham and castrated animals among timepoints in pain scores, cortisol concentrations and leukograms. LCC decreased significantly in castrated animals at day 7 compared to baseline values, thus indicating an increase in stress level. A significant difference beween groups was observed in LCC values 7 days after ring application ($p<0.0001$). Pain indicators such as serum cortisol, behavioural observation, scrotal temperature and clinical evaluation were unaffected after ring castration. Since LCC presented a significant variation, we considered that this method might represent a more sensitive tool in case of procedures that cause mild pain.

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EVALUATION OF A NEW ANAESTHETIC PROTOCOL IN RING-TAILED LEMURS (*Lemur catta*)

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Lemur catta are endangered prosimians often kept in zoological exhibits. Chemical immobilization is most of the time compulsory to perform veterinary procedures in these animals [1]. The aim of this observational study is to evaluate the quality of sedation, induction and maintenance of anaesthesia and cardiorespiratory effects of a new anaesthetic protocol in ring-tailed lemurs undergoing bilateral orchiectomy. The study was carried out at Valcorba Zoological Park (Pozzonovo, PD, Italy) following internal ethical clearance approval. Six healthy adult ring-tailed lemurs were sedated with a combination of dexmedetomidine (0.015 mg/kg), midazolam (0.2 mg/kg) and butorphanol (0.2 mg/kg) administered intramuscularly. To perform oral intubation, alfaxalone (0.5 mg/kg) was administered intravenously over 60-seconds and further boluses of 0.2 mg/kg were given every 20 seconds if needed. The initial infusion rate of alfaxalone (0.1 mg/kg/minute) was adjusted to maintain cardiovascular and respiratory stability while performing the surgical procedure. Heart rate, ECG, SpO_2, rectal temperature, respiratory rate, $EtCO_2$ and non-invasive blood pressure were recorded every 5 minutes throughout surgery and recovery. Quality of sedation, induction, maintenance and recovery were assessed, and reaction to manipulation scored. These assessments were performed by the same observer using a descriptive scoring system specifically designed for this study. Quantitative data were analysed with two-way Anova and the Bonferroni correction using SAS statistical software (version 9.3, SAS Institute). P values <0.05 were deemed significant. Sedation at 20 minutes was scored as “profound” in all animals and no reactions to manipulation were seen. Induction and intubation were scored as “smooth and uneventful” in all animals but one, which was scored as “satisfactory”. Three out of six animals had self-limiting muscle twitching at induction. Maintenance of anaesthesia was scored as “good” in five animals. Only one speciment showed respiratory depression, thus oxygen was administered and ventilation manually assisted. Heart rate and respiratory rate decreased 15 min after the beginning of alfaxalone infusion ($p<0.05$). Alfaxalone infusion was discontinued at the end of the surgery and, although extubation was possible after 15.33±8.02 min, atipamezole had to be administered to achieve full recovery after 38±5 minutes from injection. In conclusion, the combination of drugs proposed in this study offered a reliable sedation and anaesthesia in lemurs, nevertheless careful monitoring is always recommended.

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EQUINE CHRONIC LAMINITIS: EFFECTS AND EVOLUTION WITH THE APPLICATION OF FIVE DIFFERENT SHOEING

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Laminitis, in its chronic stage, is one of the most devastating pathologies currently known in equine podiatry and orthopaedics [1].

Over the past years, a great number of different therapies have been experimented in the treatment of this condition, some of them with better results than others [2].

The purpose of this study is to evaluate and compare the effects and the evolution of this stage of the pathology in five horses, with the application of five different therapeutic shoeing. All horses received the same treatment during the acute phase [2], except for the last one which was presented too late. Each of them has a different history and different pathologic evolution: the first horse is a show jumper that was treated for more than one year (as in hospital, as at home) using ACR300 which toward the end of the study was substituted with a handmade wooden shoe; the second one is a carrying sport horse treated for almost six months (as in hospital as at home) using ACR300; the third one is a Polish draft horse used for reproduction treated only at home using Rock'n Roll shoes; the fourth one is a Shetland pony, used for pet, treated at home with Banana shoes; last one is a Romanian mixed breed horse, used for work, showing a severe chronic laminitis with perforation of the sole in all four limbs, treated with Reverse shoes.

There were collected data regarding the pain scale about all horses during the evolution of the disease and they have been evaluated using the Obel Grading System (1948) [3] and the body condition score (BCS) to allow an objective evaluation of the real improvement of the condition of the animal, between the beginning and the end of the study.

Three horses showed a sensible improvement of the condition, total remission of the symptoms at the end of the study, so they could come back to their activities; a fourth horse showed a sensible improvement of the condition as well, but after one year he had not yet reached a total remission of the symptoms, even if toward the final period of the study his conditions were visibly improving. The fifth horse, instead, at the end of the study and after a first improvement of the condition, was still fighting with the pathology.

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ROPIVACAINE VIA WOUND SOAKER CATHETER (WSC) IN POST-OPERATIVE PAIN MANAGEMENT OF AMPUTATED DOG

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Limb amputation induces a high level of post-operative pain. The use of soaker catheters for the administration of local anesthetics into the surgical wound is a relatively new technique for providing post-operative pain relief. Although local anesthetics (LA) provide excellent analgesia, their duration of action is relatively short, limiting their efficacy in the post-operative period. Diffusion catheters for intermittent administration of LA into the surgical wound may be kept in place as long as they are needed in order to block somatic sensation of the incision and surrounding muscle beds [1]. In veterinary medicine, there are few studies about the use of soaker catheters in which it was found that the technique had few complications, led to a more rapid post-operative return to walking, eating, and urination and reduced recovery [2,3].

The aim of the study was to evaluate the post-operative analgesic efficacy of the intermittent administration of LA through the WSC in canine patients who undergone surgical limb amputation. Ropivacaine (R) was used in order to evaluate possible additional benefits of this drug, actually rarely used in veterinary medicine. 20 dogs undergoing limb amputation at the Ospedale Veterinario Universitario were assigned to 2 groups: G1 received the routine post-operative analgesic protocol (buprenorphine, 10 µg/kg QUID – carprofen 2 mg/kg BID); G2 received, in addiction, boluses of R (1 mg/kg QUID) through the WSC for 24h after surgery. The rescue analgesic protocol provided for the administration of buprenorphine (10 µg/kg) in both groups. Every dog was submitted to the same evaluations. During pre-operative phase, a behavioral evaluation report (ad hoc designed) and the pain assessment (Colorado Pain Scale and Melbourne Pain Scale) have been assessed. During the surgery, HR and NIBP were monitored and recorded. Pain scores (Colorado and Melbourne) were assessed for several time post-operatively (T2, T8, T16, T24). In addition, HR and RR were measured in this phase (T0, T6, T12, T18, T24) and a descriptive evaluation of post-operating course has been made too. Written informed consent was obtained from all of dog's owners. Pre- and intra-operative data were compared by statistical analysis (SPSS Statistics Ver 25 Test of: Kruskal-Wallis, Jonckheere-Terpstra and Independent samples medians*)* in order to evaluate homogeneity between the two groups in those phases and the post-operative results in order to evaluate possible differences between groups. Statistical analysis showed no significant differences between G1 and G2 before T0 (starting point of the different protocol). G2 showed a significant pain score reduction >8h after surgery (M: 10±2 vs 4±3; C: 2±0.4 vs 1±0.4), that was not previously demonstrated in veterinary medicine, and amputees receiving peripheral administration of R had faster recovery after surgery. The use of WSC demonstrated to provide a more effective analgesia when compared to traditional systemic analgesia after limb amputation and R provided optimal analgesia without adverse effects. May be suggested that the use of the behavioural evaluation report provide more effective pain assessment in dog.

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DNA ELECTROVACCINATION WITH A CHIMERIC CSPG4 PLASMID FOR THE TREATMENT OF MALIGNANT MELANOMA IN DOGS

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Canine oral malignant melanoma (OMM) accounts for 30–40% of all oral tumors, with 80% of dogs presenting tumor invasion in different organs [1,2]. The median survival time of dogs affected by OMM is very short, being about 200 days after diagnosis, with a high mortality rate because of recurrences and metastasis [3,4]. Standard therapies are mostly surgery and/or radiotherapy for the local control of the tumor, which occurs in about 75% of treated dogs. However, the 1-year overall survival is less than 30% [3]. Systemic chemotherapy could be an option even if it has very little, if none, benefits in terms of clinical outcome [5]. Therefore, more effective treatments are still needed. In our studies, the feasibility and the anti-tumor potential of targeting the melanoma-associated oncoantigen chondroitin sulfate proteoglycan (CSPG)4 with DNA vaccines delivered by electroporation (EP) have been evaluated. We have previously demonstrated the safety and the clinical relevance of the intramuscular injection of a Hu-CSPG4 plasmid followed by EP in dogs with stage II-III surgically resected $CSPG4^+$ OMM. Hu-CSPG4 EP caused no side effects and resulted in a significantly longer disease-free and overall survival of vaccinated dogs as compared to controls [6,7]. Nevertheless, some vaccinated dogs eventually died because of metastasis. To increase the efficacy and at the same time the translational power of our approach, we employed a hybrid plasmid coding for a chimeric CSPG4 protein (HuDo-CSPG4), expected to be effective in both veterinary and human settings.

We tested the safety, the immunogenicity and the anti-tumor potential of HuDo-CSPG4 intramuscular DNA EP in mice, in stage II-III surgically resected $CSPG4^+$ OMM canine patients and in an in vitro human setting. In conclusion, our results demonstrated that the EP of HuDo-CSPG4 plasmid: i) is safe in canine OMM patients and induces high affinity antibodies effective in significantly increasing the overall survival of vaccinated dogs as compared to controls; ii) is an effective way to in vitro break immune tolerance against the self-antigen in humans, laying the foundation for moving the approach of anti-CSPG4 DNA plasmid EP to standard anti-cancer therapy in both veterinary and human clinical settings.

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RECOVERY TIME AND COMPENSATORY EFFECTS OF MUSCULOSKELETAL OVERLOAD INJURIES IN STANDARDBRED RACEHORSES

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Overload musculoskeletal injuries (MSIs) are the most common cause of day-off from training [1] and the first cause of retirement in racehorses [2,3]. Percentage of Standardbred racehorses (STBRs) returning to racing after a specific lesion, the time to return in activity and the long-term effect on performances following overload MSIs are poorly explored in harness racing. Compensatory load redistribution during trotting is demonstrated in experimental conditions [4,5], but there is no data demonstrating a compensatory effect can favour the onset of subsequent overload MSIs. The ability of the injured STBRs to meet performance expectation is increasingly important, as racing industry need dealing with costs of therapy and rehabilitation. The first objective of our research is to calculate the percentage of STBRs returning in activity after overload MSIs, and to define the time to return in activity after specific classes of injuries. We hypothesized that overload MSIs could account for a lower racing speed and a relationship of dependency between multiple overload MSIs when STBRs resumed racing activity. A retrospective observational study has been conducted over four years of training in a single racetrack. Medical records and training logbooks were reviewed. Number of retirements, time of return to activity, effect on performances and the compensatory load redistribution were studied after an overload MSI. Survival analysis was employed with the return to training as primary endpoint. Performances were analysed in 30 races after injuries. Localization of overload MSIs was analyzed to detect any compensatory effect when racehorses resumed training.

Considering overall injuries in 356 racehorses, the 38.7% (95% CL: 0.34, 0.43) of STBRs suffering an overload MSI abandoned the agonistic career. The median time of return to activity was 180 days (95% CL: 165, 210). Horses with post-traumatic osteoarthritis were characterized by a greater hazard of return than horses with stress fractures (hazard ratio (HR) = 4.8, 95% CL: 3.5, 6.7) and with tendon/ligament strains (HR = 4.1, 95% CL: 3.1, 5.4). We observed a trend of increasing racing speed despite the injury events. A compensatory effect was identified analyzing the distribution of second MSIs in comparison to the first (n=138 cases; Chi- squared, $P<0.01$). Secondary MSIs are more likely localized at the contralateral limb (estimated proportion (EP) 0.37, 95%CL 0.30, 0.45), diagonal limb (EP 0.30, 95%CL 0.23, 0.38) and same limb (EP 0.23, 95%CL 0.16, 0.30) than to the ipsilateral limb (EP 0.08, 95%CL 0.05, 0.14). Our study contains accurate estimates of prognostic data associate to overload MSIs in STBRs.

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EFFECTS OF INTRAMUSCULAR MIDAZOLAM IN COMBINATION WITH ALFAXALONE IN PIGS

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Since pigs are difficult to restrain, intramuscular (IM) induction represents the most widely used method of anaesthetic administration, commonly by a combination of several drugs [1]. The ideal anaesthetic protocol in pigs should provide fast and deep sedation, adequate analgesia and muscle relaxation with minimal cardiovascular and respiratory depression [2]. This study aimed to determine whether a combination of IM midazolam in combination with alfaxalone can induce safe and satisfactory restraint in pigs. Fourteen healthy mixed-breed male pigs, involved in another experimental study, weighing 25 to 35 kg were used. Food was withheld for 12 hours and water for 30 minutes prior to induction of anaesthesia. Before the administration of anaesthesia, baseline heart rate (HR), respiratory rate (RR) and rectal temperature (RT) were recorded. The animals were anesthetized IM with a combination of midazolam (0.5 mg/kg) and alfaxalone (5 mg/kg) mixed in the same syringe. The drugs were administered IM by injection into the neck behind the base of the ear by using a 19 gauge needle connected to a line extension. Pain on injection was scored using a modified scale from Michou et al. [3] and Santos et al. [4] with four levels: no pain; mild pain (movement of tail and turning of head towards injection side); moderate pain (light grunts and attempts to remove needle); and severe pain (strong vocalization and attempts to escape, requiring vigorous physical restraint). A 4-point scale was used to evaluate the quality of sedation (score 0, poor: no sedation, standing; score 1, sufficient: quiet but standing and reactive to manipulation; score 2, good: sternal recumbency and inability to move; score 3, very good: lateral recumbency with central depression). The time to achieve lateral recumbency were recorded. HR, RR, RT and arterial oxygen saturation (SpO_2) were monitored at 5, 10, 15 and 20 minutes after inoculation. The data were analysed with simple one-way analysis of variance (ANOVA). A p-value <0.05 was considered statistically significant. No animals showed pain on drug injection. The quality of sedation was scored as good to very good (five animals scored good, and nine scored very good). The time (mean±SD) from the end of injection to lateral recumbency was 315±127 seconds. During recumbency, none of the animals showed excess salivation or apnoea. There were no significant differences in HR, RR, or RT when compared to the baseline values and SpO_2 remained within normal limits for all animals. The results of this study showed that IM alfaxalone in combination with midazolam at the dose used induced safe and satisfactory restraint in pigs and provided faster onset of recumbency and deeper sedation, with minimal side effects and may be considered a reliable premedication in pigs.

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USE OF UC-II (UNDENATURATED TYPE II COLLAGEN) IN MANAGEMENT OF OSTEOARTHRITIS IN DOGS: A CLINICAL TRIAL

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Osteoarthritis (OA) is a chronic disease that requires a multimodal therapeutic approach. The use of non-steroidal anti-inflammatory drugs (NSAIDs) is a cornerstone in the treatment of the disease. Undenatured type II collagen (UC-II) [1] reduces the inflammation by acting on joint immunity with an alternative mechanism of NSAIDs. The aim of the trial was to test the efficacy of UC-II alone or in combination with cimicoxib [2], for treatment of OA in dogs. The hypothesis is that UC-II can enhance the effect of cimicoxib on the mobility of patients with OA. In order to test our hypothesis, dogs diagnosed with OA from the Surgery Unit of the Section of Veterinary Clinics and Animal Production of the Department of Emergency and Organ Transplantation of the University of Bari were recruited. All dogs underwent orthopedic and radiographic examination and assessment of the impairment of mobility, through a questionnaire completed by the owner (Liverpool Osteoarthritis in Dogs, LOAD) [3]. Subjects meeting the inclusion criteria were randomly divided into 3 treatment groups: cimicoxib (2 mg/kg die) for 30 days (CIMI Group), one UC-II tablet /day for 30 days (UC-II Group), and the combination cimicoxib (2 mg/kg die) and UC-II (one tablet/die) for 30 days. The LOAD score obtained before (T0) and 30 days (T30) after the beginning of the pharmacological treatment, was evaluated for the purpose of the study. For all the parameters collected the mean and the standard deviation or the median and the range were calculated. The LOAD scores at T0 and T30 were compared with the Anova one way test ($P<0.05$). Until now, 45 dogs were enrolled: 13 CIM, 20 UC-II and 12 CIM + UC-II. There was a significant reduction in LOAD score between T0 and T30 within each treatment of similar magnitude among the three groups(CIMI = 31.8%, $P<0.001$; UC-II = 32.7%, $P = 0.013$; CIM + UC-II = 31.7%, $P=0.009$). Preliminary results of the study show a similar effectiveness of the 3 treatments in reducing the degree of impairment of mobility in dogs with OA. UC-II, while not showing a synergistic effect with cimicoxib, provided a comparable clinical efficacy to the NSAID itself.

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INFLUENCE OF ARTERIAL LINE PLACEMENT ON BLOOD PRESSURE READING: PRELIMINARY RESULTS IN HORSES

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Invasive blood pressure (IBP) is the gold standard for arterial pressure measurement. It allows continuous and more accurate beat-to-beat pressure monitoring, and enables the rapid detection of hemodynamic changes, essential in emergency situations [1], such as hypotension, which is crucial especially in equine anesthesia. The commonest method for placing an arterial line describes the insertion of the catheter at 30-45° angle into the vessel against the pulsation, then a pressure transducer will connect it to a monitor device, and the periodic pressure pulses produced by the heart will generate electrical signals amplified and displayed by the monitor [2]. Sometimes in horses this standard approach of arterial catheterization is not comfortable for the anesthetist due to the patient position required by surgery. Nevertheless, as no scientific evidence exists, both in human and veterinary literature, which demonstrates the reliability of the standard technique, the aim of this study was to compare 2 methods of IBP measurement: with the standard placement of the catheter against the flow (AF) and with the catheter placement according to the flow direction (DF). Thirty-one horses undergoing general anesthesia in dorsal recumbency, either for elective or emergency surgery, were included. A 22Gx25mm catheter was placed in each facial artery: one AF and one DF. Both arterial lines were connected to the same pressure transducer through a 3-way-stopcock, which allowed a quick deviation of the blood flow in order to record IBP measurement at both arteries simultaneously. For each horse we recorded IBP values (systolic [SAP], diastolic [DAP] and mean [MAP]) collected from both catheters every 5 minutes with a random order (i.e. first AF then switched to DF and vice versa for the following measurement) interposed by 1 ml flushing of the arterial line with heparinized solution and 10” of pause to display the new waveform on the monitor.

The evaluation of the agreement between the methods by Bland-Altman analysis adjusted for repeated measures indicated that small biases existed: 5.6, 3.3, and 2.6 mmHg for SAP, MAP and DAP, respectively. The limits of agreement between these two methods were -13.2-25.2, -8.6-15.2, and -8.0-13.2 mmHg, respectively for SAP, MAP and DAP.

Moreover, Generalized Linear Models showed that SAP, MAP and DAP, either evaluated as AF or DF, were not influenced by preoperative Hct and total protein.

MAP is normally used to modulate the correct depth of anesthesia. The detected differences between the 2 methods were not clinically relevant suggesting that the catheter direction is not important for a correct blood pressure reading. Nevertheless, a larger sample group can yield more reliable study results.

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EVALUATION OF THE EFFECTS OF ROBENACOXIB OR UNDENATURATED COLLAGEN TYPE II (UC-II) ON THE MOBILITY IMPAIRMENT INDUCED BY OSTEOARTHRITIS IN DOGS ASSESSED BY THE LIVERPOOL OSTEOARTHRITIS IN DOGS SURVEY

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Osteoarthritis (OA), also known as degenerative joint disease, is a chronic low-grade inflammatory disease in dogs and it is the most common source of chronic pain. Animals with osteoarthritis are treated with multimodal approach. Non-steroidal anti-inflammatory drugs (NSAIDs) are the current gold-standard pharmaceutical therapy for dogs with OA. Robenacoxib is an NSAID with several properties of interest for use in dogs [1]. It is registered for the treatment of pain and inflammation associated with orthopedic diseases, showing safety also for long period of therapy. Undenatured type-II collagen(UC-II), from chicken sternum, markedly reduced pain in dogs through the modulation of the immune system response and thereby improved their quality of life [2]. The aim of this study WAS to compare the efficacy of UC-II and robenacoxib for the therapeutic management of OA in dogs. Our hypothesis was that Robenacoxib would be more effective than UC-II in improving mobility impairment associated with OA. To test this hypothesis the L.O.A.D survey (Liverpool Osteoarthritis in dogs) [3] was proposed to owners of dogs undergoing the two treatments. Client-owned dogs affected by OA, conducted at the Surgical Unit of the Section of Veterinary Clinics and Animal Production of the Department of Emergency and Organ Transplantation of the University of Bari, were enrolled in this study. All patients, underwent a complete physical examination, x-ray and orthopaedic examination, and were randomly divided into two treatment groups: Robenacoxib (1mg/kg die) for 30 days (R group) and UC-II (one tablet/die) for 30 days (UC-II group). Based on inclusion criteria, 44 patients were enrolled in this study: 24 (R group) and 20 (UC-II group). The load score obtained before (T0) and 30 days (T30) after the beginning of each treatment was evaluated and compared by the Friedman and ANOVA test ($p<0.05$). For all data collected (age, weight, rx score, LOAD T0, LOAD T30) the mean and the standard deviation or median and range were calculated. There was a significant reduction in LOAD score between T0 and T30 with a similar magnitude among the two groups (R group 31,5%, $P<0.001$; UC-II group 32,7%, $P=0.013$).The results of this study show a similar effectiveness of both treatments in reducing the degree of mobility impairment induced by OA in dogs. UC-II provided a comparable clinical efficacy to the Robenacoxib.

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PLANAR BONE SCINTIGRAPHY AND CT FINDINGS IN DOGS WITH FORELIMB LAMENESS

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Scintigraphy has been used for many years in veterinary medicine, due to its high sensitivity, for the localization of lameness of unknown origin in horses and for the assessment of thyroid/skeletal neoplasia in dogs. In the last few years bone scintigraphy (BS) has become increasingly used in dogs for the localization of occult lameness, when clinical examination and radiographic exam are inconclusive [1]. This study describes BS and computed tomographic (CT) findings in dogs referred for monolateral forelimb obscure lameness, for which a precise localization had not been found by clinical examination nor radiographic exam (no abnormalities at all, mild radiological abnormalities which could not be related to the grade of lameness or symmetrical bilateral alterations). Eight dogs matched inclusion criteria: 3 mixed breed, 1 Bernese mountain dog, 1 Amstaff, 1 Labrador retriever, 1 Australian shepherd and 1 Boxer. BS images showed intense IRU (Increased Radiopharmaceutical Uptake) of elbow joint in 6 cases; these findings coincided to CT alterations of proximal ulna in 5 dogs (mostly located in the medial coronoid process - MCP region: bone density alterations, evidence of fragmentation, new bone formation). In one of these cases, an intense IRU was observed in correspondence of the region of the flexors attachment, with no concurrent abnormalities on CT examination. These findings were suggestive of an obscure form of flexor enthesopathy. In one case we observed diffuse and intense IRU of the carpus joint; this coincided with arthrosis and the presence of a subchondral cyst. One dog showed only mild IRU of the elbow joint, not compatible with the degree of lameness. Because of lack of significant IRU, CT and MRI examination were performed and revealed the presence of an expansive lesion in correspondence of the brachial plexus roots compatible with PNST (Peripheral Nerve Sheath Tumor). BS' high sensitivity allowed the localization of the lameness thanks to the assessment of functional bone state, as already stated in literature [2]. However, its low specificity required additional imaging (CT, MRI), targeted on the region identified on scintigraphic examination. In our experience, the combined use of functional and morphologic diagnostic imaging techniques (bone scintigraphy and computed tomography-magnetic resonance) has been helpful to reach a definitive diagnosis. Further studies, with an increased sample size, are needed to evaluate whether there is a correlation between bone density changes and grade of IRU in limbs affected by different pathologies.

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PULSE PRESSURE VARIATION CANPREDICT THE HEMODYNAMIC RESPONSE TO PNEUMOPERITONEUM IN DOGS: A RETROSPECTIVE STUDY

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Pneumoperitoneum (PP), in laparoscopy, may cause hemodynamic alterations, including reduction of venous return and cardiac output [1-2]. The purpose of the study was to evaluate whether pulse pressure variation (PPV) can be a predictive parameter of these hemodynamic effects during pneumoperitoneum in dogs. To test our hypothesis, we have retrospectively evaluated cases of laparoscopic surgery in which the PPV was monitored. Twenty healthy patients were included, premedicated with acepromazine (10 µg kg^{-1}) and methadone (0.3 mg kg^{-1}) IM, induced with propofol (5 mg/kg IV) and maintained with inhalant isoflurane. After induction and intubation, a palmar artery was cannulated. All dogs were mechanically ventilated with a tidal volume of 15 ml/kg, an inspiratory to expiratory ratio of 1:2 and a respiratory rate adjusted for the end-tidal carbon dioxide ($EtCO_2$) level. Five minutes before (baseline) and ten minutes after (P10) the PP, were recorded: heart rate, mean arterial pressure, stroke volume, cardiac output, pulse pressure variation (PPV, %)and systemic vascular resistances, using the "Pressure Recording Analyting Method" technique [3]. Cardiac index (CI) was calculated by correlating cardiac output to patient's body surface area. Based on the cardiac index variation after the beginningof PP (Baseline Vs P10), dogs were classified as "sensitive" (S group, CI≤15% compared to baseline) and "no-sensitive" (NS group, the remaining dogs). All data were reported as mean +/- standard deviation. Shapiro Wilk test was used for the evaluation of normal distribution. The comparison between hemodynamic data of the two groups was performed with the one way ANOVA test. Moreover, the Receiver Operating Characteristic curve (ROC curve) and PPV cut-off to identify the S and NS were obtained, maximizing the specificity and sensitivity of the test [4]. For the all data $p<0.05$ was considered statistically significant. The results showed that 11 dogs (55%) had a reduction of CI at P10 ≥15 % (S group).The CI at P10 in S group was significantly lower (2.97±1.4 L/min/m2) than baseline in the same group (4.32±1.62 L/min/m2) and compared to P10 in NS group (4.51±1.41 L/min/m2). The PPV value at baseline was significantly higher, in S group (22.4 ± 6.1%) compared to the NS group (10.9 ± 3.3%). The ROC curve analysis showed a cut off value of PPV>16% to distinguish between S and NS (AUC=0.970, P=0.0001, Sensitivity=90.91% and Specificity=100%). In conclusion, PPV may be a valid predictor of hemodynamic abnormalities in laparoscopy. A PPV value >16% predicts patients that may have an important hemodynamic impairment at the creation of PP, and thus should receive hemodynamic stabilization before starting PP.

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PERIBULBAR BLOCK IN EQUINE ISOLATED HEADS. DEVELOPMENT OF A SINGLE NEEDLE TECHNIQUE AND TOMOGRAPHIC EVALUATION

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Peribulbar block (PPB) has been used in humans as a safer alternative to retrobulbar block (RBB). PBB, depends on the diffusion of anaesthetic solution into the muscle across the connective tissue and it is performed introducing the needle within the extraconal space. The advantages are fewer complications and palpebral akinesia. In Veterinary Medicine few studies describe this technique in dogs (Shilo-Benjamini et al., 2017) and cats (Shilo-Benjamini et al., 2013). The aim of the study is to determinate, in equine specimens, feasibility of inferior PBB with single needle injection, by using contrast medium (CM), and to evaluate thought Computed Tomography (CT) the distribution of the injected volume and regional anaesthesia likelihood. PBB was performed in 10 orbits. The mixture injected consisted of 20 ml of physiological solution and iodinated CM at 25%. Each periorbital area underwent three CT scans. A basal acquisition to assess the needle position before the injection, a second and third scan were performed immediately after injection, and after application of pressure on the periorbital surface area to promote CM diffusion. The injectate distribution at the base and within the extraocular muscle cone (EOMC) and around the optic nerve was evaluated and scored based on Shilo-Benjamini's work of 2017. The mean minimum distance between the tip of the needle and the optic was 2,23 mm ±0,2. The mean volume distribution before pressure application was 23.56 cm^3 ± 2.58 and after pressure application was 27.56 cm^3 ± 4.8. The CM median distribution around the optic nerve at the base of the EOMC was of 117° prior pressure and 189° after pressure. The CM distribution within the EOMC was present in 1 orbit prior pressure and in 3 orbits after pressure. The CM distribution at the base of EOMC was considered unlikely to provide regional anaesthesia in 2 orbits, possible in 3 orbits and likely in 5. In the present study, intraconal distribution was not consistent. For this reason, the likelihood of achieving regional anaesthesia was evaluated at the EOMC base where through the optic foramen the oculomotor, trochlear nerve, ophthalmic branch of the trigeminal nerve, and the abducens travel to reach the orbit together with the optic nerve. Whereas the maxillary branch of the trigeminal nerve passes through the foramen rotundum (Carastro 2004). Therefore, despite the lack of intraconal distribution if the EOMC base had good distribution then it was considered likely to provide regional anaesthesia. This approach needs to be evaluated in clinical trials to assess its feasibility and effectiveness in locoregional anaesthesia; moreover, further investigations on equine PBB are mandatory with higher volumes of injectate and different approaches.

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INTRA ARTICULAR STANOZOLOL FOR TREATMENT OF SUBCHONDRAL BONE CYSTS IN HORSES

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Equine subchondral bone cysts (SBCs) can be an incidental radiographic finding but can be a cause of lameness (1). An important factor in cyst formation seems to be cytokine-mediated inflammation after trauma or failure in local ossification (2). SBCs mainly occur in medial femoral condyle (MFC), metacarpal/tarsal and digital bones.
Many treatments have been proposed with the aim to reduce clinical signs, radiographic cyst size and promote subchondral bone formation resurfaced by articular cartilage or fibrocartilage (3).
Stanozolol is a synthetic derivative of testosterone with anabolic and/or androgenic activity. It stimulates the cartilaginous tissue by means of production of collagen and other components of the cartilage matrix, as indicated by some histological studies and clinical reports (4,5). Aim of this study is to assess the ability of stanozolol to produce clinical and/or disease-modifying effects in horses affected by SBCs. Client-owned horses were selected for inclusion in the study. All horses show a SBC in one or more joints with clinical signs associated or not. Horses with clinical signs were treated arthroscopically with an intracystic injection of stanozolol (5mg). Horses that did not show clinical signs were treated with two intra articular (IA) stanozolol injection (5mg) at a distance of 15 days. Control visits and radiographic examination were scheduled on days 0 and 60 after the last treatment. Eight horses were enrolled in the study: four had SBC located in the MFC of one limb, of these horses only two were lame (4/5 and 2/5 on AAEP scale respectively). Two horses have SBC bilaterally in the MFC without lameness. One horse had a SBC in the right distal radius with lameness (3/5 in AAEP scale). One horse had the cyst located in distal metacarpal bone (lame 3/5).
Of the two lame horses with cyst in the MFC, both showed an improvement of one grade of lameness in AAEP scale, only one have a radiographic improvement in cystic radiopacity and marginal sclerosis. One the two horses with SBCs located in the MFC not associated with lameness had radiographic changes in term of decrease of bone lucency. The other horse with cyst in MFC of one leg had no changes in cystic radiological aspect. For what concern horses affected by SBC located in MFC of both legs, one had important radiographic changes in decreasing the dimension and lucency of both SBCs, one had an increase in the size of the SBC in left femur and no change in the other leg. The horse with the cyst located in metacarpal bone showed no lameness at the control visit, the radiological aspect of the cyst was the same. The horse with the distal radius involved presented no lame at the control visit without radiological changes. This study demonstrated that in horses affected by SBC, IA treatment with stanozolol, administered at a dosage of 5 mg for each joint can be effective in treatment of lameness and joint pain. Regarding the radiographic improvement, it occurred in half of the horses (4 on 8 horses). Stanozolol therefore could be considered as a therapeutic option to prolong the athletic career of horses suffering from SBCs.

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RADIOGRAPHIC STUDY OF THE THORACOLUMBAR SPINE IN THOROUGHBRED YEARLING

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Impingement or overriding of the spinous processes of the thoracolumbar spine a frequent cause of back pain and a common cause of poor performance in horses. Radiographic changes can be varied and abnormalities can be detected in many clinically normal horses [1]. The aim of this study was to investigate the frequency of thoracolumbar spinous processes abnormalities in Thoroughbred yearlings free from back pain. Twenty-nine Thoroughbred yearlings (aged 14-18 months) referred for several reasons underwent radiographic examination of the back from T7 to L3. Images were obtained in sedation with the horse standing squared and with the neck in a neutral position. Three radiographs were obtained with a computed or digital radiographic equipment; retrospectively, images were evaluated for narrowing, increased radiopacity, presence of radiolucent areas and remodeling of the spinous processes as well as number of affected spinous processes; impingement was recorded as well. A radiographic score (0-3) was assigned using a readapted grading system from a previous publication [2]. Statistical analysis was performed using the software Jasp; a descriptive statistic was used to report the number of the affected spinous processes, grade of the lesions and overall lesions scores. A Spearman's correlation coefficient (rs) was used to test correlation between the four radiographic scores and between radiographic scores and the number of affected spinous processes; Friedman ANOVA was used to investigate the differences in the grade of the lesions and gender between the interspinous spaces. Significance level was set at $p<0.05$.

A total number of 406 interspinous spaces were evaluated; the number of spinous processes with radiographic abnormalities varied from 0 to 14 per horse, with a median of 4, 8, 2, 9 for narrowing, increased opacity, radiolucency and modelling, respectively. Total radiographic score for each horse ranged from 0 to 82 (median 41). Overall the most commonly affected interspinous space was T15-T16, except for narrowing (T16-T17). Modelling was the most frequent abnormality (56.3%) recorded with an overrepresented Grade 1. Increased opacity and narrowing were diagnosed in 51.4% and 27.5% of interspinous space, respectively, most commonly as Grade 2. Radiolucent areas were detected in 22.9% and overrepresented by Grade 3. Impingement of spinous process was present in 5.6% of the interspinous space, most commonly T16-L1. There was a positive correlation between the radiographic scores of the abnormalities evaluated ($p<0.05$; $0.47<rs<0.85$). Positive correlation was also found between radiographic score per horse and number of affected spinous process ($p<0.001$; $0.78<rs<0.87$). Significant difference was found between gender and grade of the lesions ($p<0.001$) and also between grade of the lesion and interspinous spaces ($p<0.001$).

In conclusion, the study demonstrated the presence of radiographic lesions and impingement in Thoroughbred yearlings with the caudal thoracic spine T14-L1 most severely affected.

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MINERALIZED MASSES OF THE ILIOPSOAS MUSCLE RESEMBLING HUMAN CALCIFIC MYONECROSIS IN TWO CATS

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Calcific myonecrosis is a rare post-traumatic complication characterized by the replacement of muscles with central liquefaction and peripheral calcification resulting in a central cystic mass in the muscle [1]. Although its pathophysiology has not been completely elucidated, it is postulated that these lesions most likely result from post-traumatic ischemia and cystic muscle degeneration. All cases reported in the human literature involve the lower limb; almost all patients had an history of remote trauma, ranging from 10 to 64 years before presentation [2]. To our knowledge calcific myonecrosis has not been reported in veterinary medicine.

In the present report we describe the radiographic and computed tomographic features of a large retroperitoneal mineralized mass, arising from the iliopsoas muscle, in two male castrated adult cats (5 and 13 years old). They were referred for abdominal mass. Both patients had a remote history of trauma (road traffic accident) 4 and 11 years earlier; after trauma one cat underwent femoral osteotomy and nephrectomy, the second one underwent femoral osteotomy and abdominal hernia repair. Radiography and CT scan were performed; ultrasonography was performed on cat 1. The retroperitoneal mass arose from the right iliopsoas muscle in both cases; as a fusiform mass with peripherally oriented plaque-like amorphous calcifications in cat #1 and as a large ovoid lobulated mass with mineralized periphery and some septa in cat #2; both presented a marked cavitary component. In cat #2 the large size of the lesion caused ventral dislocation of the abdominal organs. Post-necropsy histological examination revealed the presence of necrotic skeletal muscles, with calcific deposition involving also arterial walls. Focal aggregates of hemosiderin-containing macrophages were present. This central part of the lesion was delimited by a dense fibrous connective capsule with osseus metaplasia. No evidence of neoplasia was found.
A retroperitoneal mass with calcification has a wide range of differential diagnoses, including several benign and malignant conditions. The non-tumoral benign conditions include exuberant fracture callus, post-traumatic calcified chronic expanding hematoma, myositis ossificans and calcific myonecrosis [3]. In the present cases, the presumptive diagnosis of calcific myonecrosis was based on the radiographic and tomographic findings, histopathology and the remote history of trauma. In humans calcific myonecrosis presents with distinct radiographic features [2]. Calcification of soft tissue is thin with a plaque-like or linear configuration organized around the margin of the lesion with infolding septa. Contrast-enhanced CT clearly shows the rim-like distribution of calcification with homogeneous density, suggesting central or peripheral fluid retention. In myositis ossificans the mineralization of the muscle is focal, homogeneous, without muscular necrosis; furthermore, earliest radiologic signs of myositis ossificans appear within 11 days to 6 weeks after injury followed by subsequent ossification.

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KIDNEY-TO-AORTA ULTRASOUND MEASUREMENTS IN WHIPPETS

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Kidney size has diagnostic and prognostic value. Since CT and MRI require general anesthesia and are expensive, radiography and ultrasonography (US) represent the preferred techniques in Veterinary practice. Radiographic kidney size is obtained by relating renal length to the L2 body length: a ratio of 2.5 – 3.5 is considered normal. Ultrasonography, other than the profiles, allows to assess internal structure and blood perfusion and can be performed on awake patient, without any risk. The main limit of US is that its accuracy is operator dependent. Therefore, any available quantitative information are useful to reduce subjective assessment. Although several authors tried to relate renal measures to bodyweight or body surface area, to date, the renal US size is still subjectively assessed. Recently, a method in which kidney length (KL) is related to the aortic luminal diameter (AoD) has been proposed (1). The main disadvantage of this method is the wide range of normal values (5.5-9.1) which determines a poor sensitivity and specificity. Therefore, in order to assess a narrower range of KL/AoD normal values and to increase its clinical importance, only one breed - the Whippet - was considered. Differences between right and left kidneys and influence of sex, weight and age were also investigated. Furthermore, longitudinal and transversal scans of AoD were compared. Thirty-six whippets (16 males, 20 females), ranging from 10 months to 14 years old, mean bodyweight 14.12±2.38 kg, clinically normal and without any US renal lesions were included. All US studies were performed on awake dog, in right and left lateral recumbency or in standing position. The US images were obtained using a 3.5-10 MHz microconvex probe and acquired in dorsal and transversal scans. The KL and width (KW) were measured on dorsal scan while depth (KD) on transversal one. The AoD was measured from the left side, both in transversal (AoDT) and longitudinal (AoDL) scans. Measurements were made at the maximal luminal diameter dilatation just caudal to the left renal artery origin. Mean, median, minimum, maximum, SD and 95% C.I. for the KL, KD, KW, AoDT, AoDL, KL/AoDT and KL/AoDL, for each side and for pooled data were calculated. Paired t Student’s test was used to compare right and left side. Mann-Whitney test was used to compare the sexes. Pearson correlation and Spearman's rank correlation were used to correlate bodyweight and age, respectively. Bland-Altman plot was used to compare AoDL and AoDT. P was set at <0,05. The 95% C.I. of KL/AoD was narrower than that reported (6.3-7 instead of 5.5-9.1) and showed no differences between sides and sexes. Side did not affect kidney size while males had larger kidneys than females. Kidney size was positively correlated to the bodyweight. In spite of no significant differences and a strong correlation between AoDL and AoDT, the KL/AoDL was significantly higher than KL/AoT and the Bland-Altman plot showed a greater bias for the AoDT. In conclusion, in Whippets, a KL/AoD lower than 6.3 means reduced renal size, while greater than 7, increased renal size. The KL/AoD showed no differences in the two sides and, moreover, although a marked sexual dimorphism, no differences between the two sexes.

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MAC-SPARING EFFECT OF TRANSDERMAL FENTANYL IN SEVOFLURANE-ANESTHETIZED SHEEP

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Systemic opioids provide intra-operative antinociception, which may result in variable reduction of the minimum alveolar concentration (MAC) of sevoflurane (SEVO). Transdermal administration of fentanyl allows for more consistent plasma concentrations for up to 72 h compared to that of intermittent boluses of various analgesic medications [1]. We hypothesized that the administration of transdermal fentanyl will significantly reduce MACSEVO in sheep. Nine healthy female Sardinian sheep, aged 5 (range 3-10) years and weighing 34.0 (3.7) kg, were enrolled and anesthetized twice, seven days apart. The study protocol was approved by the Institutional Animal Care and Use Committee at the University of Sassari (CIBASA; protocol number 23/052-3-07-2012) according to Italian legislation (D.Lgs. 27 gennaio 1992, n.116). Animals were face-mask induced with SEVO in O2 and immediately mechanically ventilated. MACSEVO was determined in duplicate with a supramaximal noxious stimulus an electrical current (5 Hz/1 ms/50 mA) delivered to the lateral metacarpus for 60 s or until gross purposeful movement was observed [2]. After first determination, the skin surface over the medial aspect of the antebrachium was clipped, thoroughly cleaned and dried prior to application of a transdermal fentanyl patch (Duragesic, Janssen Pharmaceuticals; 75 µg/h), secured in place with an auto-adhesive bandage. After a mean of 15.1 (1.8) h post patch application, animals were re-anesthetized for determination of MACSEVO in the presence of fentanyl (MACSF) employing the same protocol. All variables were tested for normal distribution by a Shapiro-Wilk test. Parametric data are reported as mean (standard deviation, SD) and non-parametric data as median (interquartile range, IQR). Student's t test and Wilcoxon Signed-Rank test were employed for parametric and nonparametric data sets, respectively to detect statistically significant differences between groups. A two-tailed P-value <0.05 was accepted as indicating statistically significant differences. The mean fentanyl dose in each sheep was 2.2 (0.28) µg/kg/h. The MACSEVO as determined previously was 2.67 (0.30)%. The measured MACSF was 1.99 (0.32)% and thus on average 25.6 (8.1)% lower than MACSEVO (P<0.001). The mean heart rate recorded during MACSF determinations (113 beats/min) was significantly higher than during MACSEVO measurements (107 beats/min), P=0.008. Similarly, the end tidal CO_2 partial pressure was higher in MACSEVO (43 mmHg) compared to MACSF (41 mmHg) (P<0.001). Invasive blood pressure recordings, temperature, or respiratory rate were not statistically different. All sheep recovered smoothly upon discontinuation of SEVO administration.

The results confirm that transdermal fentanyl results in a marked reduction (25.6%) of MACSEVO in sheep, 15 h after patch application without compromising cardiopulmonary function during anesthesia and is well tolerated as an adjunctive agent. The differences observed in HR and ETCO2 between groups were clinically irrelevant despite statistical significance.

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ULTRASOUND ELASTOGRAPHY OF THE CRICOARYTENOIDEUS LATERALIS MUSCLES IN CLINICALLY NORMAL HORSES: INITIAL EXPERIENCES

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Recurrent laryngeal neuropathy (RLN) most commonly affects the left side of the larynx and it is characterized by a distal axonopathy, resulting in progressive monolateral denervation atrophy of intrinsic laryngeal muscles (1). The cricoarytenoideus dorsalis muscle (CAD) is an abductor of the arytenoid cartilage and vocal fold, and its dysfunction results in a significant reduction in airflow, severe exercise-induced hypoxemia and an abnormal upper airway sound (1). The diagnosis of RLN is primarily based on a history of poor performances, associated with an abnormal upper respiratory noise, external examination and palpation of the larynx, and upper airway endoscopy (2). Nevertheless, in early stages of RLN ambiguity of clinical and endoscopic findings can lead to misdiagnosis. Although atrophy of the CAD results in significant clinical signs, the adductor muscles of the arytenoid cartilage are affected earlier and more profoundly (1). The cricoarytenoideus lateralis muscle (CAL) is an adductor of the arytenoid cartilage, situated between the thyroid lamina and the arytenoid cartilage; it is not externally palpable or visible with endoscopy, thus an earlier dysfunction is often clinically undetected. CAL can be visualized ultrasonographically in normal horses and recent studies proved the diagnostic sensitivity of CAL ultrasonography also for RLN (1,2). Ultrasound strain elastography (EUS) is a recent technique used to evaluate the mechanical proprieties of muscle tissue, based on low-frequency compression of the tissue applied via the hand-held ultrasound transducer, causing axial tissue displacement (strain), which is then calculated by comparing the echo set before and after the compression (3,4). The purpose of this study was to determine the feasibility of EUS on CAL muscles in healthy horses. Twelve CAL muscles from 6 horses were assessed using B-mode and EUS. All horses were sedated and examined in standing position, with mild controlateral neck extension. The hair was not clipped and alcohol was used as an acoustic coupling agent. Sonography was performed with a linear array transducer (9 MHz) by two different operators. Each structure was examined in transverse and longitudinal planes for the caudolateral window of the larynx, as previously described (1). Semi-quantitative analysis of CAL muscles was performed, comparing the strain ratio between a reference area, represented by the thyroid cartilage, and different regions of interest of CAL (3 in transverse and 5 in longitudinal plane). Mean and standard deviation of Elasticity Index (EI) and Strain Ratio (SR) of each region were calculated, and Mann Whitney-U test was performed to assess interobserver agreement (SpSS). CAL was easily imaged in all examined horses. Both examiners were able to correctly perform EUS examination, obtaining high quality images during at least 3 compression-relaxation cycles, and results showed interobserver statistical significative comparability. EI and SR of CAL were 2.68 ± 1.85 and 1.48 ± 1.30 on transverse plane, and 2.21±1.60 and 1.24±1.44 on longitudinal plane. The results of our study indicate that the CAL muscle can be assessed by mean of EUS, but further research is required to make such results diagnostically relevant.

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COATING WITH PECTIN-HONEY HYDROGEL DOES PREVENT MESH ADHESIONS IN A RAT MODEL OF ACUTE HERNIA

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In surgery the traditional approach to the abdomen includes a medial or ventral incision. The most frequent complication is the incisional hernia, that causes pain, bowel obstruction, formation of adhesions, with the inevitable consequence of the re-intervention, and prolonging the time of recovery for patients [1]. Incisional or ventral hernia can occur after abdominal surgical procedure in a high percentage of patients. Several strategies are under investigation to reduce this complication and to avoid a re-intervention [2].
Polypropylene mesh uncoated and coated with pectin-honey hydrogel have been evaluated in the present study in order investigate the adhesion formation and fascia healing in a rat model. All procedures were approved by the Bioethical Committee of the University of Turin and by the Italian Ministry of Health (number 262/2015-PR). Forty male Sprague-Dawley rats were included in the study. The animals were randomly divided into two groups using a free online calculator: group C (control) and group T (treatment) with 20 rats for each group. A 4-cm midline incision was made in the abdominal wall and a full thickness 1 cm x 1 cm abdominal fascial defect was created. Polypropylene mesh (group C) and polypropylene mesh coated with PHH (Group T) were then fixed directly on the edges of the defect using interrupted suture 4-0-USP polypropylene. The skin was closed with continuous suture 3-0-USP nylon. After 30 days, the presence of adhesions, measurement of inflammation were histological evaluated at necropsy. The cyclooxygenase 2 was analyzed and evaluated.
In all rats, the examination of the wounds permitted to see the absence of site infection or presence of intra-abdominal abscess. In group C, 11 of 17 (64.7%) animals developed adhesions between omentum and viscera, while in group T only 5 rats of 14 (35%) demonstrated the same adhesion (p=0.48). C group shown also adhesions between the omentum and liver, while in T group only 1 rat demonstrated the same lesion. In C group, 9 animals developed adhesion between the distal jejunum and the omentum, while in T group only 1 animal did. In C group, 1 animal developed adhesion between the omentum and the median ligament of bladder. In T group, 3 animals developed adhesions between omentum and linea alba. Significantly upper grades of inflammation in T group were seen at the margin of the granuloma induced by the mesh (p=0.048, p=0.0009, respectively). The inflammation in the host tissue and the maturation of the tissue were similar in two groups. The immunohistochemical expression of COX-2 was 1.824 (±0.727) mean (±SD) in group C and 2.286 (±0.611) in group T. While the intensity of the COX2 expression was 2.286 (±0.611) and 1.786 (±0.578) in group T. The differences were not statistically different between groups.
In conclusion, the pectin-hydrogel does not prevent adhesions formation but improved the peritoneal healing and induced the rapid regeneration of tissue within the mesh. However, further investigations are needed in order to establish whether these results are transferable to other models and genuine cases, and to establish the true relevance of this treatment modality to the prevention of postoperative adhesions in the peritoneum.

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A RETROSPECTIVE STUDY OF SURGICAL CORRECTION OF 18 CASES (16 DOGS) OF EVERSION OF THE NICTITATING MEMBRANE

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Everted third eyelid cartilage is a common ocular disease in young, large breed dogs. The inappropriate third eyelid margin position impairs optimal tear film distribution and drainage and may contribute to conjunctivitis and exposure keratopathy. This condition was presumed to be congenital in certain breeds of dogs. Possible unequal growth rates of the bulbar and palpebral cartilage surfaces and/or an unequal growth rate of the adherent conjunctiva were hypothesized as contributing factors [1,2]. Different techniques for restoration of the anatomical position of the everted nictating membrane have been reported. However, few clinical reports evaluated postoperative results and relapse rate. The purpose of this study is to describe and evaluate surgical treatment of third eyelid cartilage eversion in dogs, with or without concomitant nictitans gland prolapse. A retrospective analysis of cases of eversion of third eyelid cartilage in dogs, between January 2010 and December 2017 was performed. The sex, breed and age at the time of diagnosis were recorded. The presence of other concurrent ocular diseases and surgical treatment were also recorded. Follow-up information was obtained from medical records and subsequently by telephone conversation with the owner. Sixteen dogs representing 18 everted third eyelid cartilage were included in this study. The sex distribution included 9 intact males and 7 intact females. Six dogs were Neapolitan Mastiffs, three were English Bulldogs, two were Great Danes, two were Boxer, with one each of the following breeds: Dachshund, Cane Corso and Rhodesian Ridgeback. Ages ranged from 3 months to 8 months. Only two dogs were bilaterally affected. Surgical treatment provided the excision of 2 mm of the folded cartilage, through posterior conjunctival approach. The same approach was used to perform a Morgan Pocket technique, also in dogs without prolapse of the nictitans gland. Incisions were finally apposed by simple continuous suture with pds 5/0 until 1-2 mm of incision's end. In one dog a double approach was performed: the excision of the folded cartilage through anterior conjunctival approach while the Pocket technique through posterior conjunctival approach, however, mild inflammation of the third eyelid was observed. All dogs had good results in terms of cartilage correction with no recurrence. In conclusion, the association of Morgan Pocket technique to the excision of folded cartilage may be considered in the dogs with everted cartilage, in order to prevent the prolapse of the gland and probably the relapse of the eversion. Bulbar approach to the scrolled portion of cartilage, in the dog has the advantage that dissection is easier with less adhesion of the cartilage to overlying conjunctiva. Although it was suggested [3] that corneal damage may occur from resulting scarring if approach through the bulbar aspect is chosen, the association of Pocket technique may be beneficial in reducing the possibility of traumatic corneal scarring in the postoperative period.

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Scienze Cliniche:SIRA

EFFECT OF AMNIOTIC PROGENITOR CELL MICROVESICLES ON FREEZING OF IN VITRO BOVINE PRODUCED EMBRYOS AND ON PREGNANCY RATE AFTER EMBRYO TRANSFER

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In mammals, the exchange of signals between the mother and the embryo is essential for the development and implantation of the embryo [1]. In cattle, the absence of communication between a receptive endometrium and a viable embryo has been reported as an important cause of low pregnancy rates [2]. These considerations led to the hypothesis that there should be a paracrine communication between the mother and the embryo. Microvesicles (MVs) are involved in communication mechanisms based on microRNAs active in the regulation of gene expression at transcriptional and post transcriptional level in crucial biological processes such as gametogenesis, fertilization, implantation and embryonic development [3]. In this context, after comparative study with endometrial derived MVs, we evaluated the most efficacy of MVs derived from amniotic cells on embryo development and demonstrated that MV-exposed embryos show a larger number of cells constituting the inner cell mass, greater viability, higher expression of *GPX1* gene (protective against lipid peroxidation) and lower expression of *BAX* gene (involved in apoptosis processes) compared to control [4]. After these in vitro results, we evaluated the effect of MVs on bovine embryo survival rate after cryopreservation and on pregnancy rate after embryo transfer of fresh or cryopreserved MV-exposed embryos. Embryos were produced in 18 replicates from 3.812 oocytes using routine protocol. Presumptive zygotes were randomly transferred in SOFaa (control, CTR) or cultured by adding $50x10^6$ MVs/ml in the SOFaa on day 5 post fertilization [4]. The embryo developmental rate was evaluated at day 7 (blastocyst stage) and, after that, the embryos were transfer fresh, in cows synchronized by Cloprostenol, or cryopreserved.
Results showed that blastocyst rate was 34.62±1.32% in CTR and 34.27±1.71% with MVs. After cryopreservation, embryo survival was statistically different ($P<0.05$) between CTR and MVs (32.70±6.26 and 43.51±5.73, respectively). The pregnancy rate was statistically different ($P<0.05$) after embryo transfer of fresh embryos, with 66.67% (20/30 cows) of pregnancies for MVs and 36.67 (11/30) for CTR. The transfer of cryopreserved embryos provided again statistically ($P<0.05$) different result: 36.67% (11/30) for MV-embryos versus 10% (3/30) for CTR. Our data show that pregnancy rate of fresh and cryopreserved MV-embryo is higher compared to CTR. These results indicate that the higher expression of *GPX1* gene, due to the presence of MVs, probably limits the damage of lipid peroxidation by improving the survival rate following cryopreservation, while the less expressed *BAX* gene limits damages due to apoptosis. In conclusion, amniotic derived MVs probably play a biological role in the interaction between the embryo and the endometrium providing a more resourceful environment.

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EFFECTS OF OCHRATOXIN A ON NUCLEAR AND CYTOPLASMIC MATURATION OF OOCYTES OBTAINED FROM PREPUBERTAL LAMBS

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OchratoxinA (OTA) is a major mycotoxin produced by several species of *Aspergillus* and *Penicillium* fungi and has been reported as an ubiquitous natural contaminant found in food and feed [1]. OTA plays reprotoxic, embryotoxic and teratogenic as well as nephrotoxic, neurotoxic, immunotoxic and carcinogenic activity as reported in either laboratory or farm animals [2]. Major mechanisms of action include inhibition of protein synthesis, toxic effect on mitochondrial (mt) function and calcium homeostasis with consequent oxidative stress, apoptosis induction and DNA adduct formation [2]. The aim of the present study was to evaluate the effects of OTA on nuclear and cytoplasmic maturation of oocytes from pre-pubertal lambs. Cumulus-oocyte complexes (COCs) were recovered at local slaughterhouses from the ovaries of prepubertal lambs (less than 6 months of age). During in vitro maturation (IVM) [3], COCs were exposed to 10 µM OTA, a concentration reported as effective in a previous study in the mouse [4]. Control conditions were: vehicle controls (IVM medium with 1% methanol) and standard controls (IVM medium without vehicle). After IVM and the removal of cumulus cells, the oocytes were stained with MitoTracker Orange CMTM Ros, 2′,7′-dichlorodihydrofluorescein diacetate and Hoechst 33258 and fixed in 2% paraformaldehyde solution in PBS. Metaphase II oocytes were analyzed by laser scanning confocal microscopy for assessing their cytoplasmic maturation indicated by mt distribution pattern [2]. Data were analysed by Chi-square test and differences were statistically significant when $P<0.05$. A total of 218 oocytes were analyzed. Lack of vehicle-related effects was noticed (23/37, 62% vs 62/96, 65%, for oocytes cultured with or without the vehicle; $P>0.05$). OTA caused a slight ($P>0.05$) reduction of the maturation rate (39/85, 46% vs 23/37, 62%, for exposed and controls, respectively). Instead, it affected oocyte bioenergetic status, as it reduced the rate of oocytes showing healthy perinuclear/pericortical mitochondrial distribution pattern (4/39, 10% vs 9/23, 39%, $P<0.05$). These data indicate that OTA, in the exposure conditions used in the present study, hinders nuclear and cytoplasmic maturation in prepubertal lamb oocytes.

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BEAUVERICIN DISTURBS NUCLEAR AND CYTOPLASMIC MATURATION OF PREBUPERTAL SHEEP OOCYTES

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Beauvericin (BEA) is a mycotoxin produced by several *Fusarium* species and a common contaminant of food and feed. Due to its ionophoric properties it can disturb mitochondrial function, and enhance reactive oxygen species (ROS) production and membrane lipid peroxidation [1]. It has been reported that BEA may affect the quality of oocytes by reducing granulosa cell function in pigs [1]. Here we determined the effect of BEA on meiotic competence and cytoplasmic maturation of oocytes from pre-pubertal sheep. Cumulus-oocyte complexes (COCs) recovered at local slaughterhouses from the ovaries of sheep younger than 6 months were underwent in vitro maturation (IVM) [2]. COCs were exposed to BEA concentrations selected on the basis of previous studies (0.5, 1, 3, 5 μM) [3]. COCs cultured in IVM medium with 0.02% DMSO (vehicle) were used as controls. After IVM, cumulus cells were removed and oocytes stained with MitoTracker Orange CMTM Ros, 2′,7′-dichlorodihydrofluorescein diacetate and Hoechst 33258 and fixed in 2% paraformaldehyde in PBS. Oocytes at the metaphase II stage were analyzed by confocal laser scanning microscopy for their cytoplasmic maturation expressed as mitochondria (mt) distribution pattern [2]. Data were analysed by Chi-square test and differences were considered to be significant when $P<0.05$. A total of 464 oocytes were analyzed in four replicates. BEA, at 5 μM, was found to reduce the maturation rate (45/94, 47.9% vs 59/93, 63.4%, for exposed and controls, respectively; $P<0.05$) whereas it was not effective at the lower tested concentrations (58/94, 61.7%; 45/93, 48.4%; 47/90, 52.2% for 0.5, 1 and 3 μM, respectively; $P>0.05$). In addition, BEA at 5 μM affected the bioenergetic status of oocytes, as it increased the rate of oocytes showing abnormal mt pattern (5/45, 11% vs 0/59, 0%, for exposed and controls, respectively; $P<0.05$) and reduced the rate of oocytes with healthy perinuclear/pericortical mt pattern (20/45, 44% vs 39/59, 66%, for exposed and controls, respectively; $P<0.05$). No effects were noticed on mt pattern at lower tested concentrations (37/58, 64%; 25/45, 56%; 29/47, 62%, for 0.5, 1 and 3 μM respectively; $P>0.05$). These data indicate that BEA, in the exposure conditions used in the present study, hinder nuclear and cytoplasmic maturation in prepubertal sheep oocytes.

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ENCAPSULATION OF OVINE CUMULUS-OOCYTE COMPLEXES IN TAILORED ALGINATE MICROBEADS – TOWARDS MODELING OF OVARIAN FOLLICLE ORGANOIDS

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Organoids are artificially assembled cell masses developed to reproduce organ functionality as the source tissue [1] and recently proposed in assisted reproduction technologies [2]. The aim of this study was to establish a 3D in vitro maturation (IVM) culture system, in the juvenile sheep model, by using the bioprinting technology, in view of modeling ovarian follicle organoids. Three experiments were conducted for: 1) defining a time interval allowing COC scheduling for encapsulation and IVM by using the Earle' s/Hank' s M199 (EH medium) holding procedure; 2) establishing the dataset to produce tailored alginate microbeads allowing inclusion of intact COCs and 3) comparing the effects of 3D versus 2D IVM culture on oocyte nuclear maturation rate. In experiment 1 (Exp. 1), COCs recovered from the ovaries of slaughtered juvenile sheep underwent 24h or 48h EH holding [3] and IVM [4]. COCs cultured immediately after retrieval were used as controls. After IVM, oocytes found at the metaphase II stage were analyzed for bioenergetic status expressed as mitochondria (mt) distribution pattern [4]. In Exp. 2, the spherical hydrogel generator (Sphyga) [5] was used to generate COC-including microbeads and variations of needle diameter and alginate concentration were analyzed. In Exp. 3, the meiotic stage of oocytes cultured in the 3D vs 2D system were compared. Data were analysed by the Chi-square test (significance at $P<0.05$). In Exp. 1, 318 COCs were analyzed (4 replicates). No deleterious effects on nuclear maturation rate were observed after 24h EH holding (54/108, 50% vs 55/101, 54.5% for treated and controls, respectively; $P>0.05$) whereas 48h significantly affected maturation rate (33/109, 30.3% vs 55/101, 54.5% for treated and controls, respectively, $P<0.001$). No significant effect of EH holding on mt pattern was found on either 24 or 48h EH holding (32/55, 58.2% vs. 25/54, 46.3% vs. 15/33, 45.5% for control, 24hEH and 48hEH, respectively; $P>0.05$). In Exp. 2, working parameters of Sphyga to get reproducible COC-including microbeads mimicking their 3D morphology were set up. In Exp. 3, 132 oocytes were analysed (3 replicates). No differences were observed in oocyte maturation rates in 3D vs 2D system (48/67, 71.6% vs 55/65, 84.6% for 3D and 2D, respectively; NS). In conclusions: 1) 24h EH holding allows to preserve ovine COC viability and competence; 2) microbead size and viscoelastic properties are sensitive parameters to obtain efficient COC encapsulation; 3) the established 3D IVM system allowed to obtain oocyte maturation at comparable rates than conventional 2D system.

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IN VITRO DEVELOPMENTAL COMPETENCE OF HORSE OOCYTES WITH DIFFERENT CUMULUS MORPHOLOGY

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In vitro production of equine embryos by intracytoplasmic sperm injection (ICSI) is gaining interest, and both commercial and research applications have rapidly increased worldwide. Oocyte collection from excised ovaries is generally performed using one of two methods: aspiration and scraping. Aspiration has been found to give a lower recovery rate [1] and to yield oocytes largely denuded of cumulus, when compared to scraping [2]. For commercial programs, oocytes are typically recovered from live mares using transvaginal ultrasound-guided aspiration. Despite the high number of oocytes with only corona radiata (CR) collected by aspiration (in vivo or ex vivo), most studies classify horse oocytes simply as having a compact (CP) or expanded (EX) cumulus, without considering oocytes with only CR. In the only study [3] classifying horse oocytes as having CP, EX, or partial cumulus investments, no data on embryo production were available. The aim of this study was to investigate the embryo developmental ability after ICSI of horse cumulus-oocyte complexes (COCs) presenting only CR as compared to CP and EX COCs. Horse oocytes were collected by follicular aspiration of abattoir-derived ovaries. After classification into EX, CP or CR COCs, they were in vitro matured for 26 h in DMEM-F12 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 50 ng/ml epidermal growth factor, 100 ng/ml insulin-like growth factor 1, 0.1 IU/mL pFSH-LH (Pluset). At the end of the maturation period oocytes were denuded and classified as mature, immature or degenerate. MII oocytes were fertilized by piezo-drill ICSI using frozen-thawed semen from the same stallion, and in vitro cultured in SOF medium for 7.5 days. Culture medium was refreshed every 3 days and on day 6 of culture 5% FBS was added. At day 7.5 of in vitro culture, embryos were stained with 1 µg/mL bisbenzimide fluorescent dye (Hoechst 33342) to assess the number of nuclei and classify them. Maturation rate, cleavage rate and morula/blastocyst rates were recorded; data were statistically analysed using a Chi Square test (IBM SPSS Statistics 23) and significance was assessed for $P<0.05$. The experiment included 14 replicates. A total of 611 oocytes were used. Overall maturation rate was 60.2%. MII, immature and degenerate oocyte rates were not statistically different ($P>0.05$) among different COC morphologies (MII 61.7% vs. 57.6% vs. 59.3% for EX, CP and CR COCs respectively). Cleavage rate was lower ($P<0.05$) for CR (42.1%) compared to CP (55.6%) but not significantly different from EX (54.3%), while morula/blastocyst development after 7.5 days of culture was similar ($P>0.05$) among groups (12.0% vs. 8.9% vs. 14.7% of injected oocytes for EX, CP and CR COCs respectively). In conclusion, even if CR COCs show a lower cleavage rate after ICSI, their developmental ability is similar to CP and EX COCs, demonstrating that they can be used as a useful source of embryos in the horse.

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AN UNUSUAL CASE OF DISORDERS OF SEXUAL DEVELOPMENT IN ARABIAN HORSE 2N=64, XX, SRY-NEGATIVE

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"Sex reversal syndrome - SRS" is a congenital condition in which there is a complete or incomplete gonadal development but a discordance between genetic, gonadal and phenotypic sex [1,2]. The term Disorder of Sexual Development (DSD) has been proposed for all congenital abnormalities in which the chromosomal, gonadal or anatomical sex development is atypical [3]. In the horse, several types of DSDs have been described, including mosaic shapes, as follows: (i) 63, XO; (ii) 64, XX, SRY-negative; (iii) 64, XY, SRY-positive; and (iv) 64, XY, SRY-negative [4]. This work describes a DSD in a 3-year-old Arabian mare. At the clinical examination, this mare showed ambiguous external genitalia and non-typical female behaviour (nervous temperament, aggressiveness, masculine attitude). The distance between anus and vulva was shorter than normal, vulvar lips were dorsally fused except for the lower neckline showing a blind ending, from which a penis-like structure protruded. The ultrasound examination revealed the presence of cervix, uterus, hypoplastic uterine horns and small gonads with an echogenicity similar to a testicular parenchyma. Blood samples have been used for hormonal dosage and cytogenetic and genetic analyses. Blood testosterone levels ranged from 0.4 to 0.6 ng/ml. Cytogenetic analysis showed a normal female karyotype (2n=64, XX), while PCR amplification of SRY and ZFY genes revealed the absence of Y chromosome. At necroscopic examination, we found internal genitalia arising from the genital ridge in the form of masculine type structures while those deriving from the Mullerian ducts in the form of feminine type structures. In addition, we found an infundibular portion of the fallopian tube at the cranial pole of the gonads. This is a first case in equine species of DSD 2n=64, XX, SRY-, with simultaneous presence of male (hypoplastic testicles, epididymal portions and a penis-like structure) and female (cervix, horn and body of an hypoplastic uterus) genital structures. The absence of the Y chromosome suggests to hypothesize that other genes, besides those present on the Y chromosome, are involved in sexual differentiation in the equine species.

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HANDLING THE TRANSITIONAL MARE: COMPARISON BETWEEN HCG AND GNRH FOR THE INDUCTION OF THE FIRST OVULATION OF THE BREEDING SEASON

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In equine reproduction, phases of non-cyclicity such as winter anestrus and spring transition coincide with the beginning of the breeding season. For this reason, it is advisable to anticipate the resumption of cyclicity in mares after winter anestrus. Although artificial photoperiod treatment is still the best method to hasten the first ovulation [1], it is quite difficult to apply in large herds stabled outdoors. Treatment with dopamine antagonist was reported as able to hasten the first ovulation in transitional mares [2]. Induction of ovulation in cyclic mares is possible with hCG and GnRH [3,4] but transitional mares seem to fail to respond to the induction of ovulation with GnRH more frequently than cyclic mares [4]. The aim of this study was to compare the efficacy of hCG and GnRH for the induction of the first and the second ovulation of the year after treatment of transitional mares with dopamine antagonists. Twenty-one healthy mares, between 3 and 17 years of age, in deep winter anestrus were stabled in paddocks under natural photoperiod. Reproductive tracts were monitored by ultrasound and oestrus behaviour was checked by the exposure to a teaser stallion. At the evidence of at least two growing follicles ≥ 25 mm and ultrasound oestrus appearance of the uterus or oestrus behaviour, mares were treated with 50 mg/100kg of Levosulpiride (Levopraid, Teofarma, Pavia) IM until ovulation or for a maximum of 21 days. Mares were divided in two groups (hCG and GnRH), homogeneous for age, body condition score, reproductive career and timing of treatment. Ovulation was induced at the evidence of a growing follicle ≥35 mm, with 2500 IU of hCG (Corulon, MSD ANIMAL HEALTH Srl, Segrate) IV or 1 ml of GnRH (Suprefact, SANOFI, Milano) SC. Ultrasound was performed daily until detection of ovulation. In the first cycle of the year, 4/9 (44%) and 6/9 (67%) mares responded at the induction of ovulation with GnRH and hCG, respectively (P>0.05), and 3/9 (33%) and 8/9 (89%) responded in the month of March and April, respectively (P=0.05), regardless to the use of GnRH or hCG. In the second cycle of the year, no differences were seen for the drug employed or for the month of treatment. In conclusion, mares' response to the induction of the first ovulation of the year seemed more dependent on the month of induction than on the treatment, GnRH or hCG. The repetition of the study in a larger number of animals will produce more reliable results.

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YOUNG WILDLIFE REHABILITATION RECORDS OF A NORTH-WEST ITALY CENTRE (CANC)

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CANC (Centro Animali Non Convenzionali) is a ward of the Ospedale Veterinario Universitario of the Dipartimento di Scienze Veterinarie dedicated exclusively to wild and exotic animals. It is active from 2010 and every year it deals with more than 4000 patients of different ages, species, and pathologies, divided in about 3000 wild subjects and 1000 that have an owner. From 2012, a management information system (MIS) and an electronic medical record (EMR) were activated and, in all these years, data of about 15,622 hospitalized subjects were collected. The EMR ensures a better data administration and, at the same time, helps veterinary and student in diagnosis and feeding management of these very peculiar animals.

The most critical patients are surely newborns and very young wild subjects because they have the same management and clinical issues of the adult-ones and the additional troubles due to the young age; the aim of this paper is to refer the CANC's results.

During the years 2012-2017, 3745 young patients were hospitalized at CANC. Of the 3745 recovered animals, 1151 were released (32.45%) and 2396 (67.55%) died (1993 – 56.19% - deceased and 403 – 11.36% - euthanized). These data are quite different from those of the recovered adults (n= 11772): 4441 (37.73%) alive and 7331 (62.27%) died (3921 – 33.31% - deceased and 4310 – 28.97% - euthanized).

There is a remarkable difference between animal classes regarding survival rates (SR). In birds (n = 2567) SR was 34.44% (n = 884) vs a mortality rate (MR) of 65.56% (n = 1683; 1385 – 53.95% - deceased and 298 – 11.61% - euthanized); whereas, in mammals (n = 980), SR was 27.24% (n = 267) and MR was 72.76% (n = 713; 608 – 62.04% - deceased and 105 – 10.71% - euthanized).

The data show that although the bird management is surely more laborious than in mammals (e.g. they need to be feed at least 8 times daily vs a peak of 5-6 in mammals) the SR in birds is quite higher than in mammals (34.44% vs 27.24%). It must be emphasized that milk feeding in some mammals (e.g. *Pipistrellus* sp. or *Sylvilagus floridanus*) is laborious and time-consuming.

Regarding the admission causes of these kind of patients to CANC, categories like “starvation” or “no pathology” are predominant and these causes are quite similar in birds and mammals, instead in adult-ones are prevailing traumatic pathologies (fractures and or injuries caused by praying or car-crashes).

The reduced release rate in young subjects, is similar to that reported from other quoted centers in the world, should be due to narrow knowledge levels of food schedules in these species for lacking of literature [1-3]. However, the CANC experience shows how often people try to breed orphans, mammals in particular, without any experience using inappropriate food, for this reason the center sometimes collects animals when the clinical conditions are critical and the release rates are very poor.

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HAIR CORTISOL, DHEYDROEPIANDROSTERONE AND PROGESTERONE CONCENTRATIONS IN CROSSBRED BEEF COWS FROM CALVING TO 100 DAYS POSTPARTUM

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Dehydroepiandrosterone (DHEA) and cortisol (C) are hormones secreted by the adrenal cortex after stress stimulation, and they are related to resilience and allostatic load. Hair steroids measurement has recently received increasing attention for measurement of chronic stress in dairy cows, as it offers the advantages of being noninvasive, fast, and able to indicate steroids levels over long periods [1]. Around the time of calving, cows experience a suite of stressful events, including regrouping, diet changes, parturition, and the onset of lactation; during the transition period, it has been demonstrated that primiparous cows behave differently and are more likely to experience negative health outcomes compared to multiparous cows [2]. The objects of this study were to evaluate C, DHEA and progesterone (P4) hair concentrations in crossbred beef cows from calving to 100 days post partum (pp), and to assess possible differences related with parity (primiparous vs multiparous cows). Six primiparous and five multiparous pregnant beef cows from a tethered stall were enrolled. Hair samples of the 11 cows were collected during the autumn/winter season by shaving at calving (T0) and every 20 days for five times (T1-T5), only on the re-growth area. Hair C, DHEA and P4 were analyzed by RIA [1]. No differences were detected in the hair re-growth among animals. Statistical analysis showed higher C concentrations in primiparous cows at calving (T0) and at 20 days pp (T1) compared to all the subsequent samples ($p<0.05$). C levels at T0 and T1 in primiparous cows (6.7±3.37 pg/mL and 7.9±3.16 pg/mL, respectively) were higher than in multiparous cows (4.2±3.76 pg/mL and 4.1±2.36 pg/mL, respectively) ($p<0.05$). No other differences were detected within each group or between the two groups of cows regarding C, DHEA and P4 concentrations. These results suggest that C hair concentrations in cows are affected by parity. The higher C levels registered at calving and at 20 days pp in primiparous cows indicate a greater stress level before and around parturition compared to multiparous cows. Considering that the animals enrolled in this study did not undergone major changes of management or diet, this difference is probably related with the fact that calving was experienced for the first time by the pregnant heifers. Cortisol levels did not change from sample T2 (40 days pp) to the end of the study, suggesting the capacity of the heifers to recover from calving stress. Similarly, pluriparous cows did not show any relevant increase in C levels around calving, possibly because of their resilience. DHEA and P4 hair concentrations were not affected by parity neither by time; both these hormones are secreted also by the placenta in the pregnant cow [3], so the concentrations observed in their hair also derives from a placental synthesis. The present study suggests that hair C can be useful for measurement of the allostatic load in cows, and that primiparous cows undergo a higher stress level around calving compared to multiparous cows.

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INDUCTION OF ABORTION IN 7-12.5 MONTHS OLD MUCCO PISANO HEIFERS: A CASE REPORT

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Puberty is defined as the process of acquiring reproductive competence, that is ability to accomplish reproduction successfully [1]. No information is present about age at puberty in Mucco Pisano heifers, but it is reported that a percentage between 17 and 60% attains puberty before 12 months of age [2,3]. Anyway, the most common reason for inducing abortion in cattle is to terminate pregnancy in feedlot heifers [4], using a PGF2alpha analogue with or without desamethasone, depending mostly on gestational age (before or after the 150th day of pregnancy) with an efficiency of the 95% in 3-9 days [3]. This study describes the case of an herd of feedlot heifers left to be bred by bullocks of the same age, submitted to ultrasonographic pregnancy diagnosis and the subsequent abortion rate of the pregnant heifers treated with Alfaprostol. At the “Parco Naturale di Migliarino San Rossore Massaciuccoli” around sixty days after the isolation from males of the same age, 13 Mucco Pisano heifers, aged between 210 and 376 days, have been submitted to trans-rectal ultrasonographic pregnancy diagnosis (Mindray DP-30 equipped with a 4-7 MHz linear probe). A dose of 1.5 mg/100 kg of bodyweight of the PGF2alpha analogue Alfaprostol (Gabbrostim, VETEM Spa, Agrigento, Italy) was administrated in heifers diagnosed pregnant at ultrasound. Pregnancies have been checked by ultrasound 8 days later to ascertain abortion. Heifers have been monitored for 30 minutes after Alfaprostol injection to evidence undesirable side effects. Seven/13 heifers (54%) resulted pregnant between 65 and 80 days at ultrasound, no differences between age of pregnant and non-pregnant heifers (289.4±42.2 and 264.3±59.5 days) have been found ($P>0.05$). No pregnancies, fluid or foetal remnants have been evidenced by ultrasound, 8 days after the Alfaprostol administration. “Mucco Pisano” heifers submitted to pregnancy diagnoses were separated from the males between 5 and 10.5 months of age, as well as undesiderable side effects. The high percentage of heifers diagnosed pregnant (53%) could be an indication of the precocity of this breed, confirmed by the fertility of the equal in age bullocks responsible of these pregnancies. A dose of 1.5 mg/100 kg of bodyweight of alfaprostol was able to induce the 100% of abortion before 8 days after administration without side effects [5].

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HIGH FIELD MRI OF THE REPRODUCTIVE STRUCTURES IN THREE SPECIES OF CHELONIANS: PRELIMINARY OBSERVATIONS

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Diagnostic imaging plays an essential role in chelonian medicine and reproductive diseases. The physical examination results strongly hindered by the presence of plastron and carapace. Indeed, radiography and ultrasonography, although often used in reptilian medicine, are still limited in the case of chelonia [1]. Thus, for a complete evaluation of the coelomatic structures it would be desirable the use of others advanced techniques such as TC, MRI and endoscopy. Endoscopy has the disadvantage of being invasive, requiring the sedation of the animal. TC does not necessarily need an anesthetized animal, however because of the presence of carapace TC does not allow the attainment of an excellent evaluation [2]. On the contrary, through MRI a better evaluation of the coelomic structures could be done. However high cost and long times of execution are nowadays limiting the use of this technique [1]. The aim of this study was to evaluate the normal appearance of the coelomic structures of chelonian in MRI, highlighting in particular if reproductive organs could be appropriately studied through this technique and furthermore if MRI could be useful to determine turtles sex and diagnose reproductive disorders. The purpose of Authors was also to elaborate a protocol fast enough to allow to obtain good quality images in non-anaesthetized animals. We considered 16 clinically healthy chelonians (7 males, 6 females, 3 sexually immature) belonging to 3 different species: *Trachemys scripta scripta* (3), *Testudo marginata* (3), *Testudo hermanni* (10). Scans were performed in non-anaesthetized animals, and each animal has had at least one scan. Exams were performed using an RM apparatus equipped with a 1.5 Tesla Superconductive Magnet (Philips Multiva®, Philips Healthcare, Milan, Italy). The following sequences were used: T1 Turbo spin echo (T1 TSE) in axial and coronal planes, T2 Turbo Spin Echo (T2 TSE) 3D acquired in sagittal plane then reconstructed in three planes of space, and a Short Tau Inversion Recovery (STIR) in axial plane. All the studies were then evaluated with a dedicated software (OsiriX, 32 bit, Pixmeo, Geneva, Switzerland). Testicles were identified in two adult males, and presented variable characteristics at different times of the year. Ovaries have been recognized if mature follicles were present. In sexually immature animals, gonads were not detectable. Heart and big vessels, lungs, liver, spleen, stomach, kidneys and intestine have been clearly identified and evaluated. Pancreas, adrenal glands and ureters were not detectable in any of the animals considered. T1 TSE on the axial or dorsal plane and T2 TSE 3D on the sagittal plane, allow to get diagnostic images. Although RMI does not seem to be a useful method for turtles sexing [3], it has shown to be an important tool for a detailed study of the coelomatic structures and gonads evaluation in adult chelonians, providing diagnostic images with very low acquisition times.

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ELECTROCARDIOGRAPHIC FINDINGS IN BITCHES AFFECTED BY CLOSED PYOMETRA

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Pyometra is the commonest disorder of reproductive tract affecting intact bitches over 8 years old [1]. Clinical signs are associated to accumulation of purulent fluid in the uterus and, in severe cases, to the onset of endotoxemia, sepsis and systemic multiorgan inflammation. Myocarditis is a complication of the pyometra, and it is suspected to be a contributing factor to unexpected deaths [2,3]. However, scare information are available on the cardiac electric disorder in course of pyometra; therefore aims of the present study were (i) to describe the electrocardiographic findings; (ii) to assess the clinical relevance of electrocardiographic changes. Thirty-nine bitches belonging to different breeds and aging between 4 and 13 years old (mean 8.4 years old) with diagnosis of closed pyometra were included. The suspect of pyometra was performed on basis of oestrus cycle (luteal phase), clinical signs, laboratory findings and abdominal ultrasound examination. All bitches underwent to electrocardiographic evaluation before the surgical treatment. Briefly, a six lead ECG was recorded using 25 and 50 mm/sec paper speed and 1 cm = 1mV for 20-30 seconds. The following electrocardiographic changes were recorded: low R-amplitude in (17/39, 43.8%) ventricular premature complexes in (9 out of 39, 23%), large T (7/39, 7.9 %), ST depression and coving (4/39, 10.2 %). No ECG abnormalities were found in 5 (12.8 %). In 21 bitches ECG was normal at the moment of hospital discharge. ECG findings observed were differently associated to mechanical factors or presence of systemic disorders. Precordial low voltage of R-wave was observed in humans affected with ascites [4]. Changes had been mostly attributed to the effect of generalized fluid retention for a shift of the anatomical heart axis in response to the abdominal pressure. The occurrence of ventricular extrasystoles and alterations of ST tract may be related to the presence of endotoxins into the circulation determining systemic of inflammation, and in any cases myocardial cellular damage [5]. In 7/9 bitches presenting ventricular extrasystoles was observed leucocytosis with a left shift, neutrophilia, monocytosis, increased globulins, reflecting systemic inflammatory response. In humans, ST depression and T abnormalities were observed in ECG of patient with myocardial ischemia. Myocardial injury may present secondary to endotoxemia, disseminated bacterial infection and is considered a factor contributing to unexpected death in course of pyometra [5]. The safest and most effective treatment of pyometra is ovariohysterectomy. Surgery and anaesthesia may cause myocardial ischemia, with consequent myocardial cell damage, especially in subjects with suspected systemic inflammation. In accordance with the presented results ECG evaluation before the surgery may be essential on the correct management of bitches affecting by pyometra.

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RESVERATROL SUPPLEMENTATION OF IN VITRO MATURATION MEDIUM IMPROVES THE DEVELOPMENT OF OOCYTES RETRIEVED FROM DOMESTIC CAT OVARIES STORED AT 4°C FOR 48H

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Cat oocytes collected from refrigerated ovaries are able to mature in vitro; however, only oocytes stored for 24 h produce blastocysts after in vitro fertilization and retain competence to develop into live offspring after transfer into recipient [1,2]. Resveratrol, an important phyto-antioxidant found in grapes, mulberry, and other plants promoted in vitro embryonic development in different species. To improve the in vitro embryo production from oocytes of refrigerated cat ovaries, we explored the effect of resveratrol supplementation of maturation medium on oocyte meiotic and development competence. Ovaries were harvested from domestic queens (8 months-2 years old) during routine ovariectomy. Cumulus-oocyte complexes were collected from ovaries stored in PBS at 4°C for 24h and 48h and matured in vitro (IVM) in presence (+) or absence (-) of 5 µM resveratrol. Oocytes from fresh ovaries were matured in vitro as control. At the end of IVM, groups of oocytes were stained with Hoechst 33342 (1µg/ml) to evaluate nuclear maturation. In vitro matured oocytes were fertilized (IVF) in synthetic oviductal fluid (SOF) containing 6mg/ml BSA with frozen-thawed spermatozoa. After IVF, the presumptive zygotes were cultured in SOF with 4mg/ml BSA and 1% MEM non-essential amino acids. On day 3 after IVF the embryos were transferred to SOF supplemented with 10% fetal calf serum and 2% MEM essential amino acids. On day 2 and day 7 of culture, respectively, the number of embryos cleaved and developed to the blastocyst stage was determined. Data were analyzed by using chi-square test followed by Fischer exact test for pairwise comparisons.

There were no significant differences in the maturation and cleavage rates of oocytes among the groups, irrespective of resveratrol supplementation. The blastocyst rate on cleaved embryos (blastocysts/cleaved) from 48h stored oocytes and matured with resveratrol (n=14/28, 50%) was higher (p<0.05) than that of 48h stored oocytes matured without resveratrol (n=4/31, 31%) and similar to that of control (n=22/36, 61.1%). On the other hand, resveratrol supplementation didn't affect blastocyst rate in 24h stored group (n=18/32, 56.2% vs. n=19/32, 59.4%, without vs. with resveratrol respectively).

In conclusion, the addition of resveratrol has allowed the development of blastocysts even from 48h refrigerated ovaries.

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EFFECTS OF CADMIUM SUPPLEMENTATION DURING IN VITRO MATURATION ON CUMULUS CELL TRANSCRIPTOME

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Cadmium (Cd) is one of the most important environmental pollutants in industrialized countries. We previously reported that Cd supplementation, at nanomolar concentrations, during in vitro maturation (IVM) affects cumulus-oocyte complex (COC) viability and oocyte fertilization in adult and prepubertal sheep [1]; however, Cd action mechanisms need to be further investigated. The aim of the present study was to determine whether exposure to nanomolar Cd concentration during IVM affects the transcriptome of cumulus cells (CCs). COCs were recovered from the ovaries of slaughtered prepubertal lambs (less than 6 months of age). In vitro maturation (IVM) [1] was performed in single medium drops of 10 microL and COCs were exposed to 100 nM Cd whereas those cultured in absence of Cd were used as controls. At the end of culture time, CCs of both groups were removed and only those isolated from matured oocytes, showing the first polar body extruded, were pooled (CCs from 25 in vitro matured COC/sample) and cryopreserved until analysis. A total of 10 samples, 5 Cd-treated vs 5 controls, were processed. Each sample underwent RNA extraction, libraries preparation and deep transcriptome sequencing (RNAseq) on the NextSeq500 Illumina platform. Raw reads were checked for quality by FASTQC and aligned onto the reference genome (Oar_v3.1 from UCSC) using STAR (PMID: 23104886). Differentially expressed genes were detected using the DESeq2 R package (PMID: 25516281), selecting genes with a log2 fold-change higher than 1.5 or lower than -1.5 and uncorrected p-value lower than 0.05 (due to low variability among RNAseq groups). Although no differences in maturation rates have been identified, several differential expressed genes (DEGs) by Cd treatment, were found. Some of these DEGs are involved in the regulation of metal ions transport, such as metallothionein (MT1A) and solute carrier family 30 member 2 (SLC30A2), both involved in Zinc transportation required for progression and completion of meiosis. These results expand our understanding of the complex molecular mechanisms of response and defense to Cd exposure in CCs in the sheep model and provide new insights for unveiling the biological mechanisms underlying the effects of Cd on cellular communication between the cumulus and the oocyte. Further studies are needed to understand if this could potentially influence embryonic development.

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EXPOSURE TO LOW-DOSE X-RAY RADIATION BEFORE IN VITRO MATURATION AFFECTS OOCYTE BIOENERGETIC/OXIDATIVE STATUS IN THE SHEEP MODEL

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Exposure to X-ray radiations has been reported among causes of infertility, both in humans and farm animals but their action mechanisms are only partially known [1]. Although it has been known from a long time that radiotherapy induce permanent ovarian failure [2], little is known to date, about the effects of X-ray radiation doses, adsorbed in radiological diagnostic procedures, on oocyte viability. The aim of the present study was to investigate, in the sheep model, the effects of low doses X-ray radiation (≤100 milligrays, mGy) on nuclear maturation and bioenergetic/oxidative status of prepubertal lamb oocytes. Cumulus-oocyte complexes (COCs) were collected from the ovaries of slaughtered prepubertal lambs (less than 6 months of age), held in Earle's/Hanks' M199-based medium (EH medium, [3]) at room temperature and transported to the "CNR-Radioactivity" division of the IZSPB. Samples underwent controlled X-ray exposure to a single dose of 10, 50 or 100 mGy by using a RS-2400 biological irradiator. Non irradiated samples were used as controls. At the end of X-ray exposure, COCs were held overnight in EH medium at 25°C and cultured for IVM [4]. After culture, COCs underwent cumulus cell removal, were stained for nuclear chromatin, mitochondria (mt) and reactive oxygen species (ROS) with specific fluorescent probes (Hoechst 33258, MitoTracker Orange CMTM ROS and 2',7'-dichloro-dihydro-fluorescein diacetate [4]) and fixed with 2% paraformaldehyde solution in PBS. Those oocytes showing a metaphase II (MII) plate with the first polar body were analyzed by confocal microscopy for mt/ROS fluorescence intensity (arbitrary densitometric units) and colocalization (overlap coefficient). Data were analyzed for meiotic stage (Chi-square test) and bioenergetic/oxidative status (Student's t-test). Differences were considered to be significant when $P<0.05$. Oocyte maturation rates did not differ between exposed and control oocytes (66.6%, 32/48, 71.4%, 40/56 and 60.6%, 37/61 for 10, 50 and 100 mGy respectively vs 63.6%, 35/55; $P>0.05$). Concerning the effects on bioenergetic/oxidative status of MII oocytes, low-dose X-ray exposure significantly reduced mt activity (200.9±69.0, 171.2±57.3 at 50 and 100 mGy, respectively vs 310.7±90.7; $P<0.01$) and ROS levels (342.9±197.0, 319.1±131.7 at 50 and 100 mGy respectively vs 445.9±163.7; $P<0.05$), with no effect on mt/ROS colocalization (0.75±0.13, 0.68±0.17 at 50 and 100 mGy respectively vs 0.74±0.14; $P>0.05$). No differences ($P>0.05$) were found in samples exposed to 10 mGy compared with controls. In conclusion, exposure to low-dose X-ray radiations does not affect the maturation rates of prepubertal lamb oocytes at any examined dose. However, 50 and 100 mGy affected the bioenergetic/oxidative status of matured oocyte, in terms of reduction of mt activity and ROS levels, indicating reduced oocyte viability. Further studies are needed to evaluate the developmental potential of irradiated oocytes.

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EVALUATION OF THE ANALGESIC EFFECT OF THE FENTANYL PATCH DURING OVARIECTOMY AND THE POSTOPERATIVE PERIOD IN BITCH

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For several years now, routine surgical procedures such as ovariectomy are performed as outpatient surgery. Post-operative care turns out to be much more comfortable for the patient than hospitalization, particularly at home in reducing the risk of nosocomial infections. However, this approach rises the problem of the administration of analgesic drugs by the owners at home. The potent opioid fentanyl is market in the form of a patch (DURAGESIC®, Jannsen), which fentanyl is gradually released for approximately 72 hours after application (1). The aim of this study was to evaluate the convenience and the analgesic efficacy of this patch, in comparison to other analgesic protocols usually utilized in ovariectomized bitches.
This study involved 20 privately-owned bitches, ovariectomized at the Veterinary Hospital of the Department of Veterinary Medicine (Bari). Only bitches housed without other domestic animals were selected, to allow the owners to perform a strict control in the postoperative period. The animals (healthy and from 2 to 5 years of age) were randomly divided into two 2groups. In both groups after premedication with acepromazine, anesthesia was induced by propofol and maintained with isofluorane. The following parameters were monitored during the procedure in all animals: heart rate, electrocardiogram, EtCO2, pulse oximetry, blood pressure and body temperature. The analgesic protocols in the Fentanyl group and Control group were different. The Fentanyl patches were applied 12 hours before the surgery. Transdermal fentanyl patches were placed on the neck skin, which had been previously shaved and disinfected. The size of individual patch was chosen based on the patient's body weight: <10 kg = 25 mcg/h; 10-20 kg = 50 mcg/h; 20-30 kg = 75 mcg/h, and 30-40 kg = 100 mcg/h (2). The patches were affixed with adhesive tape, and left on site for 72 hours after application (3). In the control group standard analgesic protocol pre-surgically was with methadone and oral administration of analgesic drugs at home was administered (robenacoxib). In the clinic, after the dog awakened from anesthesia, the behavior and level of pain/discomfort were evaluated using the Glasgow Pain Scale. The owners filled out a questionnaire with a numerical scale (from 0 to 10) to assess their dogs behavior and the owners satisfaction, regarding the management of their animals postoperative. All the animals in the study hade an uneventful recovery from anesthesia. The owners of the bitches in Fentanyl group were more satisfied with post-operative management than were the owners of the control group (administration of drugs orally). More pain was reported in the control group, related to bitches refusing oral medication. The bitches in the Fentanyl group tolerated the Fentanyl patch well, without any side effects noted. The data obtained from this study shows that the fentanyl patch is a valid aid for effective analgesia post-ovariectomy in bitches.

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CLINICAL EVALUATION OF THE EFFICACY OF TWO DIFFERENT ANALGESIC PROTOCOLS DURING PRESCROTAL ORCHIECTOMY IN DOGS

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Orchiectomy is a common surgical procedure in small animal practice. In spite of the relative technical simplicity of execution, orchiectomy requires a serious approach to perioperative analgesic management. Neuroaxial (epidural) and loco-regional (infiltration of the incision line and testicular parenchyma) techniques of anaesthesia have been reported to improve the quality of sedation and/or general anaesthesia (1). The purpose of this study was to evaluate the analgesic efficacy of administration of ropivacaine hydrochloride by ultrasound-guided injection into the spermatic cord, together with infiltration of the same anaesthetic into the prescrotal incisional line (4). Fifty privately-owned dogs presented for clinical orchiectomy were randomly divided into two groups of 25 each (ROP group and Control group). The dogs were approximately 2-4 y of age and 15-25 kg body weight. They received the same combination of drugs for sedative-analgesic purposes: 3 mcg/kg of dexmedetomidine + 0.25 mg/kg of methadone (2,3). Subjects in the ROP group were administered only ropivacaine, injected into the tissue around the spermatic cord at the level of its starting point from the superficial inguinal ring, and also injected into the incision line (0.2 ml/kg of ropivacaine 0.5% at each injection site). In the Control Group (25 dogs), 20 min after sedation, propofol were administered i.v. to induce general anaesthesia. Parameters taken into consideration were haemodynamic stability, the need for additional administration of hypnotic and/or analgesic drugs in the intraoperative period, and the need for analgesics in the postoperative period. Dogs in the ROP group showed a greater hemodynamic stability intraoperatively (considering heart rate, electrocardiogram, pulse oximetry and blood pressure) compared with the Control group, and did not react on surgical stimulation. Dogs in the ROP group and in Control group had no requirement for hypnotics during the procedure. Immediately after surgery, evaluation of pain by the Glasgow pain scale demonstrated that both group did not present signs of pain. In the postoperative period at home, owners reported no signs of pain or discomfort in two group. The loco-regional approach used in this study has been shown to be effective in minimizing responses to the surgical stimulus, and ensured adequate analgesia in the both the intraoperative and postoperative period. This method allows the procedure to be performed under deep sedation and therefore avoids the use of general anaesthesia.

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RELATIONSHIP BETWEEN CHILLED SEMEN QUALITY/FERTILITY AND SEMINAL PLASMA BIOCHEMICAL AND PROTEIN PROFILES IN TROTTER STALLION

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The composition of the seminal plasma (SP) in the horse does not differ from that of other domestic mammals. In it, have been identified amino acids, proteins, enzymes, organic ions, lipids, fatty acids, hormones and sugars. The discovery and identification of several substances in the secretion of seminal plasma from the rete testis, epididymis and accessory glands, has opened the way to study their functionality [1]. The aim of our study was to analyze the correlation indexes among SP electrophoretic protein profile (SP-EPP) (10 classes of molecular weight - MW), SP biochemical parameters (SP-BP) (urea, glucose, cholesterol, triglycerides, creatinine, Ca, Cl, K, Na, Mg, ALP, LDH, CK and total protein), semen quality (<or> 70% motility) and fertility (<or> 50% pregnancy and foal rates after AI with chilled semen). A total of 46 ejaculates from 7 healthy and fertile stallions were used during the artificial insemination season. SP-EPP verified that the higher presence of low MW proteins is a significant indicator of poor quality of the semen while the greater presence of proteins of high MW is significant for the higher quality. Specifically, 19-22 kDa and 122-160 kDa bands seem to be the most significant proteins giving information on the worst and the best quality respectively. Other Authors reported already that proteins with 15.9 kDa and 18.2 kDa and HSP 15 (26.7 kDa) are negative indicators of semen quality [2]. Proteins with 100-120 kDa of MW or higher have the characteristics to bind heparin, some produced by epididymis, seems to have a potential role on the fertility [3]. The exception is the 3-14 kDa band that could represent the homologue of bull SP linked positively with the fertility. Finally, 72-88 kDa band include transferrine (75.4 kDa) which is an indicator of testicular dysplasia with oligospermia. Similarly, SP-BP analysis has identified several elements correlated, positively (ALP, LDH, CK, Cholesterole, PT) and others negatively (Glucose, Mg, K, Ca, Na), with the success rate of the AI. High level of alkaline phosphatase is correlated with high semen concentration and motility [4]. Total protein level decrease with the age as previously reported [5]. High level of calcium inhibits tyrosin-phosphorylase activity which has a key role on sperm capacitation while high level of K and Na induce lipoperoxidation giving sperm membrane instability [6]. In conclusion SP-EPP and SP-BP give information about semen quality and could be used to make-up the extenders for fresh and frozen sperm.

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RETROSPECTIVE STUDY OF NON-INFECTIOUS LESIONS OF THE PREPUCE AND PENIS IN THE RAM

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Ram breeding soundness evaluation is an integral part of management programs of sheep flocks. Main factors affecting the reproductive ability of rams could be, indeed, detected by andrological clinical evaluation. The incidence of penis lesions has been poorly investigated, in rams, and literature mainly report about infectious disease lesions [1]. The objective of this retrospective study was, therefore, to evaluate the incidence of different pathological conditions of the penis and prepuce in Sarda rams. Thirty-seven rams (out of 1262 animals from 126 commercial flocks, examined from 2010 to 2017, before the breeding season) were admitted to the Teaching Veterinary Hospital of the Department of Veterinary Medicine, University of Sassari (Italy) and selected for the present study because of prepuce or penile lesions. Animals were reared, by traditional management systems, in the North and centre of Sardinia region (Italy). All animals underwent clinical examination followed by a further examination of the reproductive tract. The scrotum, testes, epididymis and spermatic cords were palpated; the prepuce was closely evaluated for injuries, ulcerations, abnormal discharge or bleeding as well the skin and mucosa of its orifice. The penis was exteriorized and the glans observed for possible presence of wounds, ulceration, inflammation, and lack or necrosis of the vermiform appendix. For each animal, the type and extension of any prepuce and penile abnormality was recorded. The incidence of penile and prepuce pathological conditions was 2.93%. The most frequently observed condition (19/37 animals 51.35%) was the penile necrosis consequent to urethral calculi, the latter probably caused by the high concentrates levels in the animals' diet. Other less frequent disorders were: penile necrosis (5/37) and absence of urethral process (3/37), hypospadia (2/37), injuries of the glans penis (2/37), absence of glans penis (1/37), preputial alterations (abscesses 1/37; papilloma 1/37; skin injuries 1/37), retroversion of urethral process (1/37) and balanoposthitis (1/37). In this retrospective study, we noticed that the main non-infectious pathological conditions of the penis and prepuce in Sarda rams are a consequence of urethral calculi or of traumatic origin. Obstruction of the urethra by calculi, in addition to penile necrosis, might cause inability to urinate and uremia, rupture of the bladder and uroperitoneum with fatal end in most severe cases [2]. Traumatic conditions are generally not life threatening for animals but could temporarily, or sometimes permanently, impair fertility.

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PRELIMINARY EFFECTS OF RESVERATROL ON MOTILITY AND MEMBRANES INTEGRITY OF FROZEN-THAWED BUCK SEMEN COLLECTED OUT OF THE BREEDING SEASON

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Resveratrol exhibited protective effects against lipid peroxidation and DNA damage caused by free radicals in human sperm cells [1]. Supplementation of semen extender with resveratrol enhanced bovine [2] and buffalo [3] sperm mitochondrial activity and membrane stability, and reduced DNA damage. Conversely, no effects were observed on ram's motility and membranes integrity but a loss in mitochondrial membrane potential was reported [4]. The aim of the study was to evaluate the effects of resveratrol supplementation on motility and membranes integrity of frozen-thawed (F-T) buck semen collected out of the breeding season. Ejaculates of 4 mature bucks (n=64) were collected weekly from April to July 2017, kept at 30°C, diluted in Triladyl+20%EY and divided in 4 aliquots each supplemented with increasing concentrations of resveratrol (control, 10µM, 25µM, 50µM). After cooling to 4°C in 4h, samples were loaded in 0.25mL straws, frozen and stored in LN_2. Aliquots of semen from the 4 groups underwent the following analyses after dilution (30°C), after cooling (4°C) and after thawing (30s at 37°C): i) kinematic parameters (total and progressive motility, TM, PM; smoothed path velocity, VAP; straight line velocity, VSL; track velocity, VCL; rapid, medium, slow and static cells); ii) acrosome integrity and sperm viability (Propidium Iodide+ *Pisum sativum* agglutinin staining); iii) functional integrity of cytoplasmic membrane (hypo-osmotic swelling test, 100mOsm kg-1). Resveratrol supplementation did not affect TM and PM at 30°C and at 4°C but, after thawing, the 10µM resveratrol group showed higher TM and PM compared to control ($P<0.05$). Similar results were obtained following analysis of velocity distribution: higher percentages of rapid cells were observed in the 10µM resveratrol group as compared to control ($P<0.05$). No effects were observed on VAP, VCL and VSL and on the integrity and functionality of sperm cytoplasm and acrosomal membranes ($P>0.05$). In conclusion, resveratrol supplementation enhanced motility of F-T buck semen out of the breeding season, probably contrasting oxidative stress due to storage conditions. Further investigations about the anti-oxidant properties of this compound on sperm cells are currently under assessment.

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MATRIX METALLOPROTEINASE-2 AND -9 OF AMNIOTIC FLUID IN MARES WITH NORMAL AND HIGH-RISK PREGNANCY

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The matrix metalloproteinases (MMPs) are a family of enzymes involved in extracellular matrix remodelling. MMPs are secreted in a latent form and activated by local and infiltrating cells. MMP-2 and -9 are the most studied and have been detected in bovine, ovine and human placenta. From studies in women, MMP-2 and -9 increase in amniotic fluid (AF) in case of premature rupture of membranes and chorioamnionitis [1]. There is only one study on MMPs in the equine AF reporting an increase in the activity of MMP-2 and a decrease in the activity of MMP-9 in case of premature delivery [2]. The aim of this study was focused on MMP-2 and -9 in AF collected at parturition from mares with normal and high-risk pregnancy. AF was collected by needle puncture of the amnion within 5 minutes after its appearance through the vulva. Samples were immediately stored at -20°C and analyzed within 6 months after collection. The activity of MMP-2 and -9 was analysed by in-gel zymography (NovexTM Zymogram Plus) allowing the evaluation the activity of both latent and active forms of MMPs. Thirteen mares with normal pregnancy (Group 1) and 13 with high-risk pregnancy (Group 2) were included. In Group 2, one mare was affected by severe laminitis that caused preterm delivery at 314 days of gestation; other two mares were affected by ascending placentitis and foaled at term: one of them had been treated as suggested, while the other one had not been treated. The other 9 mares presented other forms of placental insufficiency. In Group 1 zymograms showed similar patterns indicating that MMP-2 is more active than MMP-9 in normal pregnancy at parturition. On the other hand, MMP profiles in Group 2 were variable. Differently from Oddsdóttir et al. [2], an increase in the activity of both MMP-2 and -9 was found in preterm delivery. It is worth reporting that the foal born prematurely included in this study was very small but completely mature and survived, while in the study of Oddsdòttir et al. [2] the foals died; as suggested by the authors, low level of MMP-9 in preterm delivery could be correlated to lung immaturity. The mare included in this study treated for ascending placentitis presented a zymogram similar to Group 1, but with a moderate decrease of MMP-2 activity. Conversely, in the AF of the mare with untreated ascending placentitis, MMP-9 was present mainly as active isoform. This might be related to the release of MMP-9 by leucocytes, endothelial cells, macrophages and fibroblasts during inflammation. In this case, the absence of treatment offered a spontaneous model of placentitis. Our data provide a starting point to better understand the role and the diagnostic value of MMPs in equine pregnancy, although they need to be confirmed by other data in a larger population of mares with high-risk pregnancy.

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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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ANIV
MALATTIE PARASSITARIE E SANITÀ PUBBLICA

EMERGING VECTOR-BORNE INFECTIONS IN CATS FROM CENTRAL ITALY

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Cats are exposed to a number of arthropods as fleas, ticks and sand flies, and thus to the pathogens they may transmit. However, epidemiology of feline vector-borne diseases (FeVBDs) is low investigated [1]. The present study aimed to assess the prevalence of *Leishmania infantum, Rickettsia felis and Cytauxzoon* spp. infections in cat populations living in central Italy, by molecular and serological techniques. From 2010 to 2016, 286 cats were randomly selected from catteries (n=112) and colonies (n=174) of central Italy regions (i.e Marche, Umbria, Toscana). Cats were examined for signs suggestive for FeVBDs, and peripheral blood and conjunctival swab (CS) samples were collected. Sera were analysed by IFAT to detect anti-*Leishmania* and anti-*Rickettsia felis* IgG antibodies (Ab) using commercial antigens. DNA extracted from buffy coat (BC) and CS samples was submitted to a SSU-rDNA nested (n)-PCR assay for *Leishmania* [2]. BC was assayed in a n-PCR assay for *Rickettsia* spp., amplifying fragments of the *gltA* [3], *ompB* [4] and *ompA* [5] genes, and in SSU-rDNA PCR for Piroplasmida species [6] for *Cytauxzoon* spp. No cats showed clinical signs of FeVBDs. Sixty-two (21.67%) cats were positive for anti-*Leishmania* IgG, with titers ranging from 1/20 to 1/160. Forty-five animals (15.73%) were positive to *Leishmania* CS n-PCR, whereas none of the animals scored molecularly positive in BC. Considering results obtained by IFAT and CS n-PCR a slight agreement between the 2 tests was detected. The serological assay for *R. felis* revealed 23 (8.04%) positives at low titer (1/64). No *Rickettsia* neither *Cytauxzoon* DNAs were amplified. The results of the serological and molecular survey showed a substantial prevalence of *Leishmania* exposure in the investigated cats and pointed out the limited molecular diagnostic value of BC versus CS samples. On the contrary no evidence supports the circulation of *Cytauxzoon* spp. across domestic cats in contrast with previous detection in wild cats (*Felis silvestris silvestris*) sampled in the same monitored areas [7]. The low positive Ab titres for *R. felis* in association with no DNA amplification do not allow speculations on the exposure of feline populations to this FeVBD due the cross-reactivity existing within the transitional group.

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HISTOPATHOLOGICAL AND MOLECULAR CHARACTERIZATION OF SHEEP ILEAL TISSUES WITH PAUCIBACILLARY INFECTION BY *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS*

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Paratuberculosis or Johne's Disease (JD), a contagious enteritis caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is responsible for significant losses in dairy ruminant productions. Although well characterized in cows, some aspects of the disease remain unclear in small ruminants, including the dynamics and molecular patterns of the paucibacillary to multibacillary transition. To gather new insights on disease evolution and to search for possible stage-specific biomarkers, we carried out a combined histopathological, molecular, and proteomic study of sheep ileal tissues focusing on paucibacillary MAP infection. Intestinal tissue sample repositories were available from MAP-free and MAP-infected flocks where all sheep had been screened by ELISA for anti-MAP antibodies in serum and by IS900 PCR for MAP in feces. All animals were females between 3 and 4 years of age. Upon Ziehl-Neelsen staining and histopathological evaluation, 7 paucibacillary JD samples and three negative control samples (K) collected from the terminal ileum (n=10) were selected for the study and subjected to: i) histopathological grading, ii) MAP strain typing by PCR and RFLP, iii) in-depth shotgun proteomics (liquid chromatography-tandem mass spectrometry on a Q-Exactive), and iv) label-free quantitation and bioinformatic analysis. Differential protein profiles were then validated by testing several tissue damage markers by immunohistochemistry (IHC). As a result, classification based on histopathological grading scores and on proteomic profiles provided similar indications. According to hierarchical clustering and to principal component analysis (PCA), negative and paucibacillary samples were clearly separated into two distinct clusters (JD and K); in addition, JD samples showed a further sub-clustering (JD1 and JD2). Therefore, we evaluated the differential protein expression patterns first for all JD samples vs K, and then separately for JD1 and JD2 samples vs K, to uncover differential stage-specific traits and to investigate on possible disease evolution patterns. Numerous changes in protein abundance levels were seen between all JD groups and K samples, some of which were quite pronounced and related to different proteins involved in MAP pathogenesis, including cathelicidins and other granule proteins, haptoglobin, and proteins responsible for vesicular trafficking, vacuole acidification and antigen presentation. In addition, abundant MAP proteins were also identified. The extent of proteomic changes was proportional to the lesion severity, and different protein repertoires were present in the paucibacillary condition depending on the microbial load, possibly reflecting differences in the cellular and immunological patterns activated along infection. Taken together with our previous study on multibacillary MAP infection [1], this work suggests that there is a continuum of lesion states from negative, to paucibacillary and to multibacillary MAP in sheep. The identification of stage-specific protein patterns may open the way to the definition of single markers or, more likely, of marker panels of progression, and enable a better understanding of the disease evolution and of host-pathogen interactions in this ruminant species.

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BOVINE HERPESVIRUS 4-BASED VECTOR DELIVERING THE FULL LENGHT XCT DNA EFFICIENTLY PROTECTS MICE FROM MAMMARY CANCER METASTASES BY TARGETING CANCER STEM CELLS

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Despite marked advancements in its treatment, breast cancer is still the leading cause of cancer death in women aged 20 to 59 years, due to relapses and distal metastases. Breast cancer stem cells (CSCs), are a cellular reservoir for recurrence, metastatic evolution and disease progression, making the development of novel therapeutics that target CSCs [1], and thereby inhibit metastases, an urgent need. We have previously demonstrated that the cystine-glutamate antiporter xCT (SLC7A11) [2], a protein that was shown to be overexpressed in mammary CSCs and that plays a key role in the maintenance of their redox balance, self-renewal and resistance to chemotherapy, is a potential target for mammary cancer immunotherapy. This work reports on the development of an anti-xCT viral vaccine that is based on the bovine herpesvirus 4 (BoHV-4) vector, which we have previously showed to be a safe vaccine that can transduce cells in vivo and confer immunogenicity to tumor antigens [3]. Female BALB/c mice were vaccinated twice; more in details, before (preventive model) or after tumor challenge (therapeutic model), and after the second vaccination, blood and spleens were collected from some mice, while others had TUBO-tumorsphere derived cells injected i.v. and their lungs were explanted 20 days later. We show that the vaccination of BALB/c mice with BoHV-4 expressing xCT (BoHV-4-mxCT), impaired lung metastases induced by syngeneic mammary CSCs both in preventive and therapeutic settings. Vaccination induced T lymphocyte activation and the production of anti-xCT antibodies that can mediate antibody-dependent cell cytotoxicity (ADCC), and directly impair CSC self-renewal and redox balance. Our findings pave the way for the potential future use of BoHV-4 vectors that target xCT in metastatic breast cancer treatment.

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RISK OF EXPOSURE TO AGENTS OF TICK-BORNE ZOONOSES IN AOSTA VALLEY

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Climate changes, modifications in land use and increasing wild ungulates populations are factors affecting in the latitudinal and altitudinal expansion of tick-borne pathogens in Europe [1]. Accordingly, tick bites are increasingly reported in Aosta Valley, north-western Italy. We assessed the risk of exposure of people during recreational and occupational activities in a geographical area of recent ticks invasion. We selected three hiking trails at altitude ranging 800-1200 a.s.l., characterized by oaks, leaves litter, and grassland. We collected host seeking *Ixodes ricinus* from May to July 2016 in an internal trail area (walking foothpath) and an external area (foothpath edges with high grass). The ticks have been collected from a 1 m^2 dragging cloth, from people's clothes and, occasionally, from a dog's hair, on 100 m^2 of land. On *I. ricinus* nymphs, we performed PCR for *Borrelia burgdorferi* s.l. and *Rickettsia* spp. We calculated the risk exposure by a mathematical model (E=v*c*ra) which includes the passage frequency of visitors (v), the probability of infected tick attachment (c) and the acarological risk (ra) [2]. Factor v, the probability of at least 1 visitor per hour in 100m^2, has been detected by a population survey. Factor c, the probability of contact between a person and at least 1 host-seeking tick in 100 m^2, was obtained as the ratio between the mean number of nymphs collected on people clothes and of nymphs collected on the drag. Ra, the probability of collection of at least 1 infected nymph in 100 m^2 by dragging, has been calculated with the formula ra= 1-e-pDT [3], which includes the prevalence of infection (p) and the density of ticks (DT). Moreover, a questionnaire was submitted to residents, to gather information on the exposure of people and animals to ticks. Prevalence of B*. burgdorferi* s.l. in host seeking *I. ricinus* nymphs was 40% (CI 95%:22.5; 57.5), whereas prevalence of Rickettsia spp. was 13.% (CI 95%:1.17-25.5). The mean number of nymphs, which were collected by dragging, and ra were greater in the internal area of the trail than in the external area. Nevertheless, people's exposure to microbial agents was greater in the external part, because of a higher likelihood of attachment (factor c). Therefore a more realistic exposure assessment is possible considering the dragging and walking techniques together. In addition the factor v could be evaluated more accurately in future studies. This risk assessment approach could be applied to other areas of the region to understand which are riskier, in the perspective to communicate this risk and prevent illnesses.

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ASSESSMENT OF THE RISK OF EXPOSURE OF ANIMAL FARMERS TO ANTIMICROBIAL-RESISTANT AGENTS

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Antimicrobial resistant agents (AMR) can be transmitted to farmers, from animals and the farm environment [1,2]. For prevention purposes, it is important to identify those farming practices, which are associated the greatest risk of exposure to AMR. By using fattening turkey production as a case study, we listed all of the farming phases, and analyzed all practices, which are carried out by farmers, during an entire production cycle. In the case study we included 9 fattening turkey farms distributed as follows: 5 in the province of Mantova, 3 in the province of Brescia and 1 in the province of Verona. A risk priority index was obtained for each practice, based upon information on prevalence of agents in animals and in the farming environment, and on AMR transmission routes. Scientific evidence was obtained by a systematic literature review. The priority index was calculated by a modified FMEA (Failure Modes and Effect Analysis) [3], which is composed of three components: 1) probability of an event (in our approach, the prevalence of AMR in animals and the farm environment); 2) consequences (the exposure of farmers to AMR during working practices); 3) detectability (risk perception and awareness, by potentially exposed farmers). Working practices were ordered based upon work hours, type of contact, personal safety devices, and number of animals per operator, whose weight varied across different types of AMR determinants (such as, for example, methicillin-resistant *Staphylococcus aureus*, or extended-spectrum beta-lactamase producing agents). As a final result, we obtained a risk priority index, for each work practice in the corresponding farming phase, to allow a transparent comparison across practices, considering all potential sources of transmission of AMR (environment and animals). More specifically, the adopted method allowed to calculate, in a transparent and repeatable way, risk indexes resulting from the combination of varying levels of prevalence of AMR agents across farming phases, with varying exposure intensity for farmers, during working practices. During a fattening turkey farm production cycle, vaccination of birds was characterized by the highest risk of exposure to AMR, due to direct and repeated contact with animals, by workers without personal safety devices. Removal and milling of litter ranked second in the risk prioritization, given the exposure of farmers to high dust level. Appropriate risk communication and risk mitigation measures will be produced, based upon results of this study.

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FIELD TRIAL ON THE EFFICACY OF CHLORTETRACYCLINE (ISOSPEN®) AGAINST *Cystoisospora canis* IN DOGS

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Cystoisospora canis (Apicomplexa, Eucoccidida) is the most important agent of canine coccidiosis, a worldwide parasitic disease whose clinical signs are observed almost exclusively in puppies less than 4 months of age, that showed the highest levels of oocyst shedding [1]. The control of canine coccidiosis is carried out through environmental and pharmacological prophylaxis and aims to the recovery of symptoms and persistent suppression of oocyst shedding. Clortetracycline (CL) belonging to tetracycline group, is a compound currently licensed for the pharmacological control of feline and canine coccidiosis. However, the exact mechanism of action of CL against *C. canis* is currently not fully understood. Aim of the present study was to confirm the efficacy of CL in the treatment of puppies clinically affected by coccidiosis and better define the timing of symptoms recovery, oocyst suppression and loss of viability. For this tool 40 owned puppies aged between 45 days to 3 months and suffering for clinical coccidiosis were enrolled. The animals were divided into 2 groups of 20 animals each: Group A treated with CL (Isospen®, Teknofarma) at dosage of 25 mg/kg for 15 consecutive days, and Group B treated with Toltrazuril (Procox®, Bayer) at dosage of 9 mg/kg in a single administration. Individual faecal collections were conducted at T1 (the day of treatment) and the following days T6, T9, T12, T15, T21. The faecal samples were analyzed by flotation technique using Sheather solution, faecal oocyst counts (FOCs) were detected by a modified McMaster technique [2] and viability of oocysts was assessed by coproculture in 2.5% $K_2Cr_2O_2$. The efficacies of the two treatments were assessed and compared at each time set in terms of FOCR% (Faecal oocyst count reduction), rate of oocyst loss of viability and average "faecal score" (FS) (scale from 1 to 5), basing on Waltham faeces scoring system [3]. No adverse events were observed after treatments. Ninety-five % of the animals of the A group had a fully recovery of the diarrhoeic symptoms within 12 days from the treatment, instead in B group 50% of the animals showed a persistent alteration of the FS till the end of the trial (T21). The puppies of B group tested coprologically negative since the T6 after treatment, while the animals of A group showed a total negativity since T15, even though a drastic suppression of the FOCs (FOCR: 60%) was observed as well as a lack of viability (rate of vitality: 28%) since T6. At T12 all the oocysts shedded from the A group were completely inactivated. Treatment with both toltrazuril and CL significantly reduced oocyst excretion even though with a different timing. Clortetracycline, when administered for 12 days, adequately control the environmental contamination by oocysts and early resolve the diarrhoeic symptoms.

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ANTIMICROBIAL SUSCEPTIBILITY OF COMMON BACTERIA ISOLATED FROM EQUINE UTERI: VARIATION OF EFFICACY OVER TIME

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Uterine infections, well known to be one of the most common causes of reduced fertility in mares [1], are caused by several opportunistic pathogens [2]. Antibiotic resistance has increased globally and bacterial prevalence as well as antimicrobial susceptibility patterns can change over time and among equine populations [3]. Therefore, this study was aimed at providing an update on bacterial species isolated from equine uteri and analyzing variations of antibiotic sensitivity over eight years (2010-2017). A retrospective study was conducted selecting 4445 uterine swabs collected from mares in the course of routine breeding examination or reproductive tract disorder. Subsequently to bacterial culture, the agar diffusion test was performed and interpreted according to the CLSI guidelines. Additionally, the results of prevalence and trends of susceptibility were processed and analyzed over time. Aerobic bacteria were isolated from 3245/4445 (73%) samples. The most frequently isolated microorganisms were *Escherichia coli* (892/3245, 20.1%) and *Streptococcus equi zooepidemicus* (791/3245, 17.8%). Antimicrobial susceptibility patterns of *E. coli*, *S. equi zooepidemicus*, *Pseudomonas* spp. and other bacteria belonging to *Enterobacteriaceae* family changed over the years. Overall, *E. coli* showed a slight reduction of sensitivity to gentamicin and amikacin, while quinolones maintained durable efficacy over the time. Ampicillin was the most active molecule against *S. equi zooepidemicus* but recently thiamphenicol and rifampicin increased their efficacy against this bacterium. The present epidemiological study demonstrated that the prevalence of uterine pathogens differs from previous studies [4-6]. Such a difference could be due to geographical area and antecedent antibiotic management of equine populations. However, quinolones and aminoglycosides appear to still be the first therapeutic choice for uterine infections. To conclude, antimicrobial susceptibility monitoring programs are essential to guarantee an effective antimicrobial treatment over time and consequently to enhance the reproductive performance.

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DISCREPANCIES BETWEEN IN-CLINIC ELISA TEST AND HAEMAGGLUTINATION INHIBITION FOR DETECTION OF MATERNALLY DERIVED ANTIBODIES (MDA) FOR PARVOVIRUS IN PUPPIES

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Canine parvovirus (CPV) is one of the most common cause of puppies' mortality [1]; vaccination is known to be the main instrument to protect pups against the infection [2], but the presence of maternally derived antibodies (MDA) interferes with vaccination. MDA titres ≥1:20 in pups can cause a vaccination failure, while MDA titres ≥1:80 confer protection against CPV [3]. MDA can remain until 12 weeks of age or more, waning with a linear decrease in the weeks after birth. Considering the different antibody titres in each pup and the difficulty of estimating the time of the MDA decrease, the most efficient strategy is to administer multiple core vaccinations from 6 to 16 weeks of age, as suggested by WSAVA guidelines [5]. The aim of this study was to evaluate the applicability of an ELISA in-clinic test to assess the parvovirus MDA levels in unvaccinated puppies in their early weeks of age and its agreement with the hemagglutination inhibition (HI) test, which is considered the gold standard for CPV antibody detection and titration. Serum samples of 136 unvaccinated puppies (different breed, size and sex) have been tested, along with sera of 16 regularly vaccinated bitches. Blood was collected by venipuncture of cephalic vein. Each sample was submitted to both tests: the Immunocomb VacciCheck Canine (Biogal/Agrolabo), an in-clinic modified ELISA test, which is licensed to determine the post-vaccination protection against CPV, canine adenoviruses and canine distemper virus, and the haemagglutination inhibition test (HI), which is employed in specialized laboratories for detection of CPV antibodies [4]. Considering in both tests a titer ≥1:80 as indicative of protection against CPV infection, all the regularly vaccinated bitches resulted to be protected by means of both assays, with HI titers resulting always greater than the VacciCheck ones. Conversely, significant discrepancies were obtained for the MDA titers in puppies: dogs with protective titers were 92% by HI and only 40% by VacciCheck testing, with mean titers ≥1:210 and ≥1:70, respectively. Considering the linear decrease of MDA in the first weeks of age, the high HI titers detected in most of the tested puppies are unexpected. In addition, vaccination of some puppies with low ELISA and high HI antibody titers resulted in seroconversion, thus suggesting a possible failure of HI in precisely determining the MDA levels. These findings suggest that in unvaccinated puppies VacciCheck could be more reliable than HI, that remains the choice test in vaccinated and/or infected dogs. If our results are confirmed, the in-clinic assay could be a useful tool for determining protective immunity in unvaccinated puppies and predict the best time to vaccinate them when they have no or non-interfering levels of MDA, thus drastically reducing the rate vaccination failures.

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ITALIAN DATABASE OF EXOTIC ANIMALS' POST-MORTEM RECORDS: A CALL UPON RESEARCHERS TO PARTICIPATE

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Exotic species held in captivity are a well-established phenomenon. Combined, in Italy, there are approximately 16 million exotic animals in private houses, more than 13,000 in zoos, about 2000 in circuses and an unknown number in rescue centres. Public awareness of the potential threat they pose is very limited: a global market, where humans, exotic and domestic animals may commingle, raises the risks of transmission of zoonosis or livestock diffusive diseases. Monitoring morbidity and mortality trends of infectious diseases is pivotal in a "One health" perspective. Zoos and other mixed animal collections are well suited to participation in this kind of surveillance, since they contain dozen of possibly susceptible species that can interact with wildlife, insects, humans and often livestock [1]. A clear example was demonstrated in 1999, when investigation of wild bird mortalities at New York City's Bronx Zoo led to the diagnosis of the first known occurrence of West Nile virus in the western hemisphere [2]. Routine post-mortem examination is an essential tool for detecting not only infectious diseases, but also other conditions that can reduce population health in zoos, such as lesions of rare species or husbandry related issues. Nowadays many zoos collect their data on the *ZIMS for Medical* portal, but a recent survey established that only 25% of the records were useful to determine the cause of death [3]. A portal with similar peculiarities has already been developed by the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta: PSWeb, www.izsto.it/index.php/portale-scientifico-web. This is a container of clinical cases of domestic and wildlife animals examined in depth in order to achieve a diagnosis. Thanks to this experience, aim of the present study is to create a database able to collect, maintain and display in a comprehensive and useful way post-mortem records of exotic animals. To standardize the input data, operative and diagnostic protocols will be provided. Vets of Istituti Zooprofilattici, Veterinary Schools and private will be invited to participate. The database will be free of charges and hosted online. Every user will decide which kind of visibility give to his data. This project will have multiple advantages. The Competent Authority could use the cross-institutional data to visualize trends of diseases. The database could function as an archive for researchers or institution, since data would be exportable at every moment. Moreover, experts will be put on the net to achieve a diagnosis in difficult cases.

Given that the authors would kindly ask all the researchers to participate in refine the database in the next few months. People interested in constructively collaborate may contact the authors by email at the following address cranes@izsto.it. In the long term, the authors encourage all researchers involved in post-mortem examinations of wild animals to actively use this commodity. As previously stressed, the wider the partnership, the more effective the tool will be in disease surveillance.

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MOLECULAR DETECTION OF FELINE MORBILLIVIRUS IN CATS IN NORTHERN ITALY

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Feline morbillivirus (FeMV) is an emerging virus that was first discovered in healthy and diseased stray cats in Hong Kong in 2012. The virus has also been reported within cat populations in Japan, Germany, UK, Turkey, the USA and Brazil and in one cat in Southern Italy [1]. Several reports suggested the epidemiological association of FeMV infection with chronic kidney disease (CKD) in cats. The aim of this study was to investigate the presence, examine the genetic diversity of FeMV and the relationship between FeMV infection and CKD in domestic cats in Lombardy, Northern Italy. A retrospective study on urine or kidney tissues samples from 43 client-owned cats admitted to the Teaching Veterinary Hospital between 2014 and 2017 was performed for FeMV infection and the presence of uropathy. Viral RNA was extracted from cat's samples and a fragment of the paramyxoviral L gene was amplified using a reverse transcription nested PCR assay [2]. PCR products were sequenced for virus identification and comparison. We detected FeMV RNA from three cats (7%). The three FeMV strains showed 99.2-99.6% of nucleotide identity. Phylogenetic analysis of the three FeMV-positive cats showed that the three strains clustered with FeMV strains retrieved from public database. The three FeMV strains from this study were not grouped with the previously described clusters A, B and C of FeMV but formed a distinct cluster of FeMV. The presence of FeMV RNA was observed only in urine or kidney tissues of cats without evidence of CKD. The detection of FeMV was not significantly different between CKD (0/17) and non-CKD groups (3/26). The presence of distinct genotypes of FeMV found in this study is in accordance with previous studies demonstrating that FeMV strains are genetically diverse. In contrast to previously published findings that associated FeMV infection with CKD, we did not observe a clear relationship between the presence of FeMV infection and CKD in the cats from Lombardy, Northern Italy. However, our results confirm recent studies that did not support the hypothesis that FeMV infection is associated with the development of CKD [3]. Further epidemiological studies with larger numbers of cats are needed to determine the pathogenic role of FeMV in cats.

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GENETIC VARIATION AMONG *Staphylococcus aureus* ISOLATES COLLECTED IN SARDINIA FROM HUMAN AND ANIMAL SOURCES

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In the last few decades, many bacterial species have developed resistance to antimicrobial agents that have been commonly used to treat them [1]. *Staphylococcus aureus* is one of the pathogens known to rapidly develop resistance to antimicrobial agents as new antibiotics are introduced [2]. The aim of this study was to characterize *S. aureus* from humans, and to compare their characteristics with isolates from ovine mastitis. A total of 106 *S. aureus* were recovered from different clinical specimens among hospitalized patients in intensive care unit, haematology and orthopaedics over a period of 10 months (January-October 2016) at the Sassari hospital, North Sardinia. In addition, 262 *S. aureus* isolates, collected from ovine mastitis in different provinces of Sardinia, were analysed. Animal isolates were chosen among a bank of *S. aureus* isolates used for the preparation of inactivated autogenous vaccines, according the Italian Ministerial Decree n°287/1994. Susceptibility to 12 antimicrobial agents was tested according to CLSI recommendations [3]. Some resistance genes were detected by PCR assays [4]. A total of 87 (82%) *S. aureus* from humans were resistant to one or more antimicrobials. High rate of resistance against ampicillin/penicillin (AMP/PEN, n=82), erythromycin (ERY, n=20), streptomycin (S, n=20), kanamycin (KAN, n=18) oxacillin (OXA, n=11) and tetracycline (TET, n=6) was observed. Twenty-one (19.8%) isolates were multidrug-resistant (MDR). All oxacillin-resistant *S. aureus* isolates were *mec*A positive. The SCC*mec* type I was detected in 9 out 11 *S. aureus* tested isolates while two isolates were non-typeable. Among the 20 erythromycin-resistant isolates, 11 harboured the *erm*A gene, 6 presented *erm*C gene while 3 were non-typeable. Among the 6 tetracycline resistant isolates, 5 possessed the *tet*K gene while 1 the *tet*M gene. On the contrary, only 17 (6.5%) *S. aureus* isolates from ovine mastitis were resistant with the following distribution: TET (n=9), AMP/PEN (n=3), S (n=3), gentamicin (CN, n=1) and AMP/PEN+TET (n=1). All tetracycline-resistant isolates harboured the *tet*K gene, indicating that resistance to tetracycline is mainly by efflux pumps. All isolates were characterized by *spa* typing and MLST analysis. Human isolates belonged to 44 different sequence types (STs), 16 of them were new. The most frequent STs found were: ST45 (n=11), ST7 (n=10), ST5 (n=9) and ST228 (n=8). The majority of ovine isolates (t1773, t7754 and t1532) was grouped in ST700 and CC130 (n=205) followed by 57 isolates belonged to t2678, t6060, ST 133 and CC133. This study shows that *S. aureus* isolates from human and animal sources are very dissimilar both in terms of antimicrobial susceptibility and phylogenetic clustering. Therefore, it is reasonable to speculate that there is no correlation between *S. aureus* isolates collected from clinical specimens and ovine mastitis.

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A NEW APPROACH TO CONTROLLING MICROBIAL PATHOGENS: *Bdellovibrio* AND LIKE ORGANISMS (BALO)s ISOLATED FROM MARINE AND FRESH WATER AS EFFECTIVE PREDATORS OF PATHOGENIC VIBRIOS AND SALMONELLA

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The microbial world is characterized by mechanisms of competition and predation as well as animal world. Bacterial predators are represented by Bacteriophages, Protists and Predatory Prokariotes: they can be found in every habitats as freshwaters, saltwaters, sewages, soils, plant roots and animal faeces playing an important role in bacterial ecology of natural environments, in the ecological balance and in regulation of bacterial populations in natural ecosystems. Bacteria that share this ecological role are commonly described as "*Bdellovibrio* and like organisms" (BALO), with *Bdellovibrio* being the most studied and best characterized organism in this group even if the related taxonomy is still in developing. *Bdellovibrio*'s consist in Gram negative Deltapropriobacterium able to invading the other Gram negative bacteria periplasm where multiply killing the prey. In face of the rapidly increasing number of multidrug-resistant microbes, several approaches alternative to the classic antibiotics molecules utilization are now under investigation: following this aim, the study tried to adapt natural antagonistic microbial interactions carried out by some isolated BALO's to controlling the growth of some pathogenic bacteria as *Salmonella* and *Vibrio*. In the present study, seawater and freshwater samples were collected to isolate BALOs through double-layered agar plaque assay using *V. parahaemolyticus* and different *Salmonella* species (*S. Napoli*, *S. Derby*, *S.* Typhimurium and the monophasic variant of *S.* Typhimurium, better known as 4, [5], 12:i strain) as prey bacteria. After incubation, plaques that emerge between 3 to 10 days and progressively increase in size were taken to be potential BALOs plaques. Therefore, following plaque purification, molecular and electron microscopy characterization were carried out. To verify the specificity of the isolated BALOs, other gram negative bacteria (24 different strains) were also tested by plaque assay, including *E. coli*, *Ps. aeruginosa* and *V. alginolyticus*. Therefore, the seawater sampling activity lead to the isolation of one strain (named HBXCO1) of *Bacteriovorax* sp. for *V. parahaemolyticus* and one strain of *Bacteriovorax* sp. (named M5) for all the *Salmonella* species that were tested. Both HBXCO1 and M5 appeared as highly specific towards their respective preys. Moreover, in vitro bacteriolytic activity of the bacteriovorax strains (HBXCO1 and M5) was also measured as reduction of prey cell viability (*V. parahaemolyticus* and *Salmonella* species) compared to the predator-free controls. HBXCO1 induced a decrease of *V. parahaemolyticus* viability in all the times considered, with the highest effect on the bacterial count after 24 h whereas M5 was effective to reduce the viability of all the *Salmonella* species, showing a maximum decrease of the mean log count equal to -2.54. The predatory bacteria can be suggested as biological modulators of the bacterial populations promising new perspectives of utilization and representing potential alternatives to antibiotics against infections of Gram-negative bacteria: however, further investigations are needed to translate the in vitro experiences to in vivo models.

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PREVALENCE OF *PSEUDOMONAS AERUGINOSA* AND MULTI-DRUG RESISTANT *PSEUDOMONAS AERUGINOSA* IN HEALTHY CAPTIVE-BRED OPHIDIANS

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Captive breeding of ophidians has considerably expanded in recent years, as they are often considered pet. Snakes could become spreaders of pathogenic bacteria, which can represent a serious threat to themselves and humans. One of the most representative bacteria found in snakes is *Pseudomonas aeruginosa* (PA), an ubiquitous Gram-negative bacterium, frequently present in oral and cloacal flora of healthy ophidians. It can act as opportunistic pathogen of man and animals and cause severe clinical diseases. Furthermore, PA infections treatment is often difficult due to high occurrence of antibiotic resistance [1]. Aim of this work was to evaluate prevalence and antibiotic resistance profiles of PA strains isolated from cloacal flora of healthy captive-bred ophidians, also considering the snakes reproductive status. A total of 419 cloacal swabs were collected from snakes commonly raised as pet animals: *Boidae, Pythonidae* and *Colubridae*. Antimicrobial susceptibility of PA isolates was evaluated through Kirby-Bauer agar diffusion test. Statistical analysis was performed by chi square test. The following antibiotic classes were considered: Aminoglycosides, Carbapenems, Cephalosporins, Fluoroquinolones, Penicillins and β-lactamase inhibitors, Tetracyclines, Monobactams, Phosphonic acids, Polymyxins, Sulfonamides, Phenicols. *Pseudomonas aeruginosa* was isolated from almost sixty percent of the examined samples. About thirty-five percent of the PA isolates were multidrug resistant (MDR-PA). Resistance was observed more frequently against Cephalosporins, Polymyxins and Sulfonamides. No significant differences of PA or MDR-PA prevalence were observed between active (gravid females or soon after spawning) and non-reproducing animals. The high prevalence found with this study confirms that PA can be considered part of the gastrointestinal microbial flora of healthy ophidians. However, the observed overall PA prevalence was lower than expected on the basis of other studies on captive snakes [2,3]. On the other hand, a considerable prevalence of MDR-PA was observed among PA isolates. For the clinical treatment of PA infections, wide resistance against Cephalosporins, Polymyxins and Sulfonamides antibiotic classes suggests a different choice in the routine clinical practice. However, bacteriological examinations and antimicrobial sensitivity testing are always advisable before any antibiotic treatment. As further study, the association of PA and MDR-PA prevalence with breeding conditions will be assessed, in order to evaluate management practice that may limit the risk of dissemination of the bacterium to other animals or humans.

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THE HELMINTH FAUNA OF FALLOW DEER (*DAMA DAMA*) AND RED DEER (*CERVUS ELAPHUS*) BREED IN WILD STATE IN SILA NATIONAL PARK (SOUTHERN ITALY)

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In Calabria there is one of the largest Italian natural parks, the Sila National Park, which covers 73,695 hectares and includes three provinces: Catanzaro, Cosenza and Crotone. Within it there are numerous farms of wild ungulates, in particular Fallow deer (*Dama dama*) and Red deer (*Cervus elaphus*). The internal parasites in wild deer have been reported in many Italian regions [1,2,3,4], but the data concerning the diffusion of parasites in the cervids bred in Calabria are very poor. Therefore, the aim of our research was the acquisition of recent data on the parasitic prevalence of fallow deer and red deer, bred in the Sila National Park, with the aim of giving an input to improve the management and health aspects of wild species raised. The study was conducted between Sep/Dec 2016 in all fallow deer and red deer farms of the Sila National Park. The farms have been identified both with the help of Calabria Verde and Veterinary Services of healthcare company. Following the inspections on the territory we have identified and selected, in the three provinces the active farms: 5 breeding of Fallow deer and 5 of Red deer, for a total of 128 animals: 80 Fallow deer (35 males and 45 females, average age 6 years) and 48 Red deer (15 males and 33 females, average age 5 years). For this study we have taken individual faecal samples (10g) from all animals. We have taken the biological material from the apex of faecal heap soon after the spontaneous defecation. To search for and the count of parasitic elements (eggs, larvas, oocisti) we have used Flotac dual technique [5] with two floating solutions to look for different parasitic elements. Particularly the Fallow deer were positive to Gastrointestinal nematodes 85%, *Paramphistomidae* spp. 17.5%, *Capillaria* spp. 15%, *Strongyloides* spp. 12.5%, Lungworms 10%, *Nematodirus* spp. 8.7% and *Trichuris* spp. 3.7%. While the Red deer were positive to Gastrointestinal nematodes 81.2%, Lungworms 54.2%, *Capillaria* spp. 28.8%, *Strongyloides* spp. 14.6%, *Trichuris* spp. and *Paramphistomidae* spp. 2.1%. These results, with the exception of *Paramphistomidae* spp, are in line with those emerging from other studies. It is important to underline that these trematoda have been found only in one farm where the wild species are bred in promiscuity with the domestic goats, which in our opinion are as a natural reservoir for this parasite. Indeed, carrying out the analyzes on the goats they are also positive to the *Paramphistomidae* confirming the presence of the intermediate host in the farm. Thus, confirming that the deer parasites bred together with sheep and goats, are very similar to small ruminants and therefore the deer share parasitic diseases in addition to the same pastures.

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VECTOR-BORNE PATHOGENS IN WILD CARNIVORES FROM NORTHWESTERN ITALY

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In the last 80 years we assisted to the emergence of more than 300 infectious diseases in humans, with at least 72% of these with a wildlife origin and 23% represented by vector-borne pathogens [1]. Urbanization, human encroachment into nature, increase of wildlife population and climate and habitat changes are some of the most important causes of pathogen re-/emergence [2]. In particular, an increase in synanthropic wild mammals has occurred, and among others, sylvatic carnivores have showed an important role in the life cycle of pathogens with public health and veterinary interest [3]. The aim of the present study was to evaluate the prevalence of tick-borne (*Babesia* spp., *Hepatozoon* spp., *Ehrlichia* spp., *Anaplasma* spp.) and sandfly-transmitted (*Leishmania infantum*) pathogens in carnivore sylvatic species from Northwestern Italy. Sampling was performed from 2009 to 2017, and an overall number of 238 animal spleen samples (155 foxes, 35 wolves and 48 badgers) were collected during necropsies. Extracted DNA was used as template for PCR amplification, with primer sequences and thermal conditions previously described [4,5,6,7]. Positive samples were sequenced and compared with sequences deposited in GenBank. Statistical analysis with Chi square test were performed by using R software [8]. The prevalence of *Babesia* spp. was significative higher ($p<0.01$) in foxes [p= 89.66% (CI 95% 83.66-93.63%)] and badgers [89.58% (CI 95% 77.83-95.47%)] than in wolves [40.00% (CI 95% 25.55-56.43%)]. In contrast, *Hepatozoon* spp. showed higher prevalence ($p<0.01$) in wolves [74.29% (CI 95% 57.93-85.84%)] than in foxes [5.16% (CI 95% 2.64-9.85%)], while none of the badgers tested positive for this parasite. Finally, *L. infantum* and *Anaplasmataceae* showed higher prevalence in badgers [54.17% (CI 95% 40.29-67.42%) and 60.42% (CI 95% 46.31-72.98), respectively] than in the other two species. Sequencing revealed the presence, among others, of *H. canis* and *B. canis*, causes of considerable diseases in dogs. Moreover, zoonotic species like *B. venatorum* were found. Results of this study highlighted the importance of sylvatic species in the life-cycle of vector-borne pathogens and the potential risk of transmission between wildlife and human/domestic animals.

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ASSOCIATION BETWEEN *AELUROSTRONGYLUS ABSTRUSUS* AND PULMONARY HYPERTENSION

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Cat respiratory tract can be affected by different nematodes, being *Aelurostrongylus abstrusus* the most prevalent. Cats infected by *A. abstrusus* may show a plethora of clinical signs varying from asymptomatic to mild or severe respiratory signs which among them some authors included pulmonary hypertension (PH) [1]. However, data available in literature on the association between *A. abstrusus* infection and PH are meagre. Aims of the study were (i) to investigate the association between cat aelusrongylosis and PH, and (ii) to evaluate the correlation between the number of *A. abstrusus* larvae emitted in the faeces and some clinical, echographic and radiological alterations.

Ten cats (7 males, 3 females) naturally infected by *A. abstrusus* with different larval emission rate (from 10 to 5,900 larvae per grams of faeces) were enrolled in the study. Each cat underwent clinical, radiographic and echocardiographic examination and for each one of this test a numeric scoring system was generated. Briefly, each parameter was assessed independently by the other and classified as 0 the absence of alteration, 1 slightly manifested, 2 moderately present and 3 severe alterations. Pearsons's correlation test was applied to assess the correlation between the larval emission rate and clinical, radiological and echographic changes. All the cats were not affected by other concomitant or debilitating diseases, and elder than 4 months (ranging from 4 to 72 months). The commonest non-specific symptoms was lymphoadenopaty (i.e., 6/10), while dyspnoea was observed in 4 out 10 cats. The majority of cats (i.e., 6/10) showed pathological thoracic radiograms; particularly, interstitial-nodular pattern was observed in 3 cats, while alveolar pattern and bronchial pattern were observed in 2 and 1 animals, respectively. Echocardiographic signs of PH were not recorded in examined cats, neither in pediatric patients nor in adult cats, as well as in cats with a high larval emission rate. Any correlation between the larval emission rate and the severity of clinical, radiological and echographic alterations was observed.

PH is a rare condition in cats associated, among other causes, with heartworm infection. However, so far, the pathogenetic mechanisms are still poorly understood. PH has been observed in a kitten infected by *Troglostrongylus brevior* [2] or in two kitten of the same litter affected by *A. abstrusus* [1]. The presence of PH in course of *A. abstrusus* infection could reflect the signs of other concomitant alterations of the respiratory tract that causes vasoconstriction and hypoxemia determining increased pulmonary vascular resistance. Noteworthy, the absence of significant correlation between the parasitic load and the severity of the aforementioned alterations indicates that in the determinism of disease severity a crucial role could be played by other determinant factors such as, animal age and health status as well as other comorbidity.

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EFFICACY OF A SINGLE TREATMENT WITH A TOPICAL APPLICATION OF FLURALANER AGAINST FLEAS AND *OTODECTES CYNOTIS* IN NATURALLY INFESTED CATS

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Fleas (*Ctenocephalides felis*) and ear mites (*Otodectes cynotis*) are parasites of major importance in feline veterinary practice. These ectoparasites can cause direct damage such as discomfort, pruritus, external lesions and allergic reactions [1][2]. Fleas are a constant danger for cat health for their primary pathogenic potential and for the pathogens they transmit with their blood meals or faeces. Furthermore, *Ctenocephalides felis* has been incriminated as the main vector of cat to human transmission of *Bartonella* species, including *Bartonella felis*, the causative agent of cat scratch disease [2]. The ear mite *Otodectes cynotis* is responsible for feline otodectic mange, which is very contagious and takes place directly through contacts between infested and naive animals. Otoacariosis in cats usually complicates with secondary bacterial and fungal infections such as staphylococci and *Malassezia* spp. [3]. The aim of this study was to evaluate the efficacy of a single treatment with a topical application of fluralaner against fleas and *O. cynotis* in naturally infested cats. Thirty-nine cats naturally infested with *C. felis* and *O. cynotis* were enrolled and divided in two groups: fourteen stray cats (Study 1) and twenty-five owned cats (Study 2). Cats were treated topically on Day 0 with fluralaner at a minimum dose of 40 mg/kg body weight. Both clinical, coat and otoscopic examinations were conducted on Days 7, 14, 28, 56, 84. The efficacy was calculated by comparing mean flea/mite counts on Day 0 up to Day 84 of the study. The number of live fleas found on each cat on Day 0 ranged from 1 to more than 30 (arithmetic mean live flea count = 11.9 for Study 1; 14.6 for Study 2) while no live fleas were found by Day 84. The number of live mites found on each cat on Day 0 ranged from 1 to 42 (arithmetic mean live mite count = 6.4 in Study 1; 8.9 in Study 2) while no live mites were found by Day 84. In both studies as of Day 7 no mites and no fleas were found on any cats. At each post-treatment assessment, arithmetic mean flea/mite count reductions from baseline were significant ($P<0.0001$). Topical fluralaner completely eliminated fleas and ear mites from infested cats and was 100% effective against both parasites up to 84 days after treatment. In conclusion, a single topical administration of fluralaner to cats is highly effective for controlling mixed flea and mite infestations, showing effective control for over 3 months post-treatment.

The study protocol was approved by the Animal Care Committee at the Department of Veterinary Medicine and Animal Production, University of Naples Federico II (protocol number 0041919/2017).

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BVDV2 VIRAL COMPARTMENTALIZATION IN PI INFECTED CALF

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Bovine viral diarrhea virus (BVDV), a Pestivirus belonging to the family *Flaviviridae*, represents an economically important pathogen of cattle worldwide. BVDV can be divided into two species (1 and 2) and several subtypes, and classified in two biotypes, cytopathic (CP) or noncytopathic (NCP) based on its ability to show cytopathic effect. BVDV-1 is widely distributed in Italy while BVDV-2 has been detected occasionally. Due to viral establishment before maturation of the fetal immune system, viral proteins are regarded as self-antigens and the PI calf remain immunotolerant to the BVDV strain, allowing for viral replication in all tissues and excretions without host detection. For this reason, PI are considered primary propagators of the virus as they continuously shed large quantities of virus. The aim of this study is to investigate the viral variability in different body compartments and describe the quasispecies diversity of a PI cattle through analysis of three regions of viral genome. The virus was isolated in a previous work from a PI calf identified during a serological investigation in an outbreak of BVDV2 in Piedmont region in Italy in 2014. Blood and tissue samples were collected after euthanasia. The full genome sequence obtained from cell culture and NGS sequencing was used as reference genome. Primers were designed in order to amplify three regions described in literature as more interesting: the highly variable E2 region, the region encompassing NS2-NS3 genes, containing the molecular biotype determinants as it is cleaved in two components in cp strains, and NS5 region, the replicase component. Viral RNA was extracted from different organs (spleen, thymus, retropharingeal and mesenteric lympho-nodes, ileo-cecal valve, CNS, lungs) (Qiagen RNeasy Mini kit), retrotranscribed in single stranded cDNA (Invitrogen SuperScript™ IV First-Strand Synthesis System) and used as template for three PCR amplifications with Platinum HiFi Taq DNA polymerase. Amplified PCR fragments were purified and processed for NGS amplicon sequencing with Nextera XT protocol. Paired-end sequencing was performed using Illumina MiSeq platform. The reads generated were analyzed with a resequencing approach (Geneious software) using the blood full genome sequence as reference in order to evaluate the compartmentalization in different tissues. Relationships among samples were evaluated by phylogenetic and network analyses. Even if the consensus sequences obtained by all the samples were highly similar, quasispecies was described evaluating the presence and the frequency of variants among all the reads. No clear accumulation of mutation was noted, in particular related to the NCP to CP evolution. Among all the analyzed tissues, the thymus shows the highest number of variants. Sequence analysis reveals that almost all the variants raised in the thymus are shared with other organs, but not vice versa. Indeed, some variants are presents only in some organs, attesting virus compartmentalization while the thymus shares variants with all organs. The central function of thymus in BVDV infection is well known and the results support the role of this organ as a reservoir of viral heterogeneity. The quasispecies analyses within PI cattle highlight the complex dynamics of BVDV pathogenesis and compartmentalization into the host and can increase the knowledge about viral evolution of BVDV in PI animals.

CHARACTERIZATION OF AN OUTBREAK OF *BESNOITIA BESNOITI* INFECTION IN NATURALLY INFECTED DAIRY CATTLE

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Bovine besnoitiosis, caused by *Besnoitia besnoiti*, is a (re)emerging disease in Europe, including Italy [1]. However, its economic impact is scarcely considered and generally underestimated and there are still little studied aspects concerning both the parasite and the disease. Following a natural outbreak of besnoitiosis in a dairy herd, a study was planned to characterize *B. besnoiti* infection in cattle through a multidisciplinary approach. Suspicious abortions and clinical cases of besnoitiosis were reported in a dairy farm (September 2017, Northern Italy) housing 216 Holstein Friesian cattle. Blood samples were collected; haematological and serological analyses for *B. besnoiti* antibodies using ELISA (ID Screen® Besnoitia Indirect 2.0, IDVET) and confirmatory Western Blot were performed [2]. Histology and molecular (endpoint PCR targeting ITS-1 region [3] and sequencing) analyses of tissues from a slaughtered cow with chronic besnoitiosis were carried out. Out of 59 animals resulted positive to ELISA, 50 (23%) were confirmed by Western Blot. *B. besnoiti* prevalence was higher in cows (41%) than in calves (12%); any heifer did not result positive to the infection. Considering haematological parameters, a significant shift in the differential leucocyte formula from lymphocyte to granulocyte was recorded in infected cows (Mean±S.D.: L=46.1±18.4, G=53.9±18.4) if compared to negative animals (Student's T-test, p=0.012). This finding could be helpful in clinical diagnosis, treatment and control of besnoitiosis providing information on infection pathogenesis and subsequent immune response. Histology revealed a high load of *B. besnoiti* tissue cysts in skin, vulva, muzzle, sclera, eyelid, respiratory tract, emphasizing the possibility of parasite transmission through direct contact among animals. *B. besnoiti* was confirmed by PCR in other organs (heart, liver, aorta wall, tonsils) and especially in ovary, uterus and vulva, suggesting that the infection could affect cows' fertility and pregnancy. Parasite DNA was also found in masseters posing an important question for food security. Indeed, the presence of *B. besnoiti* in muscles has never been explored, being its impact on cattle health mainly considered restricted to the skin. Even if *B. besnoiti* is not considered zoonotic, further studies are needed to clarify if it could localize in other muscles. The study suggests that to investigate the dynamics of bovine besnoitiosis is mandatory associate clinical and various laboratory tests; the genetic characterization of autochthonous isolates of the parasite and its eventual correlation with the disease outcome should also be included.

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NEOSPOROSIS IN DAIRY CATTLE: SEROLOGICAL INVESTIGATION IN TWO STUDY-HERDS

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Neospora caninum is recognized as a one of the major cause of infectious reproductive failure in cattle, possibly resulting in abortion and thus determining economic losses to both dairy and beef industries. With the aim of characterizing the abortion patterns in dairy cattle, an epidemiological study was designed in two herds recruited as case-study. Both selected farms, located in Lombardy region, performed genetic improvement of Holstein Friesians and reported cases of abortions ascribable to *N. caninum*. Blood samples were collected from 540 animals and analyzed using a commercial indirect immunofluorescent antibody test (IFAT) to determine the presence of IgG antibodies against *N. caninum* in cattle serum (MegaFLUO® NEOSPORA, Mega CorDiagnostik, Austria), considering 1:160 dilution as the cut-off and determining the end-point antibody titre (AT). Overall, 94 animals scored positive to *N. caninum* (15.5% and 18.5% in Farm.01 and Farm.02), with differences between the farms concerning AT (p-value=0.04): indeed, 38.7% and 15.6% of seropositive animals showed a >1:640 titer in Farm.01 and Farm.02. The determination of AT is a useful tool to identify cows at risk of abortions [1]. Indeed, a different pattern of abortion was depicted in the two investigated Farms. Within January 2017 and April 2018, 4 and 5 abortions from seropositive cows were recorded in Farm.01 and Farm.02, respectively, corresponding to the 28.5% and 19.2% of all recorded abortions. Abortions in Farm.01 occurred in the second trimester of gestation and cows, also used as recipient for embryo transfer, showed higher antibody titer (1:320 and 1:640) than positive aborting cows of Farm.02. In the latter farm, indeed, two cows aborted in the first trimester showing an antibody titer of 1:160, while three cows aborted in the in the second trimester with an antibody titer of 1:160 and 1:320. Subsequently, data on fertility and production were considered. The number of insemination necessary to get an animal pregnant resulted higher in seropositive animals (1.97) than seronegative animals (1.93) in both farms, as previously reported [2]. Similarly, in Farm.01 the number of day in lactation of not-pregnant cows resulted higher in seropositive (133.4) than seronegative animals (167.7). Although the association between *N. caninum* infection and milk production is still unclear [3], in Farm.01 305 days mature equivalent milk yields (MEM) was lower in seropositive (11838.94) than seronegative cows (12274.88). Concluding, neosoporosis should be always considered in dairy farms devoted to genetic improvement, regularly testing animals before fecundation or embryo transfer [3]. Serological screening with IFAT proved to be a useful diagnostic tool to identify not only herd seroprevalence but also to identify, through the determination of AT, animals at high risk of abortion.

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FIRST DETECTION OF ANTI-*BESNOITIA* SPP. SPECIFIC ANTIBODIES AND OTHER SARCOCYSTIDAE INFECTIONS IN HORSES AND DONKEYS IN ITALY

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Among *Apicomplexa* protozoa infecting equids, *Besnoitia* spp., *Toxoplasma gondii* and *Neospora* spp. represent important issues from a sanitary and zootechnical viewpoint. Besnoitiosis in equids, caused by *B. bennetti*, was historically limited to donkeys and horses in Africa; however, reports have suggested that besnoitiosis may be an emerging disease of donkeys in the United States [1]. *Besnoitia* spp. specific antibodies were detected for the first time in Europe in equids from areas where bovine besnoitiosis is endemic in Spain [2]. Anti-*T. gondii* antibodies were demonstrated in both species in serological surveys worldwide, although there is not any confirmed report of clinical disease in equids [3]. On the contrary, *Neospora hughesi* is recognized as an etiological agent of the equine protozoal myeloencephalitis (EPM), an important neurological disease of horses [4]. However, only scarce epidemiological data are available on the spread of the infections in horses and donkeys in Europe. Therefore, a serosurvey was planned to estimate the prevalence of *Besnoitia* spp., *T. gondii* and *Neospora* spp. infections in Italian equids. Serum samples from 268 horses and 18 donkeys raised in Italy were collected and serologically analyzed to detect anti-*Besnoitia* spp., *T. gondii* and *Neospora* spp. antibodies: an approach based on an initial screening by in-house ELISA followed by a confirmatory Western Blot was used. Considering the results obtained by ELISA, the presence of anti-*Besnoitia* spp. antibodies was demonstrated in 21 horses and in four donkeys. Antibodies against T. gondii and Neospora spp. were detected in 19 and 22 horses, respectively; any donkey did not result seropositive for these parasites. Considering Western Blot results, seropositivity against *Besnoitia* spp. was confirmed in six equids (P=2.1%), specifically in four donkeys (P=22.2%) and two horses (P=0.7%). Further, Western Blot analysis confirmed seropositivity to *T. gondii* in ten horses (P=3.7%); antibodies anti-*Neospora* spp. were detected by Western Blot only in one horse (P=0.4%) contemporary infected also by *T. gondii*. The study reported the first detection of *Besnoitia* spp. specific antibodies in horses and donkeys from Italy and confirmed the circulation of *Besnoitia* spp. among equids in Europe only recently evidenced. Low prevalence of *T. gondii* and *Neospora* spp. in horses raised in Italy was also recorded. The results emphasized both the risk for humans to acquire toxoplasmosis through horse meat consumption and the need of further studies to investigate the epidemiology and also for the isolation and molecular characterization of the species of *Sarcocystidae* infecting Italian equids.

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TICK-BORNE PATHOGENS IN CATTLE: A MULTICENTRIC SEROEPIDEMIOLOGICAL SURVEY IN ITALIAN BEEF CATTLE

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In cattle farming, tick-borne pathogens (TBPs) represent an important sanitary and zootechnical issue, mainly in tropical and subtropical areas. Nevertheless, reported cases are increasing also in temperate zones in Europe [1]. To update information on selected TBPs (*Anaplasma marginale, Babesia bigemina* and *Coxiella burnetii*) in beef cattle raised in Italy, a multicentric epidemiological survey was carried out in four Northern regions (Lombardy, Piedmont, Trentino Alto Adige and Liguria regions) and in Sardinia. In total, 1516 individual serum samples were collected in 63 farms, with an average of 28 animals per surveyed herd. Samples included 14 beef breeds and cross-breeds, and animals of varying age from three to 240 months. Sera were analyzed with commercial ELISAs for the detection of antibodies against the selected three pathogens. Individual and herd data were analyzed by means of generalized linear models (GLMs), using SPSS v.19.0 (IBM, USA). Prevalence of *A. marginale* was 47.7%, with the highest values detected in in Liguria (76.8%) and Sardinia (72.8%) (p-value=0.0001). Similarly, the highest prevalence of *B. bigemina* (overall prevalence: 61.5%) was found in Liguria (71.2%) and Sardinia (66.7%) (p-value=0.0001). In Northern regions, the majority of seroreactive animals to *A. marginale* and *B. bigemina* (45.1% and 86.5%, respectively) had been imported from other Italian regions or other European countries, while all autochthonous positive animals had been raised in the Northern Apennines. On the ohter hand, higher *C. burnetii* seroprevalence values were registered in cattle from Lombardy (6.2%) and Piedmont (12.4%) (p-value=0.0001), while prevalence ranged from 2 to 2.6% in the other regions (overall prevalence: 4.5%). *A. marginale* and *B. bigemina* co-infection was recorded in 31.8% of surveyed cattle, while co-infection with *C. burnetii* was recorded in 0.9% and 2.8% of cases, respectively. Increasing age was associated with a higher risk of infection (p-value=0.0001; OR=1.008-1.013). Results of the present study confirm exposure to selected TBPs in the surveyed cattle, with obvious area- and origin-related differences likely associated with the distribution range and the abundance of vector ticks (mainly *Rhipicephalus* spp. for *A. marginale* and *B. bigemina* and *Ixodes* spp. for *C. burnetii*) [2]. In a global warming context and with the increase of animal trade and exchanges between countries, TBPs should deserve more attention in sanitary programs in cattle farming [3].

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SIRNA: NEW STRATEGY FOR THE INFLUENZA A VIRUS CONTROL

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The viral recombination of influenza A virus is an impending problem, since animals act as a reservoir for the transmission of the pathology to humans. In particular, the animal species have a relevant role in the potential recombination between different influenza A virus strains. Hence, there is the need to create new molecules that can be an alternative to vaccination plans. RNA interference (RNAi) may play a key role because several studies highlight how short interfering RNAs (siRNAs) act as antiviral agents [1]. In this study, siRNA cellular clones [2] were obtained on MDCK cell line, permissive against influenza A virus. The genomic region coding for the viral nucleoprotein (NP), a well-conserved sequence among the different viral strains, was examined. The NP sequences were aligned and the strains containing siRNA target were selected. Three sequences (NP6, NP7, NP8) showed the greatest effectiveness. The siRNA clones were infected and the ability of siRNAs to inhibit the viral replication were evaluated on five avian (1067/1999 H7N1, 1082/1999 H7N1, 22A/1998 H5N9, 251/2003 H7N3, 8000/2002 H7N3) and five human (9/2014 H1N1, 79/2014 H1N1, 56/2014 H1N1, 53/2013 H1N1, 224/2015 H1N1) influenza A virus strains. To evaluate the viral titre of the different strains, the MDCK cell line was prepared in 96-well plates, infected with serial dilutions of the viruses (from 10-1 to 10-7) and incubated at 37°C + 5% CO_2 for 5 days. The viral titre was determined on the basis of the cytopathic effect observed. At the end of the incubation period, the cultures were frozen at -80°C and thawed to obtain cell cryolase. The clones showed a different sensitivity to the viral strains analyzed. Inhibition results were confirmed by hemagglutination (HA) and Real Time PCR (RT-PCR) methods. In the experiments performed, the siRNA antiviral potency appears relevant but different for each strain examined. Moreover, preliminary data related to siRNA porduction in E. coli strains (data not shown), gives encouraging results to proceed for a large-scale production [3]. However, in vivo studies are necessary to confirm the antiviral effect of siRNAs as an alternative "ready to use" therapy.

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SEVERE CLINICAL FINDINGS IN A DOG INFECTED BY *ANGIOSTRONGYLUS VASORUM*

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Angiostrongylus vasorum is a metastrongyloid nematode that primarily infects *Canidae*, especially domestic dogs and the common fox. The life cycle involves the gastropods (slugs and snails) acting as intermediate hosts. The most common clinical abnormalities are cough and dyspnea, but in some dogs the respiratory disorders may be absent but signs of other organ systems may dominate like ophtahalmological disorders, immune-mediated thrombocytopenia or disseminated intravascular coagulation [1]. The aim of this study was to describe severe clinical findings in a dog infected by *A.* vasorum. A ten months old female, intact, German Shepherd dog was referred for apathy and respiratory distress in our veterinary hospital. At clinical examination, poor general condition, dehydration, dyspnoea and blindness were present. At radiography, a focal pulmonary mixed pattern opacity, alveolar and bronchial, a moderate pneumothorax and a slight cranial mediastinum enlargement were visible. Ophthalmic examination revealed moderate buphthalmos with marked palpebral and ocular hypertension. Ocular ultrasonography revealed exudative retinal detachment, lens subluxation and, in the right eye, a vitreal linear foreign body compatible with intraocular parasite. Thorax ultrasonography showed in the right hemithorax several non-vascularized subpleural nodules (ø <1 cm) located in the caudodorsal region of the lung. No two-dimensional echocardiographic changes, and no Doppler findings suggestive of relevant pulmonary hypertension (PH) were observed. Routine coagulation parameters (PT, APTT and Fibrinogen) were delayed. Based on findings obtained, a suspect of parasitic pneumonia was made. At FLOTAC faecal examination were detected first stage larvae (LPG=324) of *A. vasorum* and the antigen test resulted as well positive. One week after first examination, the dog showed bilateral retinal atrophy, loss of retinal blood vessels and optic atrophy. Interestingly, infectious load and association with severe radiographic changes do not develop pulmonary hypertension. Alternatively, the severity of immunologic response to *A. vasorum* may be an important factor, as the immune-mediated uveitis found in the present study lead to an irreversible lesion. Increasing awareness of the importance of alternative migratory routes of *A. vasorum* in dog will improve our current understanding of the diagnosis and clinical follow-up of this infection. Therefore, veterinary practitioners should include canine angiostrongylosis in the differential diagnosis of ocular diseases.

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LEISHMANIA INFANTUM, DIROFILARIA IMMITIS AND ANGIOSTRONGYLUS VASORUM: A SEROLOGICAL STUDY IN DOGS FROM NORTHERN ITALY

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Dogs are exposed to several parasitic infections, often transmitted by vectors or intermediate hosts. In some cases, they can lead to severe diseases (acute or chronic) that are both life-threatening for dogs and a diagnostic/therapeutic challenge for clinicians. A few of them are considered (re)-emerging and their appearance could be influenced by climate changing or movement of animals from an area to another of the country. Particularly, *Leishmania infantum* (LI) *and Angiostrongylus vasorum* (AV) could be included in the category of (re)-emerging parasites of dogs, then a study was planned in order to primarily update information on their spread in northern Italy. In addition, *Dirofilaria immitis* (DI) was surveyed with a dual objective to update the epidemiological data about this parasite in this area and evaluate cross-reactions with AV that may give a misleading diagnosis with dirofilariosis. From March 2015 to February 2016 blood samples were collected from 207 healthy dogs randomly chosen from veterinary clinics and kennels located in Lombardy region. Sixty-two dogs were sampled during spring, 94 in summer, 29 in autumn and 22 in winter. The sampling included companion owned dogs (108/207), kennel dogs (83/207) and working dogs (16/207). Dog serum samples were analyzed by IFAT for determination of the presence of anti-LI antibodies, using MHOM/TN/80/IPT1 as a whole-parasite antigen fixed on multi-spot slides, and fluorescent-labeled anti-canine gamma globulin. Circulating antigens of DI and AV were detected by commercial kits (ZOETIS DiroCHEK Heartworm Antigen Test and IDEXX Angio Detect Test, respectively). Individual data of dogs and date of sampling were collected. Ten dogs out of 207 (4.83%) showed serological positivity for LI (presenting a maximum reaction titer ≥1:80); circulating antigens of DI and AV were detected in 20 (9.67%) and in one dog (0.48%), respectively. Coinfection was found in only one dog, tested positive for both LI and DI. Observed prevalence of LI and DI presented different seasonal dynamics. Lowest LI prevalence was detected in spring (1.61%), followed by the highest prevalence of summer 2015 (7.45%); intermediate prevalences were observed in autumn (3.45%) and winter (4.55%). Regarding DI, the prevalence was higher in spring (16.13%) and a progressive decrease was observed being the prevalence values equal to 8.51%, in summer, 6.90% in autumn and 0% in winter. Distribution of seropositivity to LI an DI varied according to dog attitude. Highest prevalence of LI was observed in kennel (8.43%) and working dogs (6.25%), while in companion dogs prevalence reached only 1.85%. DI appeared to be most prevalent in working dogs (18.75%), while prevalence of infection in companion and kennel dogs was similar (9.26% and 8.43%, respectively). The dog seropositive to AV was a crossbreed male kennel dog from Milano province, 1 y.o., sampled in summer. The study highlighted the exposure of selected parasites in dogs in northern Italy. LI and DI observed prevalence varied in companion, working and kennel dogs, and in different sampling seasons. In present study, no cross-reaction between AV and DI serological tests was observed.

EVALUATION OF THE INFLUENCE OF GEOGRAPHICAL INDICATION DISCIPLINARIES ON GASTROINTESTINAL PARASITE BURDEN IN CATTLE

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With more than 1 million dairy cattle [1], Northern Italy is the most important breeding area in Italy. It is characterized by different breeding systems that range from extensive to intensive breeding with geographical indication disciplinaries. Gastrointestinal (GI) parasites are some of the most important pathogens affecting dairy cows [2], and breeding system has showed to influence the parasite load and the epidemiology. The aim of the present study was to investigate the burden of parasitic infections in dairy cows and heifers from intensive breeding farms from three different areas: Parmigiano-Reggiano area, Grana Padano area and Piedmont region. The analyzed areas have three different breeding systems: for Parmigiano, grass and hay must represent the 50% of ratio, corn silage is not allowed and cows are kept indoor; in Grana Padano area, cows are kept indoor but corn silage is allowed; in Piedmont region, cattle can feed on grass and are allowed to graze on pasture. Forty-five farms equally divided among the three studied areas (15 for Parmigiano-Reggiano area, 15 for Grana Padano and 15 for Piedmont region) were analyzed. Overall, faecal samples were collected from 1304 animals (with a mean number of 15 dairy cows and 14 heifers from each stable). Faecal egg count (FEC) was performed on each sample by using MINI-FLOTAC [3] with zinc sulphate flotation solution in order to detect and quantify GI nematode eggs, *Cestoda* oncospheres and *Eimeria* oocysts. Parasite load on pasture was monitored monthly from an entire vegetative season, from April to November 2016. Briefly, larvae quantification was performed on 1 m^2 of freshly collected grass. Results were normalized on grass dry weight and expressed as larvae/kg of dry weight. Generalized linear mixed models were used to assess risk factors for parasite infections. Results showed a GI nematode prevalence of 10.35% (CI95% 8.81–12.12%) in 18 of the sampled farms, with a mean parasite load of 2.52 epg (eggs per gram) (median=0; sd=15.27). Cattle from Parmigiano-Reggiano area were significantly less infected by GI nematodes than animals from Piedmont region, while cows and heifers from the former were significantly more infected with *Moniezia* spp. than the other areas, with a prevalence of 1.99% (CI95%1.36-2.91). *Eimeria* spp. was recorded with an overall prevalence of 23.54% (CI95% 21.32-25.92) from 97.78% of the sampled farms (44/45). Heifers were significantly more infected than cows at all sites while higher prevalence was recorded in the Grana Padano area compared to Parmigiano-Reggiano and Piedmont ($p<0.001$). Larvae seasonal trend on pastures showed a peak in mid-June (93.05 larvae/kg dry weight) and again in mid-October (73.04 larvae/kg dry weight). This study showed that also dairy cattle from intensive breeding farms can be infected by GI parasites, even with low prevalence and burden. Different breeding systems show specific parasite risks linked with the use of fresh grass or hay and give helpful indications to farmers and veterinarians on how to maximize efficiency and efficacy of parasite management.

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DEVELOPMENT AND VALIDATION OF A LATERAL FLOW IMMUNOASSAY FOR THE RAPID DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS

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Canine visceral leishmaniasis (CVL) is a zoonotic infectious disease caused by *L. infantum* with severe impact on humans and animals. Infection is transmitted by phlebotomine sand-flies and several domestic and wild mammals act as reservoirs for the infection. Although *Leishmania* amastigotes parasites more than 70 vertebrate hosts, domestic dogs are considered the main reservoir for human infection. For this reason, the prompt detection of infected hosts is crucial for the prevention and control of the spread of the disease and of transmission to humans. CVL can be diagnosed by combining clinical and epidemiological parameters with parasitological, serological or molecular methods. Serology is preferred because the detection of anti-leishmanial antibodies is commonly realized by three techniques: the immunofluorescent antibody test (IFAT), the enzyme-linked immunosorbent assay (ELISA) and the lateral-flow immunoassay (LFIA). The IFAT is considered as the reference method in dogs and is used as the reference test for the validation of new diagnostic methods, though data on its diagnostic sensitivity and specificity are controversial. The ELISA is also very sensitive and specific, with the advantage of easier standardization. Both IFAT and ELISA provide quantitative results, defined as the antibody titer. However, the rapid and cost-effective detection of infected dogs is a key point in the control of infection and infection transmission. LFIA is the most popular diagnostic tool for rapid onsite assays. Its main advantage is represented by its perfect match with ASSURED criteria required for point-of-care testing (Affordable, Sensitive, Specific, User-friendly, Rapid/Robust, Equipment-free and Deliverable to end users). Nevertheless, LFIA only provide qualitative results need to be completed by quantitative information to ensure the correct management of the disease. This study describes a rapid and portable diagnostic tool for CVL diagnosis based on the LFIA technology. The specific recognition element is represented by a recombinant chimeric antigen, comprising three *Leishmania* antigens, which has been shown to be highly specific for CVL. The signal reporter is constituted by staphylococcal protein A labelled with gold nanoparticles that are used as coloured probes for the visual interpretation of the qualitative result. A total of 167 canine sera were used in the study: 37 serum samples were collected from an endemic region, while 130 samples belonged on non-endemic regions. Canine sera were characterized by analyzing them through more than one reference method (IFAT, ELISA, PCR and WB). Moreover, were tested 9 feline sera and two fox sera. The LFIA shows excellent diagnostic sensitivity (98.4%, 95% CI 91.47-99.96%), specificity (98.9%, 95% CI 94.15-99.97%) and agreement with serological reference methods for diagnosing canine CVL. The long-term stability of the LFIA device was confirmed for 6 months storage at room temperature and 4°C and the qualitative response was not affected by limited thermal stress. The use of the broad-specific protein A enables the versatile application of the LFIA to CVL diagnosis in dogs, which represent the main reservoir for human infection, and in other mammals assuring the opportunity of efficiently controlling the spreading of the infection.

SMALL RUMINANT LENTIVIRUS ISOLATION AND CHARACTERIZATION

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Small Ruminant Lentiviruses (SRLV) are heterogeneous group of viruses that cause chronic, multisystemic infections in sheep and goats. Although historically ascribed to two different viruses, strictly associated with specific clinical features and host, Caprine Arthritis Encephalitis virus (CAEV) and Visna Maedi virus (MVV) are now considered host adapted but not host specific. The group includes at least 4 genotypes and several subtypes and shows high variability of both in vivo and in vitro properties.
Many studies demonstrated that this viral heterogeneity can influence the viral-host interaction, the cell tropism, the biological properties and, not least, the diagnostic test results. Although different cell types may be infected, the major in vivo tropism of SRLV is for the monocyte/macrophages and dendritic cells while in vitro high virulence viral strains production can be obtained also in fibro-epithelial synovial and choroid plexus cells where they show the classical cytopathic effect of cell fusion (syncytia). Nevertheless, several viral strains, characterized by low pathogenic effects, showed a limited ability to replicate in fibroblast cells as well as to produce syncytia, leading to an underestimation of isolation success. Keeping in mind the heterogeneity of in vitro properties of SRLV we developed a fast and effective method for SRLV full genome characterization from spleen explants after cell culture isolation.
Blood and spleen samples were collected from 42 adult animals (16 sheep and 26 goats) during regular slaughter in two different slaughterhouses in Northern Italy. Blood samples were tested with a commercial kit for antibody screening and genotyping. Spleen derived macrophage culture was maintained over 5 passages and monitored for the appearance of cytopathic effect and Reverse Transcriptase Activity (RTA). Total RNA was extracted from the concentrated supernatant with the highest value of RTA and reverse transcribed as double stranded cDNA. The samples were sequenced with a Next Generation Sequencing (NGS) approach, using Nextera XT protocol and Illumina MiSeq platform. Data analysis was conducted following two parallel approaches, resequencing and de-novo assembling, with Geneious and Velvet softwares respectively. Twenty-two spleen explants allowed viral isolation and full genome sequencing. All the samples showed high variability of in vitro behavior. Only 13 RTA positive samples showed CPE on overgrowing fibroblastic-like cells. A positive correlation was observed between the phylogroup A8 and the absence of CPE in culture while all strains belonging to subtype B1 and B2 were fusogenic in vitro, as well as subtype A9 in a sheep and a novel subtype A in a goat. In conclusion the proposed method proved to be a fast and economically feasible approach for viral full genome characterization as well as for biological and in vitro properties study.
Despite the limited sampling area, it allows to detect both pathogenic subgroup B1 and B2, putative nonpathogenic A8 in goats, an additional A9 isolate in sheep and a novel A subgroup in goat. The success in virus isolation with this method is based on the association of three features: the choice of the spleen as target tissue for virus isolation, the use of RTA as sensitive method to detect viral replication and the use of a high throughput technology for genome sequencing.

ENDOPARASITES IN DAIRY CATTLE IN NORTHERN ITALY: STILL A HEALTH PROBLEM?

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Cattle are usually infected by gastro-intestinal nematode (GIN) infections that are important cause of production losses in pasture-based cattle production worldwide. Moreover, *Giardia* sp., *Cryptosporidium* sp. and *Eimeria* sp. are the most important protozoan parasites causing gastrointestinal problems in calves. A study was planned to characterize the parasitological status of dairy cattle in the largest area of cattle breeding in Italy in order to update epidemiological data and analyze risk factors. From March 2017 to August 2017, 10 dairy farms in Northern Italy were surveyed and 305 faecal samples from cattle were collected. The following categories were sampled: calves <6 months (n=94), heifers (n=40), pregnant heifers (n=49) and cows (n=122). Information on animal and management were collected by a questionnaire at sampling. Quali-quantitative copromicroscopic analysis was performed by a FLOTAC Dual Technique® (FDT) using two flotation solutions (NaCl, sg=1200 and ZnSO4, sg=1350) [1]. EPG (eggs/g of faeces) and OPG (oocysts/g of faeces) were determined; the presence of *Giardia* cysts was also recorded. An immunoenzymatic assay to detect *Cryptosporidium* coproantigens (RIDA®QUICK CRYPTOSPORIDIUM, R-Biopharm) were only used to test calves. *Eimeria, Strongylida* and *Trichuris* were the recovered taxa by the FDT showing the prevalence values of 47.9% (146/305), 16% (49/305) and 2.8% (10/305) respectively. *Giardia* cysts were only found on calves 30 days-<6 months aged (5/114; 4.4%); any heifers resulted infected. Heifers and pregnant heifers showed both the highest prevalence (64.1% and 92.5% respectively) and OPG values (77.21 and 112.5 respectively) for *Eimeria* spp. This finding could be related to prophylactic interventions against these protozoa that limit their circulation in the first months of life; the reappearance of *Eimeria* with higher prevalence values in both heifer categories is probably due to management causes (housing type, crowding, etc.) that promote the cycle of these parasites. Surprisingly *Strongylida* were found in 80% of surveyed farms even if their EPG values were very low (medium 0.99; min-max 0-24); in a farm the circulation of *Nematodirus* was demonstrated. No trematode eggs were recovered. *Cryptosporidium* coproantigens were detected in a few calves (7/94, 7.5%). The majority of animals infected by *Strongylida* and *Nematodirus* were bred indoor. *Strongylida* eggs were frequently found in lactating cows (49/122, 40.1%). Risk factors analysis revelead that the summer pasture is a significant risk factor both for *Strongylida* (OR=2.879) and *Eimeria* (OR=4.598). Regarding *Eimeria* spp. private water supplies were risk factors compared to public water supplies (OR=1.858). On the contrary, the unifeed ration represented a protective factor against the *Strongylida* infection (OR=0.179). The study revealed the presence of sub-clinical gastrointestinal nematode infections in dairy cattle from intensive farms that should warrants attention by veterinarians; since, a significant increase in milk yield was observed in infected heifers with subclinical infections compared with the control cattle and a treatment could be considered especially in high milk producing cows [2].

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MOLECULAR DETECTION OF BOVINE PAPILLOMAVIRUS IN THE PLACENTA AND BLOOD OF HEALTHY MARES AND RESPECTIVE FOALS

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Sarcoids are cutaneous fibroblastic tumours representing up to 90% of all skin neoplasms in horses worldwide [1]. Even if non metastasizing, sarcoids can become very aggressive locally, in addition no effective therapy is available [2]. Their etiology is considered multifactorial, but infection with Bovine Papillomaviruses type 1 and 2 (BPV-1,-2) has been implicated as major factor in the pathogenesis. The mode of BPV transmission within and between animals is still unclear: the transmission of the virus can occur horizontally between animals in close contact [3], by stable management practices [4] and flies [5], while scarce information is available about vertical transmission. For this reason, we investigated blood and placenta of mares and respective foals for the presence of the virus through DNA detection. Twelve pregnant mares, admitted to the Equine Perinatology Unit of the Department of Veterinary Medical Sciences of the University of Bologna, were involved in the study based on the absence of clinical signs or anamnestic history of sarcoid. K3EDTA blood, from mothers and foals before suckling, as well as a portion of placenta were collected after birth. DNA was extracted after digestion and separation of PBMCS from placenta and whole blood respectively. Qualitative PCR targeting a fragment of the L1 gene was performed on every sample. Our results give support to the hypothesis that the infection can be asymptomatic, with virus remaining latent in PBMCs of healthy subjects [6] as showed by 7/12 L1 positive mothers that gave birth to positive foals, in addition, the placenta of the subjects also tested positive. Sequencing of obtained amplicons demonstrated the presence of BPV-1, BPV-2 and BPV-1 EqSarc, where nucleotide substitutions were identified after alignment with reference sequences. Interestingly, the viral type was the same between mare and foal only in three cases, positive for BPV-1. Our data contribute to further knowledge on epidemiology and tropism of the BPVs and provide a starting point to better understand the transmission routes of the virus.

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SEROLOGICAL AND MOLECULAR PREVALENCE OF ANAPLASMOSIS AND PIROPLASMOSIS IN DONKEYS AND MULES FROM SICILY

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Anaplasma phagocytophilum, Theileria equi and *Babesia caballi* are tick borne parasites which cause vector borne diseases among equids worldwide. Anaplasmosis and piroplasmosis are endemic diseases in Sicily causing important losses. However, few data are available from donkeys and mules. The present study aimed to determine the prevalence of these pathogens in donkeys and mules from Sicily (southern Italy) over a period of 6 years.

For this purpose, equid samples collected from 2012 to 2017 and analyzed at the National Reference Center for Anaplasma, Babesia, Rickettsia, and Theileria (CRABaRT) and the OIE Reference Laboratory for Theileriosis and Babesiosis were included in the retrospective study. Sera and EDTA blood specimens collected from 449 donkeys and 3 mules living in Sicily were analyzed to detect IgG antibodies and DNA of *A. phagocytophilum, T. equi* and *B. caballi* using respectively commercially available IFAT kits and PCRs end point as previously described [1-2].

Overall, 1257 serological assays (447 tests for *A. phagocytophilum*, 405 tests for both *T. equi* and *B. caballi*) and 509 molecular assays (201 tests for *A. phagocytophilum*, 154 tests for both *T. equi* and *B. caballi*) were performed. Serologically, *T. equi* was the pathogen more frequently found with a prevalence of 34.32%, followed by *A. phagocytoplylum* (9.84%) and *B. caballi* (2.72%) while the *T. equi* DNA was the only one detected in the analyzed samples with a rate of 18.18%. No specimen was found positive for *B. caballi* and for *A. phagocytophilum* by PCR.

In agreement with data previously reported in Italy from horses [3] and in other countries from equids [4-5], our data show *T. equi* is the most prevalent pathogen in donkeys and mules from Sicily and is the only vector borne pathogen detected by molecular techniques. This study reports the circulation of the three pathogens in donkeys and mules and the presence of *T. equi* in 1/5 of the animals investigated.

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SICUREZZA ALIMENTARE

MODELING THE GROWTH OF *Salmonella* SPP. IN ITALIAN FRESH RICOTTA

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When post-process contamination occurs, the physical-chemical characteristics of fresh ricotta, e.g. high moisture, low salt content and pH values close to neutrality, make this product a suitable substrate for the growth of several pathogenic microorganisms, including *Salmonella* spp. Some models able to predict the behaviour of *Salmonella* spp. in many specific substrates (broth, chicken, lettuce, and pork) have been already developed, but a validated model to be applied for fresh ricotta is still not available. In this study, a cardinal parameter model was developed and successfully validated for the growth of *Salmonella* spp. in several brands of Italian fresh ricotta. For the model development, ricotta from a major Italian producer was obtained and growth data for *Salmonella* spp. were generated in three challenge tests at static temperatures (10°C to 20°C), by inoculating ricotta with a mixture of *Salmonella* spp. isolates. For the validation step, samples from the same producer and three other producers were obtained and four challenge tests at static (13°C to 20°C) and dynamic temperature profiles (10°C-14°C) were performed. The gamma type cardinal parameter secondary model covering the influence of temperature [1], pH [2] and organic acids [3] was considered. In particular, the concentrations of the undissociated form of lactic, citric and acetic acid, calculated as suggested by Mejlholm and Dalgaard [4], were used in the model equation. The validation of the model included an assessment of both the ability to predict maximum specific growth rate (μ_max) using two approaches: bias (Bf) and accuracy factor (Af), and the acceptable simulation zone (ASZ). The model for *Salmonella* spp. showed good performances with Bf of 1.10, and an average of 91% of observations within the ASZ. Comparing the performances of other existing models for this pathogen, a general underprediction of the growth rate when applied to ricotta was evidenced. The model developed and successfully validated could be applied by a high number of users with the aim to assess levels of this pathogen in ricotta under both static and dynamic environmental conditions, being useful for the dairy business operators as they cover a wide range of the brands available on the market.

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TISSUE AND SPECIES IDENTIFICATION IN MINCED MEAT AND MEAT PRODUCTS FROM ITALIAN COMMERCIAL MARKETS BY DNA MICROARRAY AND HISTOLOGICAL APPROACH

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The monitoring of animal species, unauthorized tissues and other ingredients in meat products is fundamental to guarantee food safety and maintain consumers' trust and to assure the label compliance with the real contents [1,2]. Currently, DNA microarray approach is one of the fastest-growing technologies among DNA-based analysis for species identification in meat products [3,4]. Moreover, histological examinations is an efficient and economic approach for tissue identification, able to detect bacteria, parasites and other ingredients, such as cheese and vegetables. Aim of this study was the development of a protocol as a screening test, combining DNA microarray approach to identify animal species and histological examination using haematoxylin and eosin staining (H&E) to check the correct composition and labelling of meat and meat products. One hundred-one samples of different types of bovine minced meat (without other ingredients, Group 1, n=48) and ready to cook bovine meat products (Group 2, n= 53) were collected from local supermarkets in Torino, Italy. DNA from each sample was analyzed using the Meat 1.6 LCD Array kit to screen the presence of 14 animal species. Histological screening was performed on five different aliquots taken from each sample. The results from both examined groups by DNA microarray revealed that 26 out of 101 (25.7%) samples were positive for species not declared on the product label, and pork (20 out of the 26 non-compliant samples) was the most commonly not declared species. Histological results from both examined groups, showed respectively the presence of hyaline cartilage in 31.3% and 24.6%, bone in 83.3% and 71.7%, glandular tissue in 10.4% and 7.6% of samples. In addition, histology showed the presence of bacterial colonies in 95.8% samples of Group 1 and 94.3% of Group 2 and the occurrence of inflammatory cells in 52.1% and 75.5%. A significantly higher presence of bacteria ($p<0.01$) and inflammatory cells ($p<0.01$) was detected in meat preparation compared to minced meat samples. Bacterial colonies associated to inflammatory cells were detected with a significantly higher score in Group 2 ($p<0.05$).

Examination of histological sections revealed the presence of at least one parasite in one slide per sample, which were identified as *Sarcocystis* sp., in 40 (83.3%) out of 48 samples of Group 1 and 26 (49.1%) out of 53 samples of Group 2, revealing a statistically significant association ($p<0.01$) of the finding with the groups, with the highest incidence in the Group 1.

Overall, this study confirmed that the mislabeling of meat products is not uncommon in the examined commercial markets. In conclusion, the combination of the two analysis methods, DNA microarrays and histology will increase the monitoring capacity of bovine meat food process.

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A HISTOLOGICAL BASED METHOD FOR THE DISCRIMINATION OF FRESH FROM FROZEN THAWED FISH MUSCLE OF EUROPEAN HAKE (*Merluccius merluccius*)

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Freezing process is commonly applied to prolong the seafood shelf life. However, this preservation technique produces physical-chemical modifications against muscle tissue that may lead to product's quality alterations and expose the thawed product to higher perishability and spoilage process. [1]. Thus, freezing and thawing processes have to be mandatory declared in order to guarantee both fair business-to-consumer practices and consumer's safety [2,3]. Nevertheless, deliberate substitutions of fresh with thawed fish are reported as common fraudulent incidents and numerous analytical discriminating techniques (physiological, chemical and physical) have been developed [4]. Among them, the histological method has been demonstrated as an accurate, and relatively low time and cost-consuming technique [5]. The present study aimed at providing a standard histological procedure to discriminate fresh and frozen-thawed *Merluccius merluccius* (European hake), selected as possible model of white meat fish species. A preliminary analysis was performed on 8 fresh muscle tissue specimens to define the standard morphological characteristics and to select the sampling site among three different anatomical areas (lateral line, dorsal muscle, ventral muscle). Then, 15 fresh products, sampled at different shelf-life (24h, 72h and 120h), were analyzed to evaluate the microanatomical alterations related to fish spoilage. Ninety tissue samples (30 fresh, 30 experimentally frozen at -20° C and 30 industrially frozen at -80°C) were subsequently collected to select morphological and morphometric parameters to be included in a standard operational grid. After a statistical analysis, three morphological (muscle structural score corresponding to 0= fully destructured, 1= partially destructured, 2= well preserved; presence of vacuoles; presence of intermiofibral seroproteinaceous material) and one morphometric (vacuoles per field, for which a cut off value of freezing process corresponding to 1.12 vacuoles per field was set) parameters were ultimately included in the operational grid. Accuracy and repeatability of the procedure were calculated on the analysis carried out by one expert and one in training operators on 50 randomly selected samples among the groups of the previous experiment. The final validation was conducted, by the same operators, through a blind test on 30 (13 fresh and 17 industrially frozen) additional commercial products. The procedure showed high sensitivity (97-100%) and specificity (94-100%) and a high diagnostic concordance of the two operators was observed, regardless of their operating know-how. Thus, the proposed operational grid represents a simple, reliable and low-cost check tool against fraudulent practices.

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EVALUATION OF THE ANTI-BIOFILM ACTIVITY OF A NOVEL ANTIMICROBIAL PEPTIDE AGAINST *Staphylococcus aureus*

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Biofilm formation is usually involved in increasing antimicrobial resistance, which is considered a major risk to global health [1]. Thus, the development of new molecules with antimicrobial and anti-biofilm abilities is nowadays essential. Antimicrobial peptides (AMPs) are innate immune system effector molecules presenting biocidal activity and interesting properties, such as low propensity for developing bacterial resistance, broad antimicrobial spectrum activity, synergy with common antimicrobials. For these reasons, AMPs are emerging as tools to contrast antimicrobial resistance and to control microbial biofilms [2]. *Staphylococcus aureus* is an ubiquitous pathogen able to form biofilm on surfaces of food processing plants and also on medical materials [3]. In this study, it was evaluated the anti-biofilm activity against *S. aureus* of a novel AMP (named HCAT-1) designed starting from bactenecin sequences and already characterized in terms of bactericidal action against foodborne pathogens. To this aim the strong biofilm producer S. aureus ATCC 35556 was chosen. HCAT-1 showed a marked and uncommon structural stability under several environmental conditions as determined by different spectroscopic analyses. Its antimicrobial activity was measured by using the Minimum Biofilm Eradication Concentration (MBEC) assay [4] at 24 and 72 h time exposure and 80 µmol/L concentration, against 24 h-old biofilms, starting from 10^3 or 10^5 cfu/ml inoculum. Results revealed a remarkable anti-biofilm activity of HCAT-1 against *S. aureus* with a total eradication of the biofilm structures, observed at both time-points and inoculum concentrations. These findings suggest that HCAT-1 might represent a novel ecofriendly antimicrobial agent alternative to the commonly compounds used against biofilm, although further analyses are needed in order to assess the potential application of this biotechnological solution.

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ANTIBIOTIC RESISTANCE OF METHICILLIN-RESISTANT *Staphylococcus aureus* (MRSA) FROM ITALIAN SWINE CHAIN IN PLANKTONIC AND BIOFILM FORM

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Pig herds are known to be important reservoir for methicillin resistant *Staphylococcus aureus* (MRSA), i.e. MRSA lineage ST398 [1]. MRSA is one of the most commonly identified antimicrobial-resistant pathogens worldwide [2]. Furthermore, it has been reported that some MRSA strains are able to form biofilm on different surfaces [3]. In this study, MRSA isolated from swine chain in Northern Italy were tested in their planktonic and biofilm form against the critically important antimicrobials gentamicin and enrofloxacin [4]. A total of 400 samples were collected from 50 pig finishing herds. In details: three environmental sites at each farm (150), and five carcasses per farm at slaughterhouse (250) were sampled. MRSA were identified by phenotypic analysis and a quadruplex-PCR. Biofilm-forming capacities were evaluated using a previously described method [5]. The Minimum Inhibitory Concentration (MIC) and Minimum Biofilm Eradication Concentration (MBEC) to gentamicin and enrofloxacin were evaluated using micro dilution assays [6]. In addition, the minimum concentration of antimicrobial that inhibits growth of the dispersed cells from the biofilm was also evaluated [7].

A total of 37 MRSA strains was isolated from 400 samples (9.25% prevalence). Six/37 (16.2%) isolates were moderate (1) and strong (5) biofilm producers. Among these, 2 strains (1 moderate and 1 strong) were isolated from carcasses, and 4 from environment. Resistance to gentamicin and enrofloxacin was assayed in planktonic and biofilm form. Results from MIC analysis showed that 4/6 and 2/6 planktonic MRSA strains were resistant to enrofloxacin and gentamicin, respectively. Conversely, MBEC assays on biofilm cultures showed 8- to 512-fold enhancement in resistance. In details, it was observed that sensitive planktonic isolates were able to acquire resistance, and that both antibiotics were unable to eradicate biofilms even at the highest tested concentrations. Furthermore, planktonic cells dispersed from biofilms were also found to be more resistant in 5/6 strains. In conclusion, it was observed that biofilm could act as a mechanism for the tested swine-isolated MRSA strains to get a better survival, and stress once more the importance of finding and developing new alternatives to common antibiotics to overcome the bacterial resistance issue. Further studies are needed to confirm these preliminary findings.

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DEXAMETHASONE AND PREDNISOLONE TREATMENT IN BEEF CATTLE: INFLUENCE ON GLYCOGEN DEPOSITION AND GENE EXPRESSION IN LIVER

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The European Union prohibits the use of growth promoters like sex steroids, glucocorticoids (GCs) and beta-agonists in meat production industry (Directives 96/22/EC, 2003/74/EC and 2008/97/EC). Synthetic GCs, like dexamethasone (DEX) and prednisolone (PDN), are widely used in veterinary clinical practice, but often they are illegal administered in beef cattle because of their marked capacity to improve carcass quality traits and food intake. In order to realize the growth-promoting effect, DEX or PND are usually long term administered at low dosages, below the threshold of the most common analytical methods of detection. In order to detect illegal GCs administration, new direct or indirect biomarkers should be identified. For this purpose, understanding the mechanism of action of each molecule is essential. GCs are steroid hormones that regulate multiple aspects of glucose homeostasis. They promote gluconeogenesis and increase glycogen storage in liver [1]. In this work DEX and PDN effects on glycogen deposition and expression of genes involved in glucose homeostasis were considered in bovine liver. Eighteen adult Charolaise male cattle were divided into 3 groups: group A (n=6) treated with DEX (0.70 mg/animal/day for 40 days), group B (n=6) treated with PDN (15 mg /animal/day for 35 days), and group C (n=6) was the control group. In order to visualize glycogen storage in liver samples, periodic acid-Schiff with diastase (PAS-D) staining procedure was performed. The analysis of expression of genes encoding enzymes involved in gluconeogenesis and glycogen metabolism was conducted by qPCR. To identify one or more reference genes whose mRNA expression is stable across the experimental condition, geNorm algorithm (qbase+ version 3.1, Biogazelle) was applied. PAS-D staining procedure showed mild and marked hepatocyte glycogen depletion respectively in PDN and DEX-treated animals. On the contrary a significant over expression of glycogen synthase (about 3.5-fold) and cytosolic phosphoenolpyruvate carboxykinase 1 (about 3.1-fold) was detected in liver of DEX-treated animals. FK506 binding protein 5 gene, marker of GCs activity, was down-regulated in liver after DEX and PDN treatment, as previously described in thymus [2]. Several authors described that the long-term administration of GCs leads to an increase of glucose supply to the liver for glycogen formation in human and mouse [1]. This knowledge corresponds to the present results obtained by gene expression analysis, but it is in contrast with the histological findings of the present experiment. The discrepancy of the histological results could be due to the experimental design. In particular, the treatment protocol considered a long withdrawal time (6 days) during which hepatocyte glycogen depletion could have occurred to restore the pretreatment condition. Anyway this is the first time that DEX and PDN action on glycogen metabolism was described in bovine liver and a better understanding of this pathway could be useful to identify illicit treatment in livestock.

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CHARACTERIZATION OF *Staphylococcus aureus* ISOLATED FROM HIGH SOMATIC CELL COUNT HERDS IN PIEDMONT

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Staphylococcus aureus is one of the main etiologic agents of contagious mastitis and it is able to cause significant economic losses in terms of reduced milk production and costs related to health interventions. Objective of this study was to characterize *S. aureus* strains isolated from high somatic cell counts herds in Piedmont region. Bulk tank milk samples were collected in 1,600 dairy herds (2 samples/month per 3 months) and the mobile geometric mean was evaluated in order to identify herds with higher somatic cells counts (SCC). During winter and spring periods two bulk tank milk samples were collected from a total of 79 selected herds with a SCC over 370,000/mL. Coagulase-positive staphylococci (CPS) were enumerated. Up to 3 strains were isolated from each sample, preferring those with different morphologycal characteristics. The microorganisms were then identified by mass spectrometry (MALDI-TOF). *S. aureus* strains were genotyped by RS-PCR, a method based on the amplification of the intergenic spacer 16S-23S rRNA and evaluated for the presence of Staphylococcal Enterotoxins genes. The majority of the samples (52.9%) had a concentration of CPS below the detection limit (<10 ufc/mL), while 33.1% showed CPS counts between 10 and 1,000 cfu/mL. Only in 2.9% of samples, the CPS couts was higher than 5,000 cfu/mL. A total of 181 *S. aureus* strains was isolated and molecular characterized. In this study, from the RS-PCR genotyping, 21 different genotypes were identified. The most prevalent was the GTB (No. 59), followed by its GTBI variant (No. 20) and by the GTBII (No. 14). Considering the different genotypes, including their own variants, the genotype B was identified in 50.3% of the isolated strains: 32.6% of the isolates was GTB, followed by the variants GTBI (11.0%), GTBII (3.3%) and GTBIII (3.3%). The 11.7% of the strains were found to be GTR, consisting of its variants GTRI (3.3%), GTRVI (3.3%), GTR (2.8%), GTRIX (1.7%) and GTRIV (0.6%). Considering both samplings carried out during the two seasons in the individual farms, it was not possible to isolate *S. aureus* strains in 46.8% of the farms involved in this study, although they had a high level of somatic cells in the reference period. In the 25.3% of the herds, a total of 6 *S. aureus* strains was isolated during the two samplings, while only 3 strains in the 18.9% of the farms. Only one genotype was detected in 52.4% of the herds, while in the 33.3% herds two different genotypes were isolated. In the remaining farms (14.7%), a greater number of genotypes was detected. Many strains harbored SEs genes, while 44% resulted non-enterotoxigenic. Sed-ser-sej profile was found in 21% of strains, followed by a combination of seg-sei (7%) and sea-sed-ser-sej (6%). GTB strains harbored sed-ser-sej (40%), sea-sed-ser-sej (10%), while the remainig (40%) resulted negative. This study highlighted that over 50% of the isolates was GTB, showing that this genotype is highly present in Piedmontese herds. However, it should be underlined that herds involved had high SCC (characteristic of the GTB).

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COMPARISION AMONG *Staphyloccocus aureus* ISOLATES FROM EUROPEAN AND AMERICAN COUNTRIES: GENOTYPING, DETECTION OF GENES ENCODING SUPERANTIGENS AND METHICILLIN RESISTANCE (MECA)

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Staphylococcus aureus is one of the major casual agents of intra-mammary infections (IMI) in dairy cow and it is characterized by wide variability of pathogenic and contagious properties of different genotypes [1]. This study aimed to subtype *S. aureus* isolates from Europe and America according to the frequency of genotypes and their different profiles, in order to compare them with their specific molecular characteristic. A total of 66 isolates (17 from Italy, 17 from Germany, 17 from New York State and 15 from Brazil) from 57 different farms, was collected from clinical mastitis quarter samples. These isolates, all nuc positives, were genotyped by RS-PCR [2][3]; this method, based on the amplification of the 16S–23S rRNA intergenic spacer region, is accurate, rapid and inexpensive [4]. Then, they were analyzed for genes encoding superantigens (from sea to sel and tsst), but also for mecA gene. At first, the RS-PCR analysis revealed 28 different profiles and, within these, 7 new variants of existing genotypes; in particular, they included GTBN_I, GTBN_II, GTBY_I, GTAQ_I (Brazil), GTR_XIII (Italy), GTC_V,GTI_V (New York State). Major genotypes with their variants were combined into genotypic clusters (CL) [5]; for each country, a specific genotypic pattern was found. In fact, in Italy and Germany, the most prevalent ones were CLB (29.4%) and CLR (64.7%), respectively; CLC, situated in Europe, was also widespread in New York State (70.6%), while CLBY was the most prominent in Brazil (40%). Additionally, all isolates were analyzed to investigate the diffusion of virulence factors, related to *S. aureus* pathogenicity; especially enterotoxins were identified in the majority of them. Among the 66 isolates, only 12 were non enterotoxigenic, whereas the remaining 54 isolates had at least 1 of the genes coding for A, C, D, G, H, I and J enterotoxins. In agreement with a previous study [2] showing that GTB was characterized by the presence of sea, sed and sej, our results showed that also Italian isolates were positive for these SEs. Moreover, this Swiss study revealed that GTC was positive for sec, seg and tsst; the same results were found for GTC German isolates. Furthermore, 2 different Italian isolates harboured tsst and mecA, while 4 German ones were positive for tsst and another 1 for mecA; interestingly, GTS was found only in the Italian and German isolates positive for mecA, in accordance with previous results [4]. On the contrast, the American ones harboured at least one of sea, sed, seg, seh, sei. In conclusion, this study confirms the wide variety of *S. aureus* genotypes, related to geographical origin of the isolates and to their virulence factors.

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FRAUDULENT SPECIES SUBSTITUTION IN GOAT'S MILK CHEESES

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Goat milk and its products play an important role in the economic viability of many parts of the world, including Mediterranean countries. Cheese is traditionally the main commercialized goat milk product, thanks to the nutritional, health, organoleptic and cultural values of goat milk [1]. Given its excellent nutritional properties and higher commercial value, fraudulent incorporation of cheaper bovine milk during cheese-making is common practice. Goat byproduct mislabeling, linked principally to the undeclared partial replacement of goat's milk with cow's or sheep's milk [2]. Considering that milk product mislabeling has been widely reported throughout the world and authentication of food components is one of the key issues in food safety and quality, the aim of this study was to use DNA-based methods to investigate the prevalence of mislabeling among goat-milk products. A total of 76 samples of milk products, which, according to their labels, were derived exclusively from goat's milk, were subjected to DNA extraction and purification. Real-time PCR and melting curve analysis were used to qualitatively evaluate the presence or absence of the target species [3]. The results of the molecular investigations revealed a high degree of species mislabeling in goat-milk products (81.6 %), the non-compliance's are due to the presence of DNA from bovine and/or ovine species in addition to goat. The many mislabelings may be deliberate due to technological reasons, the seasonality of the milk supply and the lower commercial values for other milks, which encourage fraudulent incorporation of bovine and sheep milk during cheese-making [4]. However, this study also indicates inefficient cleaning of the milking system and improper milking procedures, poor management of bulk tanks and inadequate separation of milking area and production/transformation areas as further factors [5]. Furthermore, the results underline the need to adopt and implement stricter quality controls and traceability procedures, including regular DNA testing, in order to guarantee milk product authenticity.

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SIMULTANEOUS QUANTITATIVE DETECTION OF ANTIBIOTICS FROM APULIAN HONEY USING A BIOCHIP MULTI-ARRAY TECHNOLOGY

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Antimicrobial compounds are used in food production to treat or prevent animal diseases. Antibiotics potentially present in honey are inappropriate, even presenting risks to human health. For this reason, the use of antimicrobial compounds in food-producing animals was banned or restricted by many countries and no market authorization may be obtained without MRLs (Maximum Residue Limits) [1]. On the other hand, no MRLs were set for honey and then only honeys free of antibiotics may be sold in the EU countries. In fact, the use of antibiotics for the treatment of honey bees is illegal in the EU, but due to the high import quota from non-EU countries, contaminated honey products may be found on the European markets [2]. The extensive use of antibiotics in veterinary medicine represented a potential hazard for human health; they may produce residues in foodstuffs causing allergic reactions, toxic effects, antibiotic resistance and damage to the central nervous system [3]. The aim of this study was to detect the presence of antibiotics in Apulian honey through the Antimicrobial array II (AM II) method. Sixty-six honey samples of nine floral origins were divided into two groups, based on the year of production. The first group, consisting of twenty-four samples of Apulian honey, was produced in 2016 while, the second one, consisting of forty-two samples, was composed by Apulian honey produced during the year 2017. Among the sixty-six honeys analyzed, as many as forty pointed out the presence of antibiotics, although many samples showed values of antibiotics lower than the limits quantifiable by the instrument. Regarding the year of production, a different use of the antibiotic substances was evident between the two years monitored. In fact, among the 24 honey samples of the year 2016, only 2 showed positivity to quinolones, however, in quantities slightly higher than the limits of quantification by the instrument. On the other hand, among the 42 honey samples collected during the year 2017, 38 highlighted the presence of at least one of the six classes of antibiotics. Local controls should be further investigated because honey, which is generally considered a natural and healthy product [4] and always widely requested on the market, may itself become a risk for the consumer health. Considering that the new EU Regulations place the consumer health as the main objective to achieve in the food chain, it is essential to check that the ban on the use of antibiotics in beekeeping practices is respected, introducing more and easy controls to ensure not only the health of the consumer but also the health of the bees themselves.

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DETERMINATION OF AFLATOXIN M_1 IN LONG-RIPENED PECORINO ROMANO, GRANA PADANO AND PARMIGIANO REGGIANO CHEESES

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The main aim of the present study was to evaluate Aflatoxin M_1 (AFM_1) contamination in three Italian hard cheeses. The acquisition of data on the occurrence of AFM_1 contamination will contribute to assess the risk for human health associated to the consumption of hard cheeses. The official method for the determination of AFM_1 in milk and cheeses is the HPLC with fluorescence detector (FLD). However, the extraction by immunoaffinity column of AFM_1 is expensive, time consuming and requires trained personnel. Therefore, an alternative method based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed (detection limit 30 ng/kg). Analyses were performed by the Regional Farmers Association Laboratory. The extraction of AFM_1 from cheese samples was conducted by the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) approach. The determination of AFM_1 was conducted on a total of 280 ripened cheese samples of which 108 Pecorino Romano PDO (PR), 40 Grana Padano PDO (GP) and 32 Parmigiano Reggiano PDO (PRG). Maturing times ranged from 8 months for PR and 12 months for GP and PRG. Cheese samples were provided by 6 sheep cheese making plant for PR and two cow's cheese factories for GP and PRG. Samples were collected monthly from three different production batches in the period from January to June. The AFM_1 was detected at concentration $\geq$30 ng/kg in 6/108 samples of PR cheese (5.6%), in 11/40 samples of GP cheese (27.5%) and 0/32 samples of PRG. The range of concentrations of AFM_1 found was between 35.1 and 90.1 ng/kg for PR cheese and between 39.1 and 96.0 for GP. None of the samples analyzed reached or exceeded the maximum limit for AFM_1 (275 ng/kg) allowed by Italian legislation, considering the concentration coefficient equal to 5.5 provided for hard cheeses. The LC-MS/MS QuEChERS used has proven to be faster, cheaper and just as reliable as compared to the official HPLC with fluorescence detector method. The present study confirms the lower risk of sheep milk cheese as compared to other cow's milk cheeses. This is consequence of differences between sheep and cows in the ingredient used for feeding, in the carry-over of aflatoxins and in the dilution effect of tank trucks milk.

[1] International Organization for Standardization, ISO 14501:1998 (IDF 171: 1998), Milk and milk powder - Determination of aflatoxin M_1 content - Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography. [2] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. [3] Nota 30652 del 17.07.2013: aflatossine M_1 in prodotti lattiero-caseari. Applicabilità dei coefficienti di trasformazione in equivalente latte nei formaggi di cui all'allegato 2 del DM 31 luglio 2001 del MIPAAF - Rettifica nota DGISAN n. 28454 del 3/7/2013.

POSTOPERATIONAL AND PREOPERATIONAL *Listeria monocytogenes* CONTAMINATION IN TWO SHEEP'S MILK CHEESE-MAKING PLANTS ENVIRONMENT

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The present study aimed to describe the pattern of *Listeria monocytogenes* contamination in the processing environment of two sheep milk cheese-making plants (A, B) of Sardinia (Italy). Objective one was to map the prevalence of *L. monocytogenes* within the facilities; objective two was to trace the pathways of cross-contamination along and between production lines; objective three was to determine the persistence of *L. monocytogenes* contamination over time. Each cheese-making plant was visited three times one month apart for the collection of environmental samples. During each visit, environmental swabs were collected at the conclusion of the processing day (POST, post-operational samples) and again after sanitation operation (PRE, pre-operational samples). Samples were collected along the two main processing lines, Pecorino Romano and ricotta cheese, from food contact and non-food contact surfaces. *L. monocytogenes* isolates were submitted to pulsed-field gel electrophoresis (PFGE). Overall were collected 211 swabs, 95 and 119 respectively from food contact and non food-contact surfaces of which 122 were PRE samples while 89 were POST samples. The prevalence of *L. monocytogenes* was of 7.3% and 2.6% in cheese-making plant A and B respectively. PRE samples prevalence was 5.7%, 11.1% in plant A and 1.5% in plant B, respectively. POST samples prevalence was 3.4%, respectively 2.5% in plant A and 4.2% in plant B. In plant A *L. monocytogenes* was recovered from whey heating, salting and ripening areas with a mean prevalence of 17.8%, 9.1% and 25.0%, respectively. While in plant B was present only in 23.1% samples of the salting area. PFGE, conducted on 24 strains, showed seven cluster of which two were observed in both facilities. In plant B two pulsotypes (II, IV) were recovered from the salting and ripening areas along Pecorino Romano production line while pulsotype II was recovered also in the whey heating area. Persistent contamination was observed only in the salting area of plant A and in the whey heating of plant B. The contamination of POST samples and the presence of common PFGE pulsotypes within and between production lines is indicative of possible cross-contamination during operation. The presence of contamination in PRE samples and the persistence over time is indicative of ineffective sanitation procedures.

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EFFECT OF CLAWS BINDING IN AMERICAN LOBSTER (*Homarus americanus*) HOUSING: PRELIMINARY STUDY OF EMOLYMPHATIC PARAMETERS

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American lobsters are crustaceans traditionally marketed live and, once caught, are subjected to different type of stressors that can affect their welfare. The European legislation does not assess specific retention requirements and the only invertebrates welfare references are the EFSA Animal and welfare Scientific panel opinion (2005) [1] and the guidelines created by the Seafish Industry Authority, a public body in the United Kingdom [2]; therefore their management is demanded to the common sense of food business operators. Keeping lobsters claws tied before and during storage is not legally required, but it is suggested to prevent animals from damaging each other. Some authors, nevertheless, affirm that this practice may prevent lobsters from showing their natural behaviour and damage the claws. Aim of the study was to evaluate the effect of claws binding on lobsters welfare by analyzing seven different emolymphatic stress parameters, vitality and weight. The study was performed in commercial aquaria of Lodi Acquaculture Research Centre, at the University of Milan. During the experiment, water temperature was 6°C; specific gravity was 1020, oxygen was 87%, un-ionized ammonia was <0.3 mg/l and nitrite <0.1 mg/l. A total of 24 lobsters of both sexes were assigned randomly to one of two experimental groups: control group was mantained with tied claws while treatment group was held with free claws. Animals were maintained unfed throughout the trial. Hemolymph samples were withdrawn from the the ventral abdominal sinus (arthrodial membrane covering the articulated base of the 5th walking leg) of each animal at arrival (T0) and after 12 h (T1), 36 h (T2), 60 h (T3) and 108 h (T4). Vitality index and weight were measured at the same sampling times. Glucose, lactate, total protein, ammonia, urea, chloride, calcium and magnesium concentration were determined. Parameters were rather constant during the whole experiment. At T1, all the parameters determined resulted significantly higher if compared to T0 ($P<0.01$); the effect of "time" confirmed the importance of lobster storage in high quality artificial seawater tanks [3].

Claws binding did not have a significant impact on vitality, weight, glucose, protein, ammonia, urea, chloride and magnesium, while calcium level was influenced by the treatment ($P<0.05$). Moreover, the absence of claws binding can contribute to enhance aggressivity in the subjects and difficulties in animal handling by food business operators.

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METHICILLIN RESISTANT *Staphylococcus aureus*: PUBLIC VETERINARIANS AS AT-RISK WORKERS

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Besides being an important foodborne pathogen, methicillin-resistant *Staphylococcus aureus* (MRSA) has been identified by a recent ECDC, EFSA and EMA joint Scientific Opinion [1] among the relevant indicators to be chosen to monitor the prevalence of antibiotic resistance among Member States of the European Union. Various studies have already shown that there is a greater risk of becoming MRSA carriers for those professionally exposed to frequent contact with animals treated with antibiotics such as, for example, farmers and veterinarians [2, 3]. The study was conducted to evaluate and possibly confirm this risk by carrying out a study among the staff member of the Local Health Unit (ASL) CN2, with the aim of improving awareness of this issue among the subjects participating in the study. The collection of samples was carried out on two dates, in November 2017, involving all the veterinarians employed by the ASL CN2. Self taken nasal swabs were collected according to the instructions provided and stored at refrigeration temperature for subsequent sending to the IZS PLV in Turin. At the same time, questionnaires were delivered to collect data on predisposing factors (frequency of contact with animals and species thereof). The swabs were processed for qualitative and quantitative analysis on Mannitol Salt Agar (MSA), Baird-Parker with rabbit plasma fibrinogen (BP-RPF) and on a specific medium for MRSA (MRSA Select®). A maximum of 5 colonies isolated from each swab were selected. The isolated strains were identified by mass spectrometry (MALDI-TOF). Each isolated strain has been tested for the presence of methicillin resistance genes (mecA and mecC) and Panton-Valentine leukocidine (pvl) gene, by PCR protocol (DTU, Copenhagen). Finally, the Kirby-Bauer method, for antimicrobic sensitivity testing, was performed on isolated colonies. A total of 25 swabs were collected. 6 were found positive for the presence of coagulase positive S. aureus (24%) and, among these, one showed a MRSA resistance pattern, confirmed by the presence of mecA gene. This pattern showed resistance to β-lactam antibiotics (ampicillin, amoxicillin, penicillin and cefoxitin), ciprofloxacin and erythromycin. Moreover, a MSSA strain showed an unusual susceptibility pattern with resistance to tetracyclines and to Sulfametoxazole / Trimethoprim association. The project represents a first contribution towards raising awareness of antimicrobial resistance among local veterinarians, which should be considered at risk workers. MRSA is increasingly reported as a cause of livestock associated infections around the world. The results of the study highlight the need to widen the survey to properly evaluate the risk for veterinarians in our region and improve the awareness among operators of the risk of bacterial resistance in the community.

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MICROBIAL EVALUATION OF ROBIOLA DI ROCCAVERANO CHEESE

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The Robiola di Roccaverano is a soft and creamy cheese produced in Piedmont. It is the only Italian artisanal cheese made with raw goat's milk which has been granted the Protected Designation of Origin (PDO). The manufacturing process of Robiola di Roccaverano requires the use of natural milk starter (NMS), without the addiction of artificial culture. The NMS is produced by culturing milk coming from previous day's milking session under non-aseptic conditions [1]. It is, therefore, an undefined complex consortium of microbes. NMS, with raw milk microbiota is responsible of artisanal cheeses' characteristics, enhancing the richness and the diversity of the product's flavors [2]. The aims of this study are to investigate the microbial composition of NMS added during the manufacturing process of Robiola di Roccaverano cheese, to monitor the microbiota evolution from milk to ripened cheese following all the steps of production and, finally, to evaluate the presence of seasonal differences in microbiota. The manufacturing process of one artisanal cheese factory was followed in each season for one year. Finally, a total of 56 samples of NMS, raw milk, curd, fresh (after 4 days of production) and matured (almost 10 days) cheese were collected. The parameters for hygiene and food safety such as *Salmonella* spp., *Listeria monocytogenes*, coagulase-positive staphylococci (CPS) and *Enterobacteriaceae* were determined according to ISO methods. Lactic Acid Bacteria (LAB), molds and yeasts were grown in specific selective media, enumerated and isolated for the identification by Random Amplify Polymorphic DNA-PCR (RAPD-PCR). The results show that no pathogens were found in the samples, except for the presence of CPS colonies in milk and cheese samples. Nevertheless, species-specific PCR confirmed the presence of *S. aureus*, the plate counts were always within the limits indicated by Regulation (CE) N. 2073/2005. Therefore, all the samples comply with the European legislative standards [3]. The results of microbiology analysis were analyzed with one-way ANOVA, where $p<0.05$ was considered significant. The CPS, *Enterobacteriaceae* and LAB bacterial count did not show seasonal differences ($p>0.05$), but molds and yeasts count for milk and fresh cheese revealed that significant seasonal differences are present. As well as significant differences was also found in LAB, mold and yeasts number within NMS, milk, fresh and ripened cheese samples of each period of the year. Preliminary results obtained by RAPD-PCR show that for the LAB the most abundant genus are *Enterococcus* followed by *Lactococcus* and *Streptococcus*. Among molds and yeasts, was showed the presence of *Geotrichum candidum, Yarrowia lipolytica* and *Kluyveromyces marxianus*. These techniques allow to obtain a great number of results useful to the producer to increase the quality of this excellent product. In the future will also be applied a new high-throughput sequencing approach to explore more in detail the microbial dynamics during cheese-making process.

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DETECTION OF BLUE PIGMENTING *Pseudomonas fluorescens* STRAINS BY PCR ASSAY

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Pseudomonas fluorescens are Gram-negative rod shaped bacteria that inhabit soil, plants, and water surfaces. While, generally, not considered pathogenic for humans, these microorganisms are able to produce extracellular enzymes and pigment, contributing to food spoilage and jeopardizing the quality of food products. Blue discoloration of mozzarella cheese, caused by strains of *P. fluorescens*, has been largely observed. Several studies have been conducted to understand under which conditions the presence of this microorganism is responsible for the discoloration [1] and the genetic determinants of the pigmenting strains [2]. This latter research highlighted the presence of a putative gene cluster in two pigmenting strains composed by accessory genes linked to the tryptophan metabolism. A study aimed to the further characterization of this putative gene cluster is undergoing in our laboratory and the WGS of 6 *P. fluorescens* strains (Genbank Bioproject: PRJNA436461) confirmed the presence of the gene cluster only in strains causing the blue discoloration. The aim of this study was to select an appropriate genetic marker for the rapid identification of blue pigmenting strains by PCR assay. Based on the sequences of the accessory gene encoding for the Tryptophan synthase beta chain of blue pigmenting strains of *P. fluorescens* deposited in Genbank, three set of primers were designed using Primer3 software. Specificity of the primers were examined in-silico using Primer-BLAST. The ability of blue pigment production of the strains included in the study was evaluated by plating on Potato Dextrose Agar (PDA) and Mascarpone Agar (MA), as described by previous studies [1]. In order to select the strains to include in the study to develop and validate the PCR assay, a set of strains identified in a previous study [3], by sequencing of rpoD and gyrB, was used. A total of 5 strains demonstrated pigment production on both media and were therefore used as positive samples. A total of 100 strains not showing blue pigment production were included in the exclusivity set: 50 *P. fluorescens* and 50 other *Pseudomonas* spp. Amplicons were sequenced to confirm their identities by Sanger sequencing. All the 5 positive strains showed the presence of the expected amplicon (400 bp), while none of the strains included in the exclusivity set was positive after the PCR. The sequencing confirmed the amplification of the desired target. The accessory gene encoding for the Tryptophan synthase beta chain of the blue pigmenting strains of *P. fluorescens* has been proved to be a promising molecular target for the rapid and specific identification of these microorganisms. This marker could be targeted by an isothermal amplification assay which might be implemented in the production environment, helping the producers to early detect the pigmenting strains and their sources.

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DETECTION OF ADULTERATION IN WHEY BUFFALO MILK PROTEINS BY CAPILLARY ELECTROPHORESIS COUPLED WITH PLS REGRESSION ANALYSIS

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The globalization and the consequent opening of the international trade promote the commercial exchanges of food products, but also their frauds, sophistications. Consequently, the need to identify food ingredients by analytical tools is increasing and the development of methods that check the ingredients became a priority. In Italy buffalo's milk and their derivatives have a great economic relevance, therefore, it is very important to defend the quality of products as Buffalo mozzarella characterized by Protected Designation of Origin. The addition of cow milk during the production of buffalo mozzarella, is a common fraud in dairy industries due to lower price and availability, throughout the year, of the cows' milk. Moreover, the addition of cows' milk affects negatively the quality of the final products, also influencing the appearance of food intolerances or allergy. Recently, Caira et al [1] overcomes the problem of false positive results of European reference method by a new proteomic approach. Previous studies demonstrate that capillary electrophoresis (CE) is able to separate, identify, and quantify the principal milk proteins [2,3] and this ability coupled by statistical analysis, as regression or partial least square regression (PLS), make it a powerful tool able to distinguish if milk fraud occurs. The aim of this work is to investigate the potential of PLS technique applied to CE analysis in order to create a robust and rapid method allowing to predict the concentration of cow milk added in fraudulent manner to buffalo milk. Mediterranean Buffaloes bulk tank raw milk samples were provided by three dairy farms sites in Calabria region (South Italy). Bovine bulk tank raw milk samples were collected from dairy farms rearing different breeds. Whey proteins were obtained by adding rennet solution (0.014% v/v) after 30 min at 37°C. Standard samples were prepared by mixing buffalo whey with cow whey from 0 to 100%, v/v (0, 1, 2.5, 5, 10, 20, 25, 30, 50, 70, 75, 80, 90, 95, 97.5, 99, and 100%) resulting in a total of 71 milk standards. CE analysis was carried out using a Minicap capillary electrophoresis system (Sebia). The CE software records variation of absorbance at 200 nm in function of migration time, converted into relative migration position, producing typical electropherograms. The results were analyzed by PLS analysis (TQ Analist software, Thermo Scientific). The determination coefficient (R2) of calibration model (n=49 samples) was excellent (R2=0.99) and the root mean square error of calibration, a measure of how well the model fits the data, was equal to 4.61. The cross-validation was performed using the leave-one-out component and the root mean square error of cross-validation, a measure of the model's ability to predict the % of cow milk in a mixture, was 6.28. The number of factors were 6 and the % of the explained variance for the model was >92%. When this regression model was applied to validation samples (n=22) the root mean square error of prediction was 8.56. We propose CE analysis coupled with PLS regression as a screening method able to predict the % of cow whey milk in cow/buffalo whey milk mixtures. Further studies are necessary to validate this approach.

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EFFECTIVENESS OF SPICED TUNISIAN OLIVE OIL ON *Anisakis* LARVAE TYPE I IN INDUSTRIAL ANCHOVY MARINATING PROCESS

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The ingestion of raw or undercooked fish or cephalopods, commonly hosts of larvae belonging to the *Anisakidae* family, is at the origin of human anisakidosis [1]. Several natural extracts, oils, essential oils and their compounds have been tested against *Anisakis* [2-3]. The aim of the study was to evaluate the effectiveness of Tunisian olive oil flavored with different spices (Laurel, Ginger, Cinnamon, Cardamon and Rosemary) against *Anisakis* larvae Type 1. Solid-phase microextraction was used as a technique for the evaluation of oil chemical composition using gas chromatograph and gas chromatograph–mass spectrometer system. All 720 *Anisakidae* larvae used for the study were identified as L3 larvae of *Anisakis* type I according to guidelines proposed by Murata et al. [4]. For the in vitro experiment 20 larvae were submerged separately in the above mentioned oils plus unflavored olive oil (control sample). The normalized mean viability (mean value of the viability score), LT100 (lethal time: time required to kill 100% parasites) and LT50 (time required to kill 50% parasites) were calculated. All experiments were carried out three times in separate conditions for a total of 360 larvae tested. Oil with Cinnamon was the most effective against *Anisakis* with LT50 = 1.5 days and LT100 = 3 days, followed by Rosemary (LT100 = 7.9 days), Laurel (LT100 = 12.5 days), Cardamom (LT100 = 14.9 days) and Ginger (LT100 = 15.6 days) oils. Cinnamon and Rosemary effectiveness can be related to the presence among their major compounds of (E)-cinnamaldehyde and 1,8-cineole respectively. These two compounds are, in fact, characterized by a strong anisakicidal activity [2]. For the in vivo experiment, Cinnamon and Rosemary oils were tested in 20 anchovy fillets previously artificially parasitized with *Anisakis* larvae. All experiments were carried three times in separate conditions. Fillets with unflavored olive oil were used as control. Thus, a total of 360 larvae were tested for the in vivo experiment. For each oil, 10 fillets without *Anisakis* were prepared with the same process for the sensory evaluation. Cinnamon was the most effective against parasites (dead after 4 days) as compared to Rosemary (7 days). For the two oils, the odor resulted pleasant as well as the taste. In conclusion, the use of these flavored oils in the industrial marinating process could represent an efficient strategy to devitalize *Anisakis* larvae.

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PANGENOME ANALYSIS AND ANTIMICROBIAL RESISTOME OF *Salmonella enterica* SEROVARS TYPHIMURIUM AND 1,4,[5],12:I:- SEQUENCE TYPE 34

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Salmonella enterica serovar 1,4,[5],12:i:- has recently emerged in food-borne epidemics of multi-drug resistance (MDR) strains associated to several outbreaks in Europe (EU) as well as in other continents. The existence of a European clone, associated to Multi-Locus Sequence Types ST34 and to R-Type ASSuT, has been described. The aim of the present study was to investigate the geographical structure and antimicrobial resistance gene patterns of a set of *Salmonella* serovars Typhimurium and 1,4,[5],12:i:- genomes belonging to ST34. A core genome gene-by-gene approach was performed on 1,254 publicly available *S.* 1,4,[5],12:i:- and *S.* Typhimurium genomes belonging to ST34 and including 148 newly sequenced genomes of Italian *S.* 1,4,[5],12:i:- isolates collected from humans, swine and pork meat between 2012 and 2014. Strains gathered in several clusters irrespectively of the year and of the source of isolation. Three subclades, STY-, WE- and NA-clade, accounting for roughly the 60% of the genomes were identified as significantly associated to Italian, United Kingdom and North American origin, respectively (Fisher's exact test; $P<0.0001$). The AMR patterns of all genomes were predicted by in silico identification of antimicrobial resistance associated genes (ARGs) from ResFinder database. Almost all genomes (96.5%) were positive for at least one ARG, whereas a limited number (45) did not show any positive match for ARGs in Resfinder database. The 63% of all ST34 isolates and the 66% of Italian ones were predicted to harbour the R-Type ASSuT. Within the 1,254 ST34 tested genomes, 55 different ARGs of the ResFinder database were found. The number of fluoroquinolone detected ARGs was 67 with qnrB gene as the most prevalent one (46). Aminoglycosides ARGs were detected 2,352 times with aph(3'')-Ib and aph(6)-Id genes as the most reported (925 and 963 respectively). Beta-lactam ARGs were rarely detected with the exception of blaTEM-1B (960). Sulfonamides ARGs were frequently detected (1,090) with sul2 (1,006) as the most identified. The frequency of detection of tetracycline ARGs was high (1,226) with tetB (1,137) as the most represented. In relation to the country of origin, Italian genomes and non-Italian genomes showed similar percentages of the most abundant ARGs (around 70-80% for aph(3'')-Ib, aph(6)-Id and blaTEM-1B genes and around 80-90% for tetB gene). However, in relation to the other ARGs, a lower percentage of ARGs was observed in Italian genomes with the exception of floR2 (7% Italian vs 5 % non-Italian genomes) and mcr genes (5 Italian genomes vs 0 non-Italian genomes). Regarding the 5 Italian *S.* 1,4,[5],12:i:- genomes found positive for mcr genes, three isolates and one isolate harboured mcr-1 and mcr-5 genes respectively and were collected from pigs between 2012 and 2014; one isolate carried mcr-4 and was collected from human in 2007. In conclusion, the identification of most prevalent ARGs might be useful to identify key genetic biomarkers of antimicrobial resistance in *S.* Typhimurium and *S.* 1,4,[5],12:i:-. Moreover, the identification of geographically segregated clades in combination with their antimimicrobial resistome gave first insights to uncover the evolution and potential routes of spread of antimicrobial resistance genes from country to country.

EVALUATION ON MGO OCCURRENCE IN APULIAN HONEY

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Honey is a natural product highly appreciated for its sweet taste and for its nutritional and therapeutic properties on human health such as antioxidant, antimicrobial, anti-inflammatory and bacteriostatic effects [1]. Biological effects of the honey are determined by its particular physico-chemical composition. In recent years, the researchers' attention has focused on a particular substance contained in some types of honey, the methylglyoxal (MGO), present in high levels of up to 800 mg/Kg in Manuka (*Leptospermum scoparium*) honey [2], identified like the component responsible for the pronounced “non peroxide” antibacterial activity [3]. Furthermore, MGO is used as a parameter for the authentication of Manuka honey. The aim of the present study was to investigate the occurrence of MGO in citrus honey collected in Apulia Region (SE, Italy) to establish relative concentrations and to recognize if this substance is useful to characterize this product. A total of 30 citrus honey samples were collected during the year 2017 from Apulian beekeepers. The samples were analyzed in triplicate in HPLC-UV with the method proposed by Mavric et al. [2] with some modifications. The results revealed low concentrations of MGO in citrus honey, ranging from 0.3 to 7 mg/kg. Although other authors observed appreciable MGO concentrations in non-Manuka honeys [4], for the Apulian citrus honeys this compound is not a suitable tool for the characterization of the geographical and botanical origin.

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EFFECT OF PROLONGED AGING ON THE CHARACTERISTICS OF MEAT FROM WATER BUFFALO (*Bubalus bubalis*)

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Buffalo meat may be a promising market due to its lipid composition, nutritive properties and excellent palatability attributes [1]. However, to increase this market it is desirable to develop new products. Aging is presented as an alternative to better the quality of meat from buffalo [2] but only few studies have treated this process in buffalo meat [3]. The aim of this study was to evaluate the effects of prolonged aging time (60 days) on the microbiological, chemical-physical and rheological characteristics of meat from water buffalo (*Bubalus bubalis*). Four animals (20–24 months of age) were slaughtered and the half-carcasses was stored at 0±3ºC for five days. Subsequently they were sectioned to obtained longissimus dorsi (LD) and semitendinosus (S) cuts. Samples were transported to the Laboratory of Food Inspection Unit of DMVPA, University of Naples Federico II, Italy. Each samples was aged in an incubator (Arredo Inox MATURMEAT®) at 1°C (Δ±2°C) and 78% (Δ±7%) relative humidity (R.H.) for 15, 30 and 60 days. pH and activity water (aw) were measured in each sample at 24h post mortem and at 15, 30 and 60 days aged. The measure of LD and S yield was calculated. Instrumental texture profile analysis (TPA), tenderness (Warner-Bratzler shear force) and meat color (CIELAB system) were measured. Moreover, total mesophilic (TVC 32 °C), coagulase positive staphylococci, *Enterobacteriaceae* (EB), *E. coli* were detected. Pathogens referable to genera *Salmonella* and *Listeria* were also researched. Nutrition declaration (European Regulation 1169/2011) was calculated. Results of texture analysis (WBS and TPA data) confirm that the buffalo meat, in particular longissimus dorsi (LD), subjected to prolonged maturation (up to 60 days) showed a high level of tenderness. The yield of LD and S cuts was 60 and 40 % respectively. Concerning nutritional value data showed an increase of the protein content. Regarding the color, the aging times did not affect the average values of lightness (L*) and redness (a*). Even if no pathogens were detected, pH and aw values (5.5 and 0.98) measured at the end of aging process were not sufficient to avoid growth of microorganisms (EB from <10 to 27ucf/g; TVC 32°C from 270 to 38000 ucf/g) on the contrary, this values should be a marked impact on the absence of yeasts and molds. Prolonged aging process implies complex changes in muscle metabolism mainly as a function of enzymatic proteolysis activated by suitable climatic conditions. However it is important to underline high weight loss and the increase of the trimming waste of sample analyzed that could be rewarded by high commercial value of this product.

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DYNAMICS OF VOLATILE MOLECULES IN RAW MILK FOR THE PRODUCTION OF PARMIGIANO REGGIANO AND GRANA PADANO CHEESES

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Milk characteristics in terms of volatile compounds can heavily influence the product organoleptic characteristics and can give indications about a number of metabolisms [1]. These features can strongly depend on feeding and the management of livestock [2]. In this perspectives, the screening of milk samples intended for Parmigiano Reggiano and Grana Padano located in Northern Italy was performed, focusing on a panel of volatile molecules. The work was carried out on a total number of 25 cows from 5 farms for the production of Parmigiano-Reggiano (PR, n=3) and Grana Padano (GP, n=2). Milk samples were collected monthly from May to September and submitted the same day to a headspace followed by a GC-MS analysis for the detection of volatiles. A panel of several volatile molecules was taken into account (aldehydes, ketones, alcohols, carboxylic acids, esters, aromatic hydrocarbons, solforates) Data were analyzed by a two-way ANOVA on logarithmic values, followed by the Tukey test for multiple comparisons. Results showed a significant influence of month and destination of milk; in particular, differences between destinations were calculated (in ng/g eq) for aldehydes (PR 24.1±45.2, GP 169.6±310.8, $p<0.05$), alcohols (PR 335.4±453.1, GP 2549.7±5223.7, $p<0.001$), carboxylic acids (PR 205.6±624.6, GP 313.8±584.3, $p<0.02$), aromatic hydrocarbons (PR 1.26±2.59, GP 0.02±0.08, $p<0.05$). Significant differences ($p<0.05$) between months were observed for all volatiles (included solforates) except for aromatic hydrocarbons. These preliminary results indicate clear differences between the two types of milk, probably due to the different feeding protocols imposed by production disciplinaries. The study of volatile molecules in milk will give important information about the physiology of milk and the evolution of dairy products - mainly fine cheeses - during ripening. These features must be extended and confirmed by the sensory analysis of raw milk and derived products, leading to a more complete characterization of milk biology and derived products.

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ANALYSIS OF THE CHEMOKINE RECEPTOR GENES VARIABILITY AND ASSOCIATION WITH SCS IN ITALIAN HOLSTEIN

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Selection for resistance to mastitis is crucial in many respects. First of all, mastitis is a major cause of economic loss in dairy farms. Moreover, a relevant problem is antibiotic resistance, defined by WHO (World Healt Organization) as one of the main causes of therapeutic failures, even though there are few relationships between use of antibiotics for the treatment of mastitis and antibiotic resistance. As regards the CXCR1 and CXCR2 genes, knowledge obtained to date has highlighted their importance in the immune response against infections of mammary gland. They code for the main CXC (chemokine, C-X-C motif) receptors of neutrophils and regulate the chemotaxis mechanism. As a result, many studies have focused on evaluating these two genes, both mapping on BTA2, to detect SNPs associated with susceptibility to mastitis [1]. The estimated breeding value (EBV) for somatic cell score (SCS) of Italian Holstein bulls born between 2002 and 2012 were considered and bulls with EBV>105 and EBV<95 were chosen. Two groups were thus formed: group A included 58 bulls with high EBV (average 108) and group B included 37 bulls with low EBV (average 92). DNA was extracted from semen and wide regions of both genes were resequenced by the NGS technique on the MiSeq (Illumina) platform at IGA Tecnology Services of Udine, which also carried out the processing of the output data and the call of variants and genotypes. Polymorphism identification was performed in R environment and the inferential analysis was carried out on the SNPs with a minor allele frequency (MAF)≥0.05. Three statistical tests, namely Wilcoxon-Mann-Whitney, Kruskal-Wallis, and heteroscedastic effects model (HEM) were performed to detect significant genotype frequency differences between the two groups. Alignment of the sequences of the investigated bulls with the reference sequence revealed the overall presence of 91 SNPs with MAF≥0.05, four of which were detected for the first time. Only two SNPs were significantly associated with EBV for SCS. The rs378981627 SNP, located on exon 5 of CXCR2, showed association using Kruskall-Wallis test, nevertheless it is a synonymous substitution. The SNP rs109694601, located on intron 1 of CXCR1, revealed a significant association with all the tests; the substitution C>T produces in silico two additional pre-miRNA sequences. Interestingly, 322 bp upstream from this point the rs41255709 SNP was previously identified [2], which showed to be associated with milk and milk fat and protein yield. This suggests that CXCR1 intron 1 is critical for health and production performances and may contain different quantitative trait nucleotides (QTN) clustered in the same region. Our results provide an additional clue about the importance of CXCR1 as a candidate reference gene in resistance to mastitis.

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HUMAN MALNUTRITION AND ANIMAL HUSBANDRY IN DEVELOPING COUNTRIES. MILK AND EGG PRODUCTION AND CONSUMPTION IN MEGHALAYA STATE (INDIA)

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Developing countries (DC) face a spread food insecurity both for quantity and quality, which justify the malnutrition, particularly in very young children and pregnant-nursing woman that have higher needs [1]. The shortage of animal food origin, particularly milk and eggs, is the main cause. Thus, livestock is one of the most important activities to be improved to reduce malnutrition [2]. Unfortunately, animal husbandry in DC has a lot of limitations, such as animal husbandry knowledge, production means and services (feedstuff, health care-vaccinations, etc.). Furthermore, the local markets are often unable to guarantee shelf life and safety of their products. Finally, rural population ignores the importance of consuming animal source foods for their high nutritional values (namely protein, vitamins and microelements) [1]. To cope this situation, in 2011 a project named "Appropriate food production: sufficient, safe and sustainable" started in Meghalaya (India) [3]. The project settled one Pilot Center (PC) that provide basic means, knowledge of husbandry (especially for dairy cattle and chicken e.g. suggestion of vaccination and use of improved breeds as Kuroiler for hens and cross-breeds with Friesian for dairy cattle) and education on appropriate diet. The purpose of this paper is to describe the current animal husbandry situation in Meghalaya, particularly the milk production and consumption level. The bred species encountered in Meghalaya are cattle, poultry, swine and goats. At the beginning of the project, it appeared that cow milk is produced with an average of 151.3+/-84.9 kg per lactation and per family. Two-thirds of the milk is consumed as milk tea (even for children) and one-third is sold. To investigate the animal husbandry situation after 4 years of project running, a properly shaped survey has been carried out in 2016-2017 and 2017-2018, interviewing 200 families in Meghalaya (half beneficiaries of the project and half not). To compare results of the two groups of families ANOVA was performed using GLM with the software SAS version 9.3. The data of the two surveys showed that a small improvement has been observed for egg production: the yearly family availability was 60 for beneficiaries vs. 50 for non-beneficiaries respectively. The cattle situation remained unchanged: the average number of cattle is similar in beneficiaries and not: 2.57 and 2.32 per family (usually 2 oxen and 1 cow). In the last two cases there are no significative differences. In general, families sell not more than 20% of the milk production, meanwhile the 80% is used in family. Regarding to the milk consumption, beneficiaries consume averagely more milk than not beneficiaries: most beneficiaries consume 101-150 L/y, meanwhile most non-beneficiaries consume 51-101 L/y. The project families have learned to use more milk, but their production is still low because little can be changed in low genetic local cows. Actual research is focused on cross breed adoption (local breed x Frisian). First results seem encouraging because cross breed cows produced 5-6 vs. 1 L/day of local breed with a very small management improvement.

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POSTERS

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REVERSAL OF AFLATOXIN B1 CYTOTOXICITY BY NATURAL ANTIOXIDANTS IN A MOUSE LIVER CELL LINE

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AFB1, a widespread feed and food contaminant, can generate highly cytotoxic and carcinogenic metabolites like AFB1-exo-8,9-epoxide upon bioactivation by drug metabolizing enzymes (DME), mainly CYP1A and 3A subfamilies [1]. Factors modulating DME are therefore expected to affect AFB1 kinetics and toxicity. For instance, a number of natural antioxidants have been reported to inhibit CYP1A1 and/or to induce detoxifying and antioxidant enzymes with the potential to reduce the generation and/or increase the inactivation of AFB1 metabolites [2-5]. Since AFs are mostly metabolized in the liver, in vitro experiments were performed using a well characterized mouse liver cell line (AML12) to evaluate the role of selected natural antioxidants (i.e. Curcumin, Curcuminoids (composed by curcumin and its metabolites, demethoxycurcumin and bisdemethoxycurcumin), Quercetin and Resveratrol) in the modulation of AFB1 toxicity. In order to determine the concentrations to be used in the co-incubation experiments, AML12 cells were incubated with increasing concentrations of AFB1 (20-60 μM), Quercetin (0.12-50 μM), Resveratrol (0.125-50 μM), Curcumin (0.15-20 μM) or Curcuminoids (0.15-20 μM) for 24 and 48h. Cells were pre-incubated with Curcumin, Curcuminoids or Resveratrol at 5μM, or with Quercetin at 15 μM for 16h, and subsequently incubated with AFB1 (20-40 μM) in the presence of each antioxidant for 24 and 48h. Cell viability was evaluated by the WST-1 or Neutral Red Uptake assays (independently three times with six replicates for each experimental condition). The statistical significance ($P \leq 0.05$) was tested using the one-way ANOVA followed by Dunnett's or Bonferroni's post hoc test. As expected, AFB1 elicited a time and concentration-dependent decrease in cell viability starting from 20 μM at 48h whereas Quercetin, Resveratrol, Curcumin and Curcuminoids showed cytotoxicity at highest concentrations. Results showed that all tested antioxidants triggered a significant protective effect on liver cells albeit to a different extent. Quercetin ($P \leq 0.001$) was the most effective one followed by Resveratrol ($P \leq 0.05$), Curcumin ($P \leq 0.01$) and Curcuminoids ($P \leq 0.05$). This study suggests that natural antioxidants could be used as promising dietary supplements to protect animals and humans against the damage caused by AFB1. Further studies are ongoing to test the protective effects of the same natural antioxidants against AFB1 in other tissue cell lines, to check the possible influence of target tissue on the reversal of AFB1 toxicity.

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SOFIVET

EXPRESSION OF GENES INVOLVED IN THE DEVELOPMENT OF ANTRAL FOLLICLES DURING THE REPRODUCTIVE AND NON-REPRODUCTIVE SEASON IN PREPUBERTAL EWES

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The antral phase of follicle growth is characterized by temporally and spatially well-organized increases in the ability to respond to gonadotropins, supporting the growth of the dominant follicle to the periovulatory stage. There is evidence that follicle selection and growth are modulated by paracrine signaling. Paracrine factors are also critical for signaling between granulosa cells and cumulus cells for ovulation and maturation of cumulus-oocyte. Among these, the Bone Morphogenetic Proteins system such as BMP4, BMP 7, BMP 15, GDF9, act through the BMPR1A and B receptors, activating the SMADs factors, intracellular effectors of the paracrine response [1]. Our goal was to study the factors involved in the modulation of follicular development during the reproductive and non-reproductive season using prepubertal sheep follicles, in which the hypothalamic-hypophysis-ovarian axis is not yet active. The antral follicles were recovered from ovaries of prepubertal 1 month-old slaughtered sheep during the breeding (from October to November) and non-breeding (from February to April) seasons. RNA was extracted using the reagent trizol and retrotranscribed using polyT primers. Selected cDNAs were quantified by sybr-green Real Time PCR [2] using specific primers for FSHr, BMP4, BMP7, BMP15, GDF9, BMPR1A and 1B, SMAD9 and Stat 5. The differences were calculated using the DeltaDeltaCt method [3]. Our data showed that some genes were expressed differentially between the antral follicles during the breeding and non-breeding season ($P < 0.01$). In particular, during the breeding season, the expression of FSHr, BMP4, BMP15, GDF9, BMPR1B and SMAD9 in the antral follicles was greater than 3.1, 5.3, 3.2, 3.0, 5.0 and 5.3 times respectively compared to that of the non-breeding season. In conclusion, our results have shown that the photoperiod plays an important role in the modulation of follicle development, improving the expression of FSH receptors and factors of the BMP family during the reproductive season. The increase in the expression of these factors is a function of the direct action of the molecules involved in the photoperiod response, of which the main actor is melatonin, and are independent of the action of sex hormones. (Supported by Migliovingensar).

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ASSESSMENT OF CORTISOL AND DHEA CONCENTRATIONS IN THE GRIFFON VULTURE (*Gyps fulvus*) FEATHERS TO EVALUATE ITS HEALTH CONDITION

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During their life, birds face several challenges which can threaten the stability of physiological functions. These situations typically trigger a cascade of endocrine secretions involving the hypothalamic–pituitary–adrenal (HPA) axis, and resulting in the release of both cortisol and corticosterone also in birds. The use of a non-invasive approach to collect samples of biological material derived from natural populations represents a greatly combination for an improvement of knowledge avoiding handling animals. Steroid concentrations in feathers are mainly acquired from the capillary around the follicle during the long period of feather grown reflecting their bloodstream concentrations. Accordingly, a chronic HPA axis activation could be assessed using molted feathers cortisol concentrations. Another hormone, dehydroepiandrosterone (DHEA), is secreted by adrenals and it could be assessed in molted feathers. This study was aimed to evaluate the feasibility of using feathers cortisol and DHEA concentrations to provide a retrospective assessment of the activity of the HPA axis in griffon vulture acquired for health monitoring purposes. For this, we introduced two groups of animals with a known health condition: one group composed by griffons that have experienced a long-term physiologically compromised period (PC group; n=8) and the other group composed by griffons physiologically not compromised (CTRL group; n=9). The feathers cortisol and DHEA analyses were carried out using RIA [1,2] adapted for feathers. The results revealed different feather hormone concentrations between the two groups of animals. The feathers cortisol had a median value higher in the PC group than in the CTRL group, with cortisol concentrations showing a central value 1.6 times higher in those animals that suffered a long-term physiological impairment. The PC group showed also (Kruskal-Wallis test) higher feather concentrations of DHEA (P=0.01) than the CTRL group. Pearson's correlation coefficients were used to examine the associations between cortisol and DHEA, showing no correlation between cortisol and DHEA feather concentrations in the PC group (r=0.18, P=0.34) and a moderate positive correlation in the CTRL group (r=0.51, P=0.011). In conclusion, our study reveals that molted feathers can be an interesting way to evaluate the physiological status of wild animals by using a non-invasive approach. Our analyses reveal that in addition to cortisol also DHEA could be evaluated to better understand the relationships between these hormones and to determine the resilience condition of wild species.

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OREXIN MODULATION OF 17B-ESTRADIOL AND P450 AROMATASE IN THE TESTIS OF ALPACA (*Vicugna pacos*)

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In the male gonad, testosterone (T) and estrogens (E), expressed as 17β-estradiol, show a key role in spermatogenesis and steroidogenesis regulation. The biosynthesis of E is catalyzed by the irreversible aromatization of T by the enzyme P450 aromatase (ARO) [1]. To date, the localization of this steroidogenic enzyme has still not been detected in the male gonad of the South American camelid alpaca (*Vicugna pacos*). In the last years, this species gained an increasing zootechnical interest for the quality of the wool also in Italy. Orexins A (OxA) and B (OxB) are two peptides discovered simultaneously by two research groups directed by Sakurai and de Lecea [2,3] in the rat hypothalamus. The two isoforms of orexins result from the same precursor protein named prepro-orexin. The physiological effects of these two peptides have been mediated by the binding with their cognate receptors called receptor 1 (OX1R) and 2 (OX2R). OX1R is highly selective for OxA, while OX2R shows equal affinity for both orexins. For this purpose, the goal of this research was to investigate: a. the presence and localization of ARO in the alpaca testis, b. the effects of orexins on E and ARO synthesis. Immunohistochemistry allowed us to detect ARO localization in the tubular and interstitial compartment of the alpaca testis. The expression of ARO in tissue extracts was established by using Western blotting analysis. Finally, the effect of orexins on E and ARO modulation was investigated by means of in vitro cultured thin testis slices which were incubated with OxA alone, OxB and OxA, and the selective OX1R antagonist SB-408124. OxA decreased the 17β-estradiol levels. This effect was abolished by the sequential addition of the selective OX1R antagonist. OxB incubation did not affect E byosinthesis. Notoriously, OxA is involved in steroidogenesis and spermatogenesis regulation of the male gonad, in normal and pathological conditions [4,5,6]. With respect to orexins on steroidogenesis, OxA induced T secretion via interacting with OX1R, presumably mediated by a reduced expression of the innate T inhibitor Müllerian Inhibiting Substance (MIS), thereby increasing T synthesis [4,5]. On the other hand, OxA is also involved in down-regulating E secretion. The underlying mechanism is most likely due to subsided ARO activity, further disabling the conversion of T to E, consequently lowering E biosynthesis and increasing the production of T in mammalian testis.

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EFFECTS OF ENVIRONMENTAL ENRICHMENT ON THE IN PIG REARED IN INTENSIVE SYSTEM

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Pigs are active animals that require a proper environment to express their exploratory behaviour. The absence of enrichment can negatively affect their behaviour and consequently their welfare [1]. In fact, the EU Directive 2001/93/EC states that pigs should be provided with proper materials to satisfy their needs for investigation and manipulation. The aim of the present study was to assess the welfare through behaviour observations in pigs reared in conditions of intensive farming and provided with three different kinds of enrichment. The investigation lasted 7 weeks and involved 75 pigs divided in 3 Groups homogeneous for live weight (34.9±2.57 kg) and age (11 weeks) and reared in boxes with chains hanging from ceiling as basic enrichment. The first Group received a further enrichment composed by logs laying on the floor (LL), the second received an enrichment composed by hanging logs (HL), the third was maintained in the usual environment (C). After an adaptation period of one week, the animals were video recorded on Monday and Thursday for 6 weeks. Every day of observation comprised two sessions of 90 minutes each, one in the morning and one in the afternoon. The scan sampling technique was used [2] with observations recorded for 30 seconds every 5 minutes; behaviours were classified as “Active” (feeding, drinking, exploring and social activity) and "Inactive” (standing and lying). Behavioural sampling [3] was performed on the observation of pig interactions. Statistical analyses were carried out by ANOVA for each behaviour considering the environmental enrichment and the period of the day as variability factors. Interactions among pigs were analysed by a non-parametric test (Wilcoxon). The Results about behaviours did not show significant differences for the parameter “Active” and “Inactive”. Significant differences ($P<0.001$) were detected among the groups on parameters related to the social interactions. Specifically, in the C Group it was observed that pigs showed a higher incidence of head to head knock and bites, while belly nosing, considered a social-interaction behaviour [4], was higher in HL Group ($P<0.001$). In addition, in HL Group the overall aggressive behavioural patterns were significantly lower ($P<0.02$). The period of the day (morning or afternoon) resulted in statistical significant differences, with pigs of all groups being less active in the morning than in the afternoon ($P<0.01$). In conclusion, the use of hanging logs was more effective than logs laying on the floor in reducing aggression within the members of the group, which improved environment and animal welfare.

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BACTERIAL CONTAMINATIONS OF CELL CULTURES CAN BE DETECTED BY FLOW CYTOMETRY

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Cell culture control for bacterial contaminations is mandatory to enable correct cell growth. Contaminated cell cultures are often subvital, showing growth delay and unreliable responses to immunological tests. Nowadays, sterility control is carried out by microbiological methods, mainly in thioglycolate and trypticase soy broth liquid media incubated at 30 - 37°C over three days, which is hardly enough to detect low-grade microbial growth or slow-growing bacteria, let alone bacteria with complex growth requirements.

Owing to the above, we decided to develop a method to detect bacterial contaminations in cell cultures on the basis of the scatter characteristics of *E. coli*.

We used non-filtered and 0.22 micron-filtered cell culture samples. Samples were clarified and the supernatant was centrifuged at 10,000 g for 3 minutes; the pellet was resuspended in sterile saline and labeled with Baclight® live / dead kit containing Syto9 and Propidium iodide (PI) fluorochromes, to detect bacterial viability by flow cytometry [1,2]. The assays were carried out in a Guava EasyCyte HT flow cytometer (Merck Millipore), using Incyte software.

By setting two gates i.e. R4 (low fluorescence) and R5 (high fluorescence) in a RED H-log x GRN H-log plot and analyzing a positive control containing only bacteria, we could discriminate between sterile and contaminated cell cultures. The morphological picture of a cell culture negative for bacterial contamination was characterized by events in R4 with limited presence in R5 and an upward oriented diagonal of events, the ratio R5/R4 being generally <1. Sample filtration profoundly alters the R5/R4 ratio which always drops to values by far <1. Also new events appear in R4, probably particles degraded as a result of the mechanical filtration stress, e.g. autofluorescent extracellular vesicles damaged after filtration, incorporating less fluorochrome and moving from R5 upper right to R4 lower left. On the other hand, in a contaminated sample ≥97% of events are concentrated in R5 as a cluster; in the contaminated, filtered sample the events cloud moves from R5 to R4 with only 1-2% of events remaining in R5. Experimental evidence indicates that contamination of cell culture flasks with bacteria in log phase growth diluted 1:2000 can be detected just after two hours of incubation. In conclusion, early detection of bacterial contaminations in cell cultures is badly needed. In this respect, flow cytometry was shown to detect bacterial growth very early, well before the three day-period of a successful microbiological assay. The cytometric approach is substantially cost-effective, also on the basis of a large prevalence of false-negative samples in bacteriological assays.

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IL-1BETA: A POTENTIAL INDICATOR OF THE TOXICITY OF AUTOGENOUS VACCINES

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Interleukin-1ß (IL-1ß) is a pro-inflammatory cytokine mainly produced by activated macrophages and monocytes. The precursor (pro IL-1ß) is located at cytoplasmic level and must be cleaved by caspase-1 to generate the mature activated form [1]. Assessment of IL-1ß production by macrophages in response to vaccine antigens could help evaluate the safety and efficacy of the vaccine-induced immune response. Macrophages were obtained after differentiation of pig monocytes from peripheral blood mononuclear cells (PBMC) frozen in liquid nitrogen. PBMC were thawed quickly at 38°C and cultured at the concentration of 6 to 10 million cells/ml in RPMI 1640 medium. The suspension was seeded in 48-well plates and incubated at 37° to promote monocyte adhesion. After 2-3 h, a medium change was made with RPMI 1640 + 10% fetal calf serum (FCS) + Macrophage-Colony Stimulating Factor (M-CSF) at a concentration of 10 ng/ml to stimulate macrophage differentiation [2]. After 4 days of differentiation, the macrophages were incubated for 24 hours at 37 °C with different dilutions of bacterial vaccine antigens. In the tests, a negative and a positive control were included. The negative control consisted of RPMI 1640 + 10% Fetal Calf Serum (FCS), only, and the positive one consisted of 50 µL/well of LPS O:111 B4 at 10 micrograms/mL in complete medium, followed by 17 µL/well of 150 mM ATP after 4 h at 37°C. At 24 h of incubation the cellular supernatant was collected and frozen at -80°C. Samples were analyzed for IL-1ß by “Duo set ELISA for Porcine IL-1ß/IL-1F2” (R&D System). Preliminary results reveal that each macrophage population shows different levels of basic activation profile, shown by the levels of IL-1ß in the negative control. Most important, the sensitivity of macrophages to vaccine antigens was shown to vary depending on their own basic activation. Non-activated cells respond effectively to the antigen, whereas activated cells display a substantial tolerance, that could also be linked to the culture period before the assay. In fact, macrophages and monocytes, that are exposed to endotoxin are rendered “tolerant” and manifest a profoundly altered response when rechallenged with bacterial endotoxin [3]. It is therefore crucial for standardization to start from a batch of low-activation cells, to standardize the period of cell differentiation and to choose a batch of suitable FCS, in order to have representative and replicable data. The proposed work aims to present the potential of this methodology in the field of autogenous vaccine efficacy and safety control. This kind of evaluation may also pave the way to new studies on the effectiveness of the immune response and the risk of toxicity of autogenous vaccines.

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CHARACTERIZATION OF THE INTERACTION OF DIVERSE VIRULENCE AFRICAN SWINE FEVER VIRUS STRAINS WITH MACROPHAGE SUBSETS

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African swine fever (ASF) is a devastating disease for which there is no vaccine available [1]. ASF virus (ASFV) has a tropism for cells of the myeloid lineage, including macrophages and dendritic cells (DC) [2]. Despite the central importance of macrophages for ASFV pathogenesis and the polarising effects of classical and alternative activation on macrophage phenotype/function, there are very few studies of the interaction of ASFV with activated macrophages. We therefore conducted an *in vitro* characterization of the interactions of porcine monocyte-derived unactivated (moM), classically (moM1) and alternatively (moM2) activated monocyte-derived macrophages with ASFV strains of diverse virulence. Monocytes were differentiated using 50 ng of hM-CSF and were then left untreated or activated with IFN-gamma and LPS (moM1) or IL-4 (moM2). Cells were infected with 1 multiplicity-of-infection (MOI) of a virulent (22653/14) or a low virulence (NH/P68) ASFV strains, along-side mock infected control. Twenty-one hours post-infection (pi) the expression of ASFV proteins and surface markers were assessed with flow cytometry. At different time pi (3, 6, 9, 12, 21 hours) total RNA was extracted and retrotranscribed, then gene expression of IFN-gamma and 17 different IFN-gamma subtypes was determined by q-PCR. We observed that both isolates infected all the macrophage subsets, however NH/P68, but not 22653/14, down-regulated MHC class I and induced IFN-gamma gene expression. These results revealed differences between ASFV strains, suggesting that virulent isolates are able to evade host immune response and promote their survival in infected pigs.

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IN VITRO IMMUNOLOGICAL PROPERTIES EXHIBITED BY DIFFERENT FRACTIONS EXTRACTED FROM THE MICROALGA *Chlorella sorokiniana* IN A SHEEP MODEL

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Marine microalgae have been proven to modulate immune system, lipid metabolism, gut function, and stress resistance [1]. Animal health conditions primarily depend on quality of the administrated feed [2]; as a consequence, feeding animals with feed enriched with extracts from natural substances, such as microalgae, could reinforce their immune and health status. *Chlorella sorokiniana* (CS) is a unicellular microalga with the most suitable source of omega (ω)-3 and ω-6 PUFA; moreover, in the market of microalgae, CS is one the most diffuse together with *Spirulina* in relation to their high content of protein and nutritional value [3,4]. *Chorella* has been proposed as botanical food for its biological activities in order to reinforce human health [5]. The objective of the present experiment was to investigate the in vitro effects of the unsaponified fraction (UP), the acetylated unsaponified fraction (AUP), the total lipids fraction (TL) extracted and purified from *Chlorella sorokiniana* (CS) on proliferative response of sheep cells and on their cytokine profile. Peripheral blood mononuclear cells (PBMCs) from sheep blood were cultured at 37°C for 24 h and treated with the UP fraction, the AUP fraction, and the TL fraction, extracted and purified from CS. Cells were activated with Concanavalin A (ConA, at final concentration of 5 µg/mL) and Lipopolysaccharide (LPS, at final concentration of 1 µg/mL). For each fraction, 0.0 mg/mL, 0.4 mg/mL and 0.8 mg/mL were tested on PBMCs. Negative Control was represented by wells with 100 µL of PBMC suspensions without mitogens. Positive Control was represented by wells containing 100 µL of PBMC suspensions treated with ConA and LPS. Cell-free supernatants from each well were collected until ELISA for the determination of IL-10, IL-1β and IL-6. Bromodeoxyuridine (BrdU) assay was performed on cells to measure cell proliferation. Extracts from CS affected sheep PBMC proliferation and cytokine production. A strong inhibitory action on proliferation was registered by UP at 0.4 mg/mL concentration showing the lowest proliferative response with respect to all the other extracts. Furthermore, UP extract at 0.8 mg/mL concentration was also characterized by an increased IL-10 production. Conversely, TL fraction at 0.4 mg/mL showed a cytokine profile characterized by increasing of IL-10, IL-6 and at a lesser extent of IL-1beta secretion. In conclusion, a biological effect of CS extracts in sheep model has been demonstrated, which makes the microalga extract eligible for a clinical trial aimed at reducing the overuse of antibiotics.

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SCIENZE CLINICHE: SICLIM-VET

EVALUATION OF SELECTED FAECAL BACTERIAL GROUPS IN DOGS SUFFERING FROM CARDIOVASCULAR DISEASES

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Gastrointestinal dysbiosis has been associated to different diseases of the GI tract (in both man and animals), but also to pathological conditions related to other organs and apparatuses [1-3]. The aim of the present study is to move a first step toward the study of faecal microbiota (selected faecal bacterial groups) in dogs presenting cardiovascular diseases (CVDs), to determine whether, similarly to human and laboratory animal medicine [4,5], interesting correlations could be found. Seven naturally voided faecal samples from 7 dogs suffering from CVD (6 valvulopathies and 1 dilated cardiomyopathy), but without gastrointestinal signs, were collected. Bacterial DNA for the study of selected faecal bacterial groups (*Lactobacillus* spp., *Bifidobacterium* spp., *Enterobacteriaceae*, *Clostridium coccoides-Eubacterium rectale* group, *Staphylococcus* spp., and *Bacteroides-Prevotella-Porphyromonas* spp.) was extracted using a modified DNA extraction method based on benzyl chloride. SYBR Green Real-Time PCR amplification were performed. For each bacterial group, the total bacterial concentration was determined using 16S rRNA gene targeted primers [6]. Our evaluation was successful in 5 out of 7 dogs, but due to the low number of patients (inclusion/exclusion criteria were very selective) it was not possible to compare statistically subgroups in our sample. However, Student's *t* test was applied to compare the mean values of bacterial concentration of each detected group in patients of the present study with those previously obtained from healthy subjects (unpublished data). *Bacteroides-Prevotella-Porphyromonas* spp. and *Bifidobacterium* spp. counts resulted significantly lower ($p<0.05$) in the CVD dogs than in the healthy subjects, while *Lactobacillus* spp. and *Enterobacteriaceae* counts were significantly higher ($p<0.05$). Data from our pilot study, even if very preliminary, suggest that the evaluation of faecal composition in dogs suffering from cardiovascular diseases is possible and may provide interesting insights in cardiovascular diseases' pathogenesis, prevention and treatment.

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EVALUATION OF DIETARY INFLUENCE AND ABILITY OF HEMOCCULT® KIT TO DETECT OCCULT BLOOD IN FAECES OF HEALTHY DOGS.

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The guaiac-based faecal occult blood (FOB) test is still widely used for colorectal cancer screening in humans. In dogs, this assay has been reported to be able to detect FOB after oral administration of 20 mg of haemoglobin/kg body weight (mghgb/kgbw) of autologous blood [1]. Unfortunately, different diets have been reported to influence guaiac-based FOB tests [2,3]. The aims of this work, using the Hemoccult® assay, were: 1) to evaluate the ability to detect FOB in healthy dogs and to assess the influence of two diets; 2) to establish the influence of the time between faecal sampling and test results; 3) to find the lowest canine haemoglobin concentration to achieve all positive tests. This work was approved by the local Ethics Committee (n.56961). Five healthy dogs were enrolled and each dog was fed initially (day1) with a meat-free protein diet (HA Purina®) for 28 days. On day6, day10, day14, day18 and day22 dogs were fed with progressive doses of autologous blood (5, 15, 20, 25 and 40 mghgb/kgbw, respectively). The faeces of each dog were tested with Hemoccult® assay the day before starting HA diet (day0) and every defecation from day4 to day28. From day29, dogs switched from HA to gastrointestinal diet (EN Purina®) with 8 days of wash-out. Thereafter, the same schedule described above was applied to each dog fed with EN diet from day35 (day before starting EN) to day63. During the study period, no extra foods were allowed, apart from fresh or whey cheeses. Two-month after, one out of 5 dogs was fed again with HA diet for 10 days and 40 mghgb/kgbw of autologous blood was administered on day5. Faeces were tested the day before starting HA diet and 6, 18 and 42 hours after the blood-added meal. For each of these latter three time points, 7 test cards were simultaneously mounted. For each set of seven cards, test cards were assessed every two days until 14-day after collection. Finally, canine whole blood (18.0 ghgb/dL) was progressively diluted in saline solution and each dilution was directly applied on a set of three test cards until a negative result was found. Only a descriptive statistic was applied to the collected data. For the first aim, a total of 185 Hemoccult® tests were examined. Twelve (6.5%) were positive and no association between positive tests and administered amount of blood was found. None of the blood-free stool specimens was positive. Regarding the second set of samples, only one resulted positive, which was collected 42 hours after the blood meal and developed 12 days after card preparation. Finally, 6.5 µghgb/mL was the lowest concentration of fresh blood able to achieve 3/3 positive tests. In conclusion, Hemoccult® was not influenced by both HA and EN diets, but its reproducibility to detect FOB in stools was unsatisfactory. Although, Hemoccult® was able to detect up to 6.5 µghgb/mL when directly added to the card, the individual blood digestion and bowel transit time might be play a role on its poor reproducibility.

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MULTICENTRIC EXTRAMEDULLARY PLASMACYTOMA OF THE LIVER IN A DOG

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Extramedullary plasmacytomas (EMPs) are benign solitary tumors frequently occurring on skin and oral cavity [1]. Other locations and multiple presentation of EMPs are rarely described and sometimes they appear aggressive based on their clinical behavior [2-4]. To our knowledge, multiple hepatic presentation of EMPs in dogs has not yet been reported. A seven-year old, intact, female Jack Russell Terrier dog presented for evaluation of mammary nodules. Abdominal ultrasound revealed a 1 cm hypoechoic nodule on the left lateral hepatic lobe (LLHL). A 4 mm punch biopsy was performed during a laparoscopic ovariectomy and a lumpectomy was performed to remove two mammary nodules. Histological examination revealed two mammary adenomas and a hepatic round cell tumor suspected to be of plasma cell origin. Neoplastic cells stained positively with Methyl Green Pyronine, confirming a diagnosis of plasma cell tumor. Complete bloodwork, serum protein electrophoresis, and urinalysis were normal. A full-body computed-tomography (CT) scan showed a 1 cm nodule on the LLHL and a 5 mm nodule on the cranial aspect of the right medial hepatic lobe (RMHL). An exploratory laparotomy was elected and an adherence between the LLHL nodule and the gastric body wall was detected. A partial gastrectomy and a partial lobectomy of the LLHL were performed to treat the left plasma cell tumor. An in-house intraoperatory fine-needle aspiration (FNA) to rule out a plasma cell tumor on the RMHL nodule and an 8 mm punch excisional biopsy were performed. Histological examination revealed a multinodular plasma cell tumor with vascular invasion and incomplete margins on the RMHL nodule. Neoplastic cells invaded the fibrous tissue connecting the LLHL nodule and the stomach. A diagnosis of multiple hepatic plasma cell tumor versus multiple myeloma was formulated. The dog was treated for 6 months with melphalan and prednisone and a fully-body CT scan showed complete remission after 10 months after diagnosis. Non-cutaneous, non-oral EMPs are uncommon tumors in dogs. The hepatic localization has been reported only once and was associated with metastases and death 20 months after surgery [5]. Even if multiple cutaneous plasmacytomas are associated with aggressive multiple myeloma [4], a multicentric oral presentation in three dogs treated with surgery alone has been associated with good outcome [3]. In the present case, a chemotherapy was elected due to the aggressive histopathological features. Neither peripheral blood cytopenia nor monoclonal gammopathy nor bone lysis were observed. Although a bone marrow evaluation was not performed, the overall clinical case and laboratory tests strongly support the diagnosis of EMP. To our knowledge, this is the first description of canine multinodular EMPs affecting the liver, and aggressive therapy seems to be associated with better results.

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ULTRASONOGRAPHIC ALTERATIONS IN DOGS AT DIFFERENT STAGE OF CKD

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Although the International Renal Interest Society (IRIS) bases on serum creatinine to stage dogs with chronic kidney disease (CKD) [1], renal ultrasound plays a fundamental role in the diagnostic and prognostic evaluation of dogs with CKD [2]. In human medicine, a significant correlation between the degree of kidney dysfunction and renal ultrasound abnormalities was documented [3]. The aims of the present study were 1) to retrospectively assess the most frequent renal ultrasound abnormalities for each IRIS stage of CKD, and 2) to evaluate correlation of ultrasound abnormalities with progression of CKD. We retrospectively included 865 dogs (January 2010 to December 2016) with diagnosis of CKD at different IRIS stage, which presented an abdominal ultrasound evaluation within 15 days from biochemistry panel. Dogs with diagnosis of acute kidney injury (AKI) or CKD dogs with no ultrasound examination or with an ultrasound examination over 15 days from biochemistry panel were excluded from the study. Dogs in IRIS stage 1 of CKD were excluded from the study, due to the low number of cases. The following ultrasound parameters were considered: renal profile, cortico-medullary junction, cortico-medullary ratio, echogenicity of the cortex, echogenicity of the medulla, echotexture, presence of cysts, mineralization, infarcts, pelvic dilation, peri-renal effusion. 337 dogs (39%) were in IRIS stage 2, 295 (34%) were in IRIS stage 3 and 233 (27%) dogs were in IRIS stage 4. The most common renal ultrasound abnormalities were related to cortical echogenicity, cortico-medullary junction and dilatation of the pelvis. With the worsening of the IRIS stage, the number of ultrasound alterations statistically increased ($p<0.0001$). According to the IRIS stage, a statistically significant difference was found in the percentage of dogs presenting alterations of the renal profile ($p=0.0185$), cortical medullary junction ($p=0.0035$), cortical medullary ratio ($p=0.0049$), cortical echogenicity ($p<0.0001$), medullary echogenicity ($p=0.0018$), echostructure ($p=0.0030$) and pelvic dilation ($p=0.0018$). No correlation between elevated Ca X P product (>60 mg2/dl2) and presence of kidney mineralization was found. The percentage of dogs presenting kidney mineralization was not statistically different among the different IRIS groups.

Despite association between elevation in Ca x P product and mortality has been demonstrated in CKD dogs [4], in our cohort of dogs elevated Ca x P product was not associated with increased risk of kidney mineralization. Although the number of renal ultrasound abnormalities increases with the progression of CKD, ultrasound abnormalities do not seem to be helpful for the clinician to discriminate the severity of CKD.

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SURVIVAL TIME ANALISYS IN DOGS AFFECTED BY MIXOMATOUS MITRAL VALVE DISEASE TREATED WITH DIFFERENT PROTOCOLS: RETROSPECTIVE STUDY

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The management of myxomatous mitral valve disease (MMVD) in dog, belonging to any ACVIM class, should be focused to obtain a good quality of life and a longer survival time [1]. The literature referred the effects of different therapies on survival time in dogs selected with restricted criteria (prospective cohort study) [2,3]. The aim of our study was to analyse the survival time in a retrospective cohort of dogs with MMVD treated with different protocols. Medical records from 2001 to 2016 were retrospectively reviewed. Inclusion criteria were: diagnosis of MMVD, ACVIM C, any weight, breed, gender, age and detailed information on treatment. 161 dogs (104 M and 56 F, mean age 12 y) were included, of whom in group 1 were 80 (Furosemide+ACE-I), in group 2 were 53 (Furosemide+ACE-I+Pimobendan), and in group 3 were 28 (Furosemide+ACE-I+Pimobendan+Spironolactone). The end-point was the cardiac death, and the Median Survival Time (MST) was calculated (Kaplan Mayer and Log rank). The influence of clinical signs, echocardiographic parameters and therapy on MST was investigated by univariate and multivariate analysis (backward Cox regression analysis), and a multiple comparison among the therapeutic groups was performed. The MST was: group 1=23.7 months (95%CI 17.6-30), group 2=17.7 months (95% CI 15-21) and group 3=16.4 months (95%CI 12-21). Multivariate analysis showed that Asx/Ao, Age and E/A had significance effects on the survival time [4]. MST in this study resulted longer than the MST reported by literature, despite the administration of more standardized protocols [2,3]. This study shows that different protocols have no influence on MST (P=0.239), nevertheless groups 2 and 3 showed more severe presentation. The lengthening of MST and a good quality of life (QoL) are significant aspects of the therapeutic strategy. Both are very important for the owners. The achievement of a longer MST in our study compared to the literature might be justified by a good compliance of the owners over time, even in the case of complex protocols (group 3) [5]. Prospective studies are needed to investigate the effects of the owners compliance besides the therapeutic protocol on the QoL and survival of MMVDiseased dogs.

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CONTRAST–ENHANCED ULTRASONOGRAPHY OF THICKENED INTESTINAL MUSCULAR LAYER IN CATS WITH INFLAMMATORY BOWEL DISEASE

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An ultrasonographic (US) pattern of thickened muscular layer (TML) of the small intestine has been associated with gastrointestinal lymphoma and inflammatory bowel disease (IBD) in cats [1]. In humans, contrast-enhanced ultrasonography (CEUS) is widely used to evaluate the vascularization of the gastro-intestinal wall in neoplastic, inflammatory and ischemic diseases [2]. The aim of this study was to describe the use of CEUS in cats with IBD associated with small intestinal TML. Six cats with gastrointestinal signs, US diffuse intestinal TML and histologically confirmed IBD were recruited. For the CEUS examination a high frequency linear array probe (L3-9 MHz) and a second-generation contrast medium (Sonovue®; Bracco diagnostic, Milano, Italy) were used. CEUS was performed following a standardized technique, as previously described [3]. For quantitative analysis three region of interests (ROI) were placed in the intestinal wall: one including all the layers from serosa to mucosal interface (“entire wall”); one including submucosal and mucosal layers (“inner wall”) and the third including only the TML. A one-way ANOVA followed by a Tukey's Multiple Comparison test were used to compare the peak intensity (PI) value of the different ROIs. P values < 0.05 were considered statistically significant. The mean thickness of the jejunal wall and the muscular layer was 3.73 mm (SD ± 0.4 mm) and 1.75 mm (SD ± 0.49 mm) respectively. On CEUS, jejunal arteries were identified and the enhancement of the jejunal wall progressed in a centripetal direction. A rapid enhancement of the serosal layer was followed by a strong enhancement of the submucosal layer progressively followed by a gradual enhancement of the mucosal layer. The TML was only crossed by several small vessels starting from the serosal layer and directed into the submucosal layer. At PI there was a homogenous increased echogenicity of the whole intestinal wall with a lack of enhancement of TML. PI of the “muscular layer” was significantly lower compared to that of the entire and inner wall ($p< 0.01$ and $p<0.001$ respectively). The lack of contrast medium enhancement of the TML is likely related to hypertrophy of the muscle cells documented histologically in our cases. Smooth muscle hypertrophy of the small intestine is commonly reported in cats with chronic enteritis and this association suggests that factors released during intestinal inflammation may also act as hypertrophy stimuli for smooth muscle cells [4]. On the basis of our preliminary experiences CEUS appeared to be useful for the characterization of intestinal TML in cats with IBD. The lack of contrast medium uptake of the muscular layer associated with intense enhancement of submucosal/mucosal layers were consistently identified in the intestinal wall of our cats.

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EFFECT OF CLIMATIC VARIATION ON HOSPITAL ADMISSION AND OUTCOME IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE AND NEW ONSET PULMONARY EDEMA

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The effect of seasonal variation on hospital admissions and outcome in humans with cardiovascular disease and congestive heart failure (CHF) has been described [1,2]. Little information is available regarding similar effects in dogs with myxomatous mitral valve disease (MMVD). Indeed, only one study evaluated the circadian and seasonal presentations of 119 dogs with congestive CHF caused by various cardiovascular diseases and manifesting with different clinical presentations including pulmonary edema, pleural or pericardial effusion, ascites, and dyspnea [3]. The aim of the present study was to evaluate the effect of the climatic variation on admission and outcome in dogs with MMVD and first onset CHF.

Ninety-six client-owned dogs with MMVD and a first occurrence pulmonary edema were included in this clinical cohort study. Recorded clinical and echocardiographic variables were cumulated and analyzed with dogs allocated into groups according to a temperature-wise manner considering the mean of the average (Tave) and maximum ambient temperature (Tmax) of the 14 days preceding hospital admission. A survival analysis was also performed.

Tmax but not Tave significantly influenced both the prevalence of CHF admission and survival of affected dogs. In particular, 44 dogs (45.8%) developed CHF during the hot climate while 21 (21.9%, $P<0.001$) dogs and 31 (32.3%, $P=0.073$) dogs developed CHF during the cold and intermediate climate, respectively. Dogs developing CHF during the hot climate lived longer (median survival time 518 days, 95% CI=159–876 days; HR=0.536, 95% CI=0.305-0.942) compared to those decompensating during the cold-intermediate climate (median survival time 280 days, 95% CI=104-456 days; $P=0.028$).

Results of the present study showed that high Tmax but not Tave in the preceding 14 days is more frequently associated with first occurrence of CHF in dogs with MMVD. Although the exact cause of seasonality in CHF outcomes is not clearly understood, dogs developing CHF during the hot climate have a better prognosis compared to those developing CHF during the cold-intermediate climate.

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PULMONARY VEIN-TO-PULMONARY ARTERY RATIO IN THE HORSE: ECHOCARDIOGRAPHIC TECHNIQUE AND REFERENCE INTERVALS

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The pulmonary vein (PV) to pulmonary artery (PA) ratio (PV/PA) has been evaluated in dogs and cats as an echocardiographic index to identify congestive heart failure and pulmonary hypertension in canine myxomatous mitral valve disease [1,2] and feline cardiomyopathies [3]. In healthy dogs and cats the PV/PA is approximately 1.0 and 0.5-0.6, respectively. However, to the investigators' knowledge, echocardiographic assessment of PV/PA in horses with and without heart disease has never been described. Therefore, we assessed the feasibility of measuring PV and PA dimensions, and sought to provide reference intervals in clinically healthy horses that could be used to assess cardiopulmonary disease status. Sixty-six healthy horses were prospectively recruited. Horses were considered healthy based on history, clinical and echocardiographic examination. The ostium of the right PV and right PA were measured at the minimal and maximal diameters from M-mode images obtained from a modified right parasternal long axis view, as previously described in the dog and cat [1-3]. The aorta was measured from the right parasternal short axis at the onset of diastole. We obtained the echocardiographic view and the PV and PA measurements in all horses. The M-mode tracing of the PV and PA in horses was characterized by multiple phasic deflections, similar to that described in dogs and cats. Reference intervals were as follows: PVmin/PAmin – 0.32 to 1.00; PVmax/PAmax – 0.40 to 0.89; PA distensibility [(PAmax-PAmin)/PAmax X 100] – 8% to 41%; PV distensibility [(PVmax-PVmin)/PVmax X 100] – 9% to 50%; PVmin:Ao – 0.11 to 0.28; PVmax:Ao – 0.18 to 0.40; PAmin:Ao – 0.23 to 0.47; PAmax:Ao – 0.35 to 0.58. Our study demonstrated the feasibility of measuring PV and PA diameters using transthoracic echocardiography and provides reference intervals for various pulmonary vascular variables in healthy horses. Whether these indices will allow clinicians to more precisely diagnose and manage horses with heart disease remains to be determined.

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EFFECTS OF *Boswellia serrata* SUPPLEMENTATION ON EX VIVO IMMUNE RESPONSES IN HORSES

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Frankincense, the gum resin of *Boswellia serrata* (BS), has been used for centuries in central Africa and in the Middle East as a remedy for many health problems, especially chronic inflammatory diseases [1]. In recent years, the extracts from the gum resin of BS have been also shown to target both the humoral and adaptive immune responses in mice [2,3]. To date, the effects of an oral administration of BS in horses has not been investigated. Purpose of this preliminary work was the evaluation of immune responses in ex vivo peripheral blood mononuclear cells (PBMCs) of horses supplemented with BS. Eight clinically healthy Italian trotters have been engaged. Animals were divided in two groups (2 males and 2 females/group, aged 2-5 years): one group received the BS complementary food (APA-CT SRL Line GREENVET, FC) in the form of a syringe paste (60 g), together with the feedstuff, twice a day (BS). The second group (CTR), at the same time intervals, received only the formulation of the paste in syringe without BS. This formulation, developed to be palatable, contained dry extract of BS titrated to 65% of Boswellic acids (5 mg of Boswellic acids/syringe for a total of 10 mg / day). PBMCs were isolated from two fresh heparinized venous blood samples (10 mL/horse) collected before the start of supplementation (T0) and 20 days after (T1). PBMCs were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE) cell tracer, pulsed or not (negative control) with either 1 μg/mL of pokeweed mitogen (PWM) or 1.2 μg/mL of phytohemagglutinin (PHA) or 5 μg/mL of concanavalin A (Conc A) and cultured for 5 days at 37°C in 5% CO_2. Flow cytometry analyses were performed on a standard FACSCalibur flow cytometer (Becton Dickinson) running the CellQuestPro software. The results of the lymphocyte proliferation assays were expressed as percentages of proliferation (LP%) and as lymphocyte proliferation index (LPI) in which LPI was calculated according to the following formula: LPI = (FV-BV)/BV*100, where FV is the proliferation value of cells pulsed with the different mitogens and BV the proliferation value of the same cells without mitogens. Data, reported as mean ± SEM were analyzed using supplementation (CTR and BS), mitogens (PHA, PWM and Conc A) and sampling time (T0 and T1: ST) or their interaction (IA) as fixed factors and IBM® SPSS Statistic 23 Software for GLM analysis. PBMC responses, at T1, significantly increased in BS group (39.3 vs 29.9, 2.2; $P<0.005$), regardless of the mitogens adopted. Furthermore, a significant IA 'supplementation*ST' was observed ($P<0.001$). Since in BS group also the not pulsed cells gave significantly higher responses, the LPI was significantly lower at T1 in BS vs CTR group (702.1 vs 1898.2, SEM 372; $P<0.05$). This preliminary study suggests that BS is able to modulate the ex vivo immune responses in equine species by increasing the immune responses in cells either pulsed or not by mitogens.

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LACTOBACILLUS ACIDOPHILUS AS A PROBIOTIC IN HEALTHY ADULT CATS

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Probiotics are defined as live microbial feed supplements, which can improve the gastrointestinal microbial balance in animals fed with this dietary supplementation [1]. The bacterial species most often used as health-promoting probiotics (i.e *Lactobacillus* and *Bifidobacterium* spp.) have the function to improve the weight of production animals. Furthermore, probiotics differ in their effects depending on the animal species considered. In literature, several studies have investigated probiotic usage in dogs [2], but few in cats [1,3]. The purpose of this report is to perform an evaluation of a feed additive based on Lactobacillus acidophilus in ten healthy adult cats.

Ten cats (7 females and 3 males) randomly assigned to two groups (C: control and T: treated), were fed with a nutritionally complete commercial diet for a period of 42 days; after the first seven days the probiotic strain was added to the T group diet for the remaing 35 days. The probiotic strain was incorporated into the diet to an inclusion concentration analyzed at $5x10^9$ CFU/kg-1. At day 0, 14, 28 and 35 each animal was monitored for its nutritional status giving it a body weight (BW) and body condition score (BCS). Fecal characteristics were assessed by fecal score (FS) and fecal moisture (FM) at day 1, 14, 28 and 35. Fecal bacterial populations were enumerated using selective bacterial culture, in particular Eosin Methylene Blue Agar to identify *E. coli* and total Coliform and de Man Rogosa Sharpe Agar for Lactobacilli (at day 7 and 28). ANOVA, Kruskal-Wallis and Wilcoxon tests were used to analyze data using a commercial statistical program (SAS® version 9.4; SAS 2013; SAS Institute Inc., Cary, NC, USA). Only $p \leq 0.05$ was considered to be significant and $p \leq 0.10$ as a trend.

Cats remained in good health conditions throughout the whole experimental period. No variation of BW or BCS was found. Fecal moisture consistency was significantly lower throughout the trial in T group than C group ($P=0.048$). A similar trend was found for the FS parameter. Even though there were no differences between treatments, Coliform populations decreased and Lactobacilli increased more in T group than C group.

Although the present study has yielded interesting findings, its design is not without flaws, in particular the low number of cats included in the trial. Despite these limitations, we found that supplementation of Lactobacillus acidophilus in healthy adult cats can improve their fecal consistency. More experiments and clinical trials are needed to better identify other probiotic bacterial species, their minimum effective dosages, the potentially adverse effects and their impact on pet well-being.

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REGIONALLY BASED LUNG ULTRASOUND EXAMINATION IN DOGS NATURALLY INFECTED WITH *ANGIOSTRONGYLUS VASORUM*: A PRELIMINARY STUDY

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Angiostrongylus vasorum (*Nematoda, Metastrongyloidea*) is a molluscan-borne parasitic nematode that has been recognized as a cause of cardiopulmonary disease and coagulopathies in dogs. Early and accurate diagnosis is fundamental but difficult because clinical, laboratory and diagnostic imaging findings are not specific [1]. Lung ultrasound (LUS) has shown potential as an important screening test and monitoring tool for the diagnosis and management of respiratory distress or with respiratory disease [2]. The purpose of this study was to establish the usefulness of regionally based LUS examination to describe the artefacts representing different states of pulmonary interstitial-alveolar infiltration or consolidation in dogs infected by *A. vasorum*. Between January and March 2018 four dogs with the diagnosis of *A. vasorum* infection (Baermann's test) were evaluated. For each dog clinical signs (i.e. coughing, tachypnea, and dyspnea), hematology, biochemistry and coagulation profiles, radiographic, and echocardiographic findings were recorded. LUS was performed before (T0), and 30 days after (T30) the treatment with imidacloprid 10%/moxidectin 2.5% (Advocate®, Bayer Animal Health), based on the Veterinary bedside lung ultrasound examination (VetBlue) protocol described by Lisciandro et al. (2014). LUS severity score: normal (0), mild (1), moderate (2), severe (3), was assigned by the evaluation of LUS artifacts (B-lines, Nodules [Nds], consolidation with aeration [Shs], consolidation with no aeration [Ts]) and their prevalence in VetBlue regions. One author-sonographer (GA) performed LUS examinations in all dogs using the same 10 C microconvex 4-10 MHZ ultrasound probe (Logiq S8 Vet GE). At T0 1/4 dogs showed severe clinical signs (coughing, and dyspnea) associated with severe radiologic changes (peripheral alveolar pattern, nodular pattern and lung consolidation affecting areas in all lung lobes). LUS score value was 3, with the prevalence of Shs and Nds findings in all regions of VetBlue examination. Three of 4 dogs showed coughing, and radiologic changes including nodular interstitial and peripheral alveolar patterns (3/3 dogs), and single areas of lung consolidation (2/3 dogs). LUS score values were 2 (2/3 dogs), and 1 (1/3 dogs). B-lines, Nds, and Shs were the prevalent LUS findings, with multiple regional distributions. No clinical signs were observed in all 4 dogs at T30, and Baermann test resulted negative. Radiographic features were substantially improved in all dogs. LUS score values were 0 (2/4 dogs), and 1 (2/4 dogs). B-lines, Nds, were the main LUS findings observed in a single location of the Vetblue examination. Based on the results of this study, regionally based Lung Ultrasound examination could represent a noninvasive diagnostic tool complementary to thoracic radiography for the evaluation, and monitoring of lung interstitial-alveolar infiltration and lung consolidation in dogs with *A. vasorum* infection.

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A RETROSPECTIVE STUDY OF THE TREATMENT AND FOLLOW-UP OF PRIMARY IMMUNE–MEDIATED THROMBOCYTOPENIA IN DOGS

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The primary immune-mediated thrombocytopenia (Idiopathic Thrombocytopenic Purpura, ITP) is a relatively uncommon hematological disorder in dogs. So far, there are only a limited number of studies about the treatment approach, the follow-up and the survival time (1-3). This retrospective study was arranged to collect in ITP cases: treatment approaches and responses during the 1st year of follow-up; the influence of therapy and dosages set-up by external referring vets in comparison to the response to the treatment prescribed in our Veterinary Teaching Hospital (VTH) in un-treated patients; the course of the disorder, follow-up, relapses and the survival of patients.

Twenty-five cases of ITP (May 2010 - December 2017) were included. Details about signalment, history including immunosuppressive treatments previously prescribed by external referring vets, and clinical signs were collected. The course of the disease, modification of therapy, relapses and survival at several times (days T0, T7, T14, T30, T60, T90, T180, and T365) were monitored and evaluated (Chi square or Fisher test). Patients treated previously for immune-mediated hemolytic anemia (n=5) or underwent to splenectomy (n=2) were separately evaluated.

Thirteen cases arrived at the VTH previously treated: 1/13 only cyclosporine 5 mg/kg/bid, 9/13 with corticosteroids, and 3/13 with combination of corticosteroids and another immune-suppressive drug (among the corticosteroid dosages used 7/13 have been treated with immunosuppressive doses and 5/13 with anti-inflammatory doses). The duration of the corticosteroids administration, the complete immune-suppressive therapy, the incidence of relapse and survival times were not statistically different between patients previously treated and those un-treated. The speed of the platelet count increase was higher during the first 7 days of treatment using corticosteroid. Indeed, if the platelet count at T0 was lower than $20x10^9$ /L, the platelet count increases by about 44 times at T7. If the platelet count at T0 was greater than $20x10^9$ /L, the platelet count increases by only 2 times at T7. The relapse of ITPs has been seen in 40% of cases, and the dosage tapering was the main cause. Only 1/25 death at about T30 was recorded. Splenectomy resulted an appropriate therapeutic option even if with low number of cases treated. The immune-suppressive therapy with inappropriate dosages did not influence the relapse or the survival of patients. On the contrary, the time for patients to respond to the treatment was extended and therefore was increased the development of adverse effects. The speed at which the number of platelets increases was depending on the basal count. The ITP cases showed a good prognosis within the initial one year of treatment and were not been influenced by the duration of therapy, drugs used, appropriate dosages or the occurrence of relapse.

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RELATIONSHIP BETWEEN BODY CONDITION SCORE AND EQUINE GASTRIC ULCER SYNDROME (EGUS) IN HORSES

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Equine gastric ulcer syndrome (EGUS) is a well-known disease of horses, with a high prevalence throughout all categories of animals. The last ECEIM consensus statement identified two clinical syndromes, one related to the squamous mucosa (ESGD) and the other to the glandular one (EGGD). Clinical signs are variable and usually involve different combination of weight loss, recurrent colic and exercise intolerance, among others, but a high number of animals are asymptomatic [1]. Despite these clinical signs, a correlation between EGUS and Body Condition Score (BCS) has not always been found in the literature [1,2]. The aim of this study was to verify a possible correlation between BCS and EGUS in a population of asymptomatic horses.

One hundred and five horses were enrolled in this prospective study. Gastroscopies were performed in all the subjects according to the literature [3] and presence and severity of gastric ulcers were recorded using the grading system proposed by the ECEIM Consensus Statement [1]. At the same time, BCS was evaluated by an experienced veterinarian according to literature [4]. Statistical analysis was carried out using “R” software (R Core Team, 2017).

EGUS was diagnosed in 80/105 animals (76%), ESGD lesions ranged from 1 to 4, EGGD was present in 16/105 horses, all positive also for ESGD. BCS ranged from 1 to 4. Statistical analysis showed no correlation between BCS and EGUS (Welch Two Sample t-test p=0.97), BCS and ESGD (Spearman p=0.54) and BCS and EGGD (Welch Two Sample t-test p=0.92). Based on these results, in our population of asymptomatic horses no correlation has been found between the presence of gastric lesions, neither on glandular nor on squamous mucosa, and BCS.

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CALVING AND EARLY LACTATION EFFECT ON METABOLIC AND HORMONAL PARAMETERS IN *BUBALUS BUBALIS*

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The increased metabolic demand leads to many changes in metabolites and hormones homeostasis during pregnancy and lactation period. Assessing blood metabolites concentration, such as Non-Esterified Fatty Acids (NEFA), β-hydroxybutyrate (BHB), glucose and insulin, could be a useful tool for providing indications on metabolic status and as a herd monitoring tool [1]. Besides, thyroid hormones determination could provide specific information on metabolism adaptation in the peripartum period for their important role in determining the cell metabolism intensity, the metabolism of lipids and carbohydrates, and the lactation course [2]. The aim of the present study was to evaluate the dynamic changes in the values of NEFA, BHB, glucose, insulin, Thyroid-stimulating hormone (TSH), Triiodothyronine (T3) and Thyroxine (T4) in buffaloes during the late pregnancy and early lactation. Also, we aimed to evaluate the relationship of the considered blood metabolites and thyroid hormones with milk yield and composition. Samples were collected from a total of 50 Italian Mediterranean Buffaloes. Blood samples were collected at -7±5 days before expected calving; +7±5; +30±5 and +50±5 days after calving. Milk samples were collected at the same post-partum time points. On serum samples, the values of NEFA, BHB, glucose, insulin, T3, T4 and TSH were evaluated. On milk, fat %, protein %, lactose %, somatic cells score (SCS), milk yield and daily milk production (DMP) were assessed. One-way analysis of variance (ANOVA) for repeated measures was used to determine a statistically significant effect of peripartum period on haematochemical parameters, and to verify the effect of calving distance on productive parameters and milk constituents. Bonferroni's multiple comparison test was applied for post-hoc comparison. Person's test was performed in order to assess significant correlations between haematochemical parameters and productive parameters and/or milk constituents. Peripartum period significantly influenced metabolic and hormonal parameters ($P<0.05$). Pearson correlation showed that milk yield was positively correlated with insulin ($r= +0.34$, $P<0.005$) and TSH ($r= +0.28$, $P<0.05$) values, negatively correlated ($P<0.005$) with NEFA ($r= -0.34$) and BHB ($r= -0.36$). Insulin was negatively correlated ($P<0.05$) with lactose % ($r= -0.26$) and SCS ($r= -0.25$). These results showed how the peripartum and lactation periods are characterized by marked changes in some metabolites concentration and in thyroid hormones values in Italian Mediterranean Buffaloes. Moreover, the relationship found between TSH values and milk yield seems to suggest a possible role of thyroid gland on the maintenance of lactogenesis. Our results highlight the importance of further knowledge on buffalo hormonal status during the transition period, to understand when a regulatory mechanisms adjustment fails predisposing the buffalo to metabolic problems.

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COMPARISON BETWEEN TWO CYTOLOGIC SYSTEMS FOR GRADING MAST CELL TUMORS IN DOGS

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Histological grading of canine cutaneous mast cell tumors (cMCT) is associated with overall survival, local recurrence rate and metastasis. Both grading systems, Patnaik (1) and Kiupel's (2), are routinely applied to histological specimens. A reliable cytological grading would be a useful tool for selection of therapy and prognosis prediction. Despite having many cytological grading systems with reported good specificity, sensitivity and accuracy (3-5), there is still no agreement among pathologists to accept one of them as a gold standard. Aim of this prospective study was to compare the ability of the cytological grading systems proposed by Scarpa (4) and Camus (5) to predict Kiupel grade. Clinically suspected and cytologically diagnosed MCTs were evaluated. Cases were included when the following criteria were present: histologic diagnosis of cMCT, Kiupel grade, adequate smear for cytological evaluation according to Scarpa and Camus. Histological grading was performed by two different pathologists. Cytological smears were blindly evaluated by one cytologist and the cytological grade was assigned according to the specific criteria and cutoffs suggested by Scarpa: (mitoses ≥1, multinucleated cells ≥3, bizarre nuclei ≥3 and karyomegaly) and Camus (granularity and/or presence of at least two of the following: mitosis, nuclear pleomorphism, binucleation or multinucleation and anysokariosis). Results were compared to Kiupel grade and diagnostic performances were calculated. Thirty-two MCTs of Kiupel low grade (n=23, 72%) and high grade (n=9; 28%) were included. Scarpa scheme correctly identified 18 low and 7 high grade cases with the following performances in recognizing high grade cMCT: accuracy 0.781, sensitivity 0.778, specificity 0.783, NPV 0.900 and PPV 0.583. Camus scheme correctly identified 18 low and 8 high grade cMCT. The additional high grade case correctly predicted by Camus compared to Scarpa was identified based on the evaluation of granularity as an adjunctive criteria. Diagnostic performances were: accuracy 0.813; sensitivity 0.889; specificity 0.783; NPV 0.947; PPV 0.615. Both methods showed good results even though accuracy was lower than previously described. The "granularity" criteria according to Camus may be useful to identify high grade MCTs. Cytologic grading of MCTs correctly predicts histologic Kiupel low grade in most cases but fails to predict many high grade cases. Thus, the proposed cytologic schemes did not appear reliable enough to substitute histologic grading. However, these preliminary results must be confirmed in a larger caseload.

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EVALUATION OF SEVERAL SUBCLINICAL MASTITIS PARAMETERS

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Mastitis induces milk losses, lower milk quality, higher treatment costs and increased the probability of premature culling and death of the cow affected by chronic mastitis [1]. Since many years, some different tests have been used for detection of subclinical mastitis, such as electrical conductivity (EC), California mastitis test (CMT), somatic cell count (SCC), teat end condition (TEC) and bacteriological examination (BE) [1]. The aim of this study was to evaluate how SCC and EC vary according to the intramammary infection (IMI) occurrence (healthy or infected by mastitogens) and to the status of TEC. Ninety-three lactating Holstein Friesian cows belonged to the same herd and 366 quarters were included. Animals evaluation and milk sampling were conducted during the milking session. Cows that showed clinical mastitis, defined as presence of udder or systemic alterations, were excluded from this study. Retrospectively, quarters were divided into healthy quarters (HQ) and infected quarters (IQ) based on BE. TEC score was performed for each quarter before the pre-milking routine [3]. Sterile samples were collected from each quarter for SCC and BE evaluation. EC was directly calculated by the commercial software of the milking machine (Afimilk, S.A.E. Afimilk, Israel). An ANOVA test was performed considering fixed effect of days in milking (DIM) (<100, 100-200, >200), parity (P) (primiparous, secondiparous, multiparous), BE (positive, negative), TEC (1, 2, >3), sampling (S) (1, 2) and random effect of animal. Quarters presented a TEC score >3 showed statistically significant higher SCC than quarters with TEC score 1 and 2, while TEC score did not show statistically significant differences with EC values. Samples BE positive showed a statistically significant higher SCC than those with BE negative. EC values did not differ between positive or negative samples for BE. SCC and EC values were significantly higher with increasing parity and DIM. High tecs might limit the efficacy of the post-dipping disinfection and predispose the bacteria colonization of the udder leading to high SCC and positive BE [3]. High tecs might be caused by the milking machine or management [3]. EC did not seem to differ between HQ and IQ. The sensitivity and specificity for detection of subclinical mastitis using EC were generally low [4]. EC in milk is influenced by temperature, fat concentration, milk solids and milk fraction [5]. This might explain the failure in separation of HQ and IQ. In conclusions, increased SCC and tecs might address to a IQ diagnosis, while EC's variations were not found in IMI. Further studies with a higher number of animals are recommended.

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WELFARE OF TRANSPORTED ANIMALS: MAIN PROBLEMS IDENTIFIED D URING OFFICIAL CONTROLS IN CALABRIA

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Over the years, the legislation on the protection of animals during transport has evolved continuously. Currently, Council Regulation (EC) No 1/2005 regulates the transport of live vertebrate animals carried out within the Community, including the specific checks to be carried out by officials on consignments entering or leaving the customs territory of the Community, and D.Lgs 151/2007 establishes the sanctioning provisions for the violation of the provisions of Regulation (EC) 1/2005 [1,2]. Despite the strict rules of the current legislation, road transport origins countless stress factors to animals. This would have a negative impact on animal welfare, leading to decrease in the quality of their products and in some cases to death of the animals.

The purpose of this retrospective study is therefore to assess the current welfare conditions during animal transport in Calabria and all related operations in the three years 2013-2015. The study was carried out through a statistical survey conducted on the data provided by the Ministry of the Interior - Department of Public Safety of the highway patrol for Calabria relating to inspections on animals, means of transport and enclosed documents, in the three years 2013-2015 in Calabria. The data obtained from the survey made it possible to verify that the control on the protection of animals during road transport and related operations, has increase over the years in Calabria. In fact, our survey shows that the checks carried out on means of animal transport, 107 in 2013, increased in the following two-year period (270 in 2014 and 268 in 2015) with a simultaneous reduction of the penalties, passed from 79 in 2013, 66 in 2014, and 69 in 2015. Furthermore, analysis of the data shows that on the total irregularities that emerged during the inspections, the most frequent were for documentation 74.8% and 12.6% for the means of transport. On the other hand, animal welfare irregularities (watering/feeding/travel and rest periods) have demonstrated a lower incidence (6.5%), the non-compliance of the transporter that did not respect the transport practices 4.2%, while the class of "Other non-compliance" reached 1.9%. The overall impact of sanctions (214) on total inspections (647) was 33.1%. Regardless of the type of transport, the persistent difficulty in satisfying the requirements of the accompanying documentation and the inadequacy of the means of transport are highlighted. In fact, these are the classes in which the non-conformities have been ascertained and which have resulted in sanctions and applications. The analysis of the irregularities detected during the inspections of the Calabria Regional Police on the protection of animals during transport in the three-year period 2013-2015, shows the need for the competent Authorities to continue in their inspection actions, as our results show that increasing controls reduces penalties.

[1] *https://eur-lex.europa.eu/legal-content/IT/TXT/?uri=celex%3A32005R0001.*

[2] *http://www.gazzettaufficiale.it/eli/gu/2007/09/12/212/sg/pdf*

COMPLETE RESPONSE OF A CUTANEOUS SQUAMOUS CELL CARCINOMA WITH ELECTROCHEMOTHERAPY IN A HORSE

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Squamous cell carcinomas (SCC) are one of the most frequent equine skin tumour. The surgical approach is considered the gold standard but sometimes a curative surgery is not possible, evaluating the tumoural dimension and/or the localization. Electrochemotherapy (ECT) is a new antineoplastic therapy that utilizes electrical field pulses to increase the cell membrane permeability to antiblastic drugs, such as cisplatin [1]. Some studies suggest it use in canine SCC [2] but more information are needed for horses for this tumour [3].

An Argentine 31 years gelding horse was referred at the teaching hospital of the Veterinary Medicine University of Turin. The horse, also affected by Pituitary Pars Intermedia Dysfunction (PPID) disease, was previously incompletely surgical treated for a SCC located in the ventral perineal area. The lesion recurred two months after and it was 5x7 cm, painful, ulcerated and inflamed. A second curative surgery was excluded considering the age and clinical condition of the patient and the neoplastic site. The ECT treatment was applied under epidural injection of 0.17 mg/kg of xilazine and 0.6 mg/kg of lidocaine.

An intra-tumoral injections of an aqueous solution of cisplatin (0.3 mg/cm^3, 27.47 mg) was followed by thirty-two electric pulses directly applied into the ulcerated area and in the 2 cm skin margins around the tumoral lesion. The horse recovered from sedation without difficulties. A slight edematous local reaction was visible two days after the treatment and it was treated orally with FANS (0.6 mg/kg of meloxicam for 8 days). No other local or systemic side effects were noted and the complete blood exam performed one month later did not report any hematologic toxicity (neutropenia, anemia o thrombocytopenia or renal toxicity). In the first month a necrotic area circumscribed the ulcerated tumor with a decrease of the local inflammation and a stabilization of the tumoral size. Two months after a single treatment, a granulation tissue replaced the necrotic area and a progressive decrease of the lesion size was evident. A clinical complete remission of the tumor was achieved 4 months after the treatment. At the last follow up control (320 days after treatment), the horse is still in clinical complete remission.

Our results demonstrate that a single ECT, also without concurrent tumor debulking, could be an effective alternative treatment for equine squamous cell carcinomas.

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USE OF CONTRAST-ENHANCED ULTRASOUND FOR ASSESSMENT OF NODULAR LYMPHOID HYPERPLASIA (NLH) IN CANINE SPLEEN

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Nodular lymphoid hyperplasia (NLH) is one of the most common non-neoplastic splenic lesions in dogs, especially in old ones, showing a splenic enlargement [1]. More recent studies have been focused on Contrast Enhanced Ultrasonography (CEUS) analysis of the spleen for establishing normal perfusion patterns and blood pool phase peculiarities of focal lesions [2-6]. Different enhancement patterns in the benign focal lesions of the spleen have been reported, with a more frequent isoechoic appearance of NLH to the surrounding parenchyma [4,5]. Morphological or functional modifications of the vascular network have been supposed to be responsible of the enhancement pattern variations in splenic lesions and in NLH a rearrangement of the splenic microvascular environment has been confirmed by histopatological examinations [7]. The aim of the study was to evaluate the qualitative and quantitative CEUS analysis of the canine splenic NLH, characterizing the CEUS pattern of this pathology on 20 clinical cases. The study was performed using a system equipped with contrast-tuned imaging technology. Mechanical Index was set from 0.08 to 0.11; the contrast medium was SonoVue®. Qualitative and quantitative assessment of the enhancement pattern of splenic NLH were performed. Cytology and histology identified 20 splenic NLH. During the wash-in phase (10-20 s) of the CEUS exam, all of the benign hyperplastic lesions assessed were isoechoic with a homogeneous pattern than the surrounding normal spleen. Starting 20-45 seconds from the contrast medium inoculation, 19/20 benign nodules became markedly hypoechoic to the adjacent spleen. Sensitivity of hypoechoic pattern for NLH was 95%. These findings should prove useful in the evaluation of focal splenic masses in dogs. Also, enhancement and perfusion patterns of NLH reported in this study seem to coincide with some neoplastic lesions of the spleen previously reported. Consequently, in clinical practice attention must be paid to the final diagnosis of canine splenic lesions using only the CEUS exam.

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2D AND M-MODE ECHOCARDIOGRAPHIC FINDINGS IN THE FIRST 5 DAYS OF LIFE IN HEALTHY FOALS

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In the first days after birth, the cardiovascular system undergoes substantial anatomical and hemodynamic changes adapting from the intrauterine to the neonatal life. The cardiovascular function can be easily monitored using echocardiography in newborn foals. To date, a few reports on normal echocardiographic reference ranges in newborn foals are present in the literature [1-3], but no data are available for Standardbred foals.

The aim of the study was to report two-dimensional and M-mode parameters during the first 5 days after birth in healthy Standardbred foals. Data were expressed ad mean±SD. Twenty healthy foals underwent transthoracic echocardiography within 24 hours (T1= 21±9hrs) and 4/5 days (T2= 106±22hrs) after birth. Body weight (BW) was recorded and two-dimensional standard images of the aortic (Ao, AoS) and pulmonary valves (PA, PAS) and M-mode of the right (RVDd and RVDs) and left ventricle (LVDd, IVSDd, LVFWd, IVSs, LVDs, LVFW) were obtained from right parasternal long and short axis views in left lateral recumbency, using a 1-5MHz phased-array probe optimized for each animal. Mean arterial pressure (MAP) and heart rate was simultaneously recorded using indirect oscillometry with the cuff positioned around the tail base. All the parameters were normal distributed and were analyzed using paired Student's T-test to verify differences between recording times and Pearson's correlation to verify relations between echocardiographic parameters and BW, HR or MAP. Main results were LVDd (T1= 51±7mm; T2= 55±6mmHg; P=0.016), LVFWd (T1= 9±2mm; T2= 10±2mm; P=0.048) and LVFWs (T1= 15±2.5mmHg; T2= 17±2mm; P=0.001) were increased over time. MAP increased over time (T1= 72.6±8.9mmHg; T2= 83.9±9.5mmHg; P=0.0006). No other differences were found between timepoints. LVDd was correlated to BW at T1 (P<0.0019; r^2=0.42) and T2 (P=0.0062; r^2=0.35), but not to HR or MAP. LVFWd and LVFWs were weakly correlated to BW at T1 (P=0.03; r^2=0.23). Echocardiographic differences found in this study could be interpreted as a consequence of hemodynamic changes (pulmonary pressure, ductus arteriosus) occurring during the first week of life. End-diastolic LV dimensions and MAP increase during the first week of life in healthy foals. Knowledge of physiologic and breed-specific changes is useful to interpret echocardiographic findings in both healthy and sick newborn foals.

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EVALUATION OF MINI-CUBE ESR (ERYTHROCYTE SEDIMENTATION RATE) IN DOGS AND CATS: PRELIMINARY RESULTS

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ESR (Erythrocyte Sedimentation Rate) in veterinary medicine has been replaced by the evaluation of some specific and sensitive markers of the acute phase of inflammation (i.e. C-Reactive Protein in dogs and Serum Amyloid A in cats) [1]. The aim of the study was to evaluate the ESR using MINI-CUBE equipment (ESR-MC) in dogs and cats. Blood samples from dogs (n=120) and cats (n=60) collected in 1 mL K3-EDTA tubes used primarily for blood counts were randomly selected. Each sample was assayed using ESR-MC (within 2 hours from sampling) and the gold standard Westergreen method (ESR-W) (within 4 hours from sampling) [2,3]. The ESR-MC was carried-out with the MINI-CUBE (DIESSE, Diagnostica Senese S.p.A., Monteriggioni, SI, Italy), an automatic continuous loading instrument analyzing up to 4 samples simultaneously, directly on the K3-EDTA tubes. Results (mm/h) were available in 20 minutes. Reference Intervals (RI) were assessed using the percentile method (2.5-97.5th) [4]. Accuracy was evaluated by Correlation test, R and Cohen Concordance test, K. Intra-assay precision (same sample measured 8 times) and inter-assay precision of ESR-MC (double reading of 80 canine and 25 feline samples) were performed and the Coefficient of Variation (CV) was calculated. Finally, the analytical Sensitivity (Se), Specificity (Sp), Positive Predictive (PPV) and Negative Predictive (NPV) values were calculated. Ten canine samples (8.4%) were ruled-out because of a flag (ERR) by the MINI-CUBE instrument (4.2%) or a diphasic pattern in ESR-W (4,2%). The canine RI of ESR-MC was ranging from 0 to 10 mm/h. Accuracy of the method was good (R=0.81, K=0.77). The agreement between the two methods slightly decreased in anemic subjects (Hct <37%) (K=0.69). Precision was excellent in intra-assay (CV=0.02) and inter-assay (CV=0.32). The analytical characteristics of ESR-MC in dogs were: Se=0.91, Sp=0.89, PPV=0.85 and NPV=0.96. Five feline samples (8.3%) were ruled-out because an ERR flag was issued by the MINI-CUBE instrument. The feline RI of ESR-MC was ranging from 0 to 11 mm/h. Accuracy was good, (R=0.85, K=0.83). Precision was excellent in intra-assay (CV=0.04) and inter-assay (CV=0.49). The analytical characteristics of ESR-MC in cats were: Se=1.00; Sp=0.83; PPV=0.87; NPV=1.00. The ESR-MC results can be obtained with the same K3-EDTA tubes used for the blood count, in short time, and at reduced costs. The accuracy is good enough to be applied in clinical settings. Further studies should investigate the ESR-MC in relation to clinical and laboratory inflammatory markers. Besides, it would be interesting to investigate if in canine and feline medicine, as in humans, ESR still has a diagnostic and prognostic value during infectious, immune and neoplastic diseases.

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CLINICAL REMISSION FROM DIABETES MELLITUS IN A 10 YEARS OLD AFRICAN GREY PARROT (*P. e. erithacus*) SUCCESFULLY TREATED WITH INSULIN THERAPY

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In avian literature the existence of true diabetes mellitus is debatable and the pathogenesis of disease in suspected cases is still unclear [1]. The goal of this study is to describe the first case of DM in a 10-years-old, male African Gray Parrot successfully treated with insulin therapy, with complete clinical remission. The patient was referred for vomit and ataxia, during the hospitalization polyuria, polydipsia and polyphagia were also detected. Laboratory results showed severe hyperglycemia (943 mg/dl), high values of ALT and CK, high level of fructosamine 466µmol/l (RR: 113-238µmol/l), mild anemia, normal amylase value 232U/l (RR: 200-384U/l) and glycosuria. In order to ruled out other causes of hyperglycemia such as pancreatitis, an endoscopy was performed, however because of bleeding, pancreatic biopsy was not performed and only no macroscopic evidences of hepatitis and pancreatitis were reported. Glycemia and glycosuria were monitored using Accu-chek Aviva and Kruuse vet-10 urine strips respectively. The suspect of DM was confirmed by the evidence of persistent hyperglycemia (>600mg/dl), glycosuria and high level of fructosamine. Treatment was set with a porcine lente insulin (Caninsulin 40U/ml diluted 1:10) at dosage of 0.1U/kg s.i.d. IM, in association with a high-fiber, low-sugar, and low-fat diet. After 3 days of treatment the glycemia was still higher than 600mg/dl and a fast-acting insulin (Humalog 100U/ml diluted 1:100) at the dosage of 0.1 U/kg IM s.i.d. was integrated to the therapeutic protocol. At day 11, since the high blood glucose fluctuations, 0,2U/kg of Humalog was injected when the glycemia was higher than 400mg/dl, while if glycemia was lower than 400 mg/dl, was injected 0,4U/kg of Caninsulin. Clinical conditions of the patient started to improve during this therapeutic protocol. At day 17, only Caninsulin was administered twice a day. At day 24 insulin therapy was stopped because, during blood glucose monitoring, hypoglycemia was detected(<80mg/dl). At day 36 the patient was discharged with periodic blood glucose monitoring; actually the patient is under strict control of the owner and has no recurrence of any clinical signs. Diabetes mellitus is a condition not completely characterized in avian medicine [2]. A presumptive diagnosis of DM in birds is based on clinical signs (polyuria, polydipsia, polyphagia with weight loss, and other nonspecific clinical signs), persistent hyperglycemia, and glycosuria [4]. Since birds can develop transient stress-related hyperglycemia, diagnosis of DM in birds should not be based exclusively on a single hyperglycemia detection [3,4]. Limited information are available regarding treatment protocol in case of DM in avian medicine. To the best of our knowledge this is the first case describe of successfully treated diabetes mellitus in an African Grey Parrot. Further studies are needed to better characterize the physiopathogenesis of DM in avian species in order to standardize the therapeutic protocol.

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SKELETAL MUSCLE METASTASES IN A DOG WITH NON-EPITHELIOTROPIC CUTANEOUS LYMPHOMA

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Cutaneous T-cell lymphomas (CTCL), epitheliotropic and, less frequently, non-epitheliotropic (NE), represent only 1% of canine skin neoplasms [1]. Muscle metastasis (MM) is rare in veterinary medicine and occur in 2-3% of the oncologic small animals. Furthermore, this localization is sporadically described only in cats [2]. A 5-year-old female American Staffordshire was referred for weakness, poor general conditions and distension of the abdomen. Three weeks before, the dog underwent surgery for nodular excision on the right shoulder region. Histopathological and immunohistochemical features were consistent with an NE-CTCL. Presence of free fluid was suspected on abdominal palpation, the laboratory works revealed only a mild leukopenia ($5.4x10^3$/µl, reference interval 6.0-17.0). Ultrasonographic (US) evaluation revealed a large amount of abdominal effusion and fluid collected was identified as a protein-rich transudate, while thoracic radiographs were within normal limits. Pre and post contrast total body computed tomography (TBCT) with 600 mg/kg of Iopamidol i.v. (Iopamiro®, Bracco Imaging S.p.A., Milan, Italy) revealed severe abdominal effusion, with heterogenous enhancing mesenteric masses and nodule lesions of soft tissue density, and infiltration of the abdominal muscular wall. Starting from the right inguinal region, from the area were the external, internal obliques and transverse muscle of the abdomen come together, a 3 cm heterogenous nodule was present. Moreover, a pattern of diffuse muscle nodules in the skeletal muscles was visible, with lesions showing homogenous, heterogenous or ring enhancement. Contrast enhanced ultrasound (CEUS) of the inguinal nodule did not add any information and showed peripheric strong contrast enhancement with a few vessels inside the lesion. Ultrasound-guided tru-cut biopsy with a 14G semi-automatic needle was taken from both inguinal muscle lesion and an intra-abdominal mass. Necrosis of muscular tissue was histologically observed. These necrotic areas were infiltrated by medium size lymphoblasts characterized by anisocytosis and large nuclei with finely distributed chromatin and irregular nuclear membrane. Neoplastic cells were CD3-positive and CD20-, CD79a- and Iba1-negative. On the basis of the immunopathological features metastatic CTCL was suspected. Skeletal muscles metastases are considered quite rare in humans and anecdotal in veterinary medicine, this is due to the fact that muscles have several protective mechanisms against metastatic invasion. When occurs, MM typically involve paravertebral muscle and hind limb, as observed in the present case. The most common primary malignancies were lung cancer, sarcomas, melanoma, renal cell carcinoma and breast cancer in humans [3] and sarcomas or carcinomas in dogs and cats [2]. In conclusion, although extremely rare, skeletal muscles should be considered as potential location of metastases of canine lymphoma.

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SCIENZE CLINICHE: SICV

EVALUATION METHODS OF ANALGESIC PROTOCOLS IN THE MURINE SPECIES

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Current use of analgesics in mice is low, but especially in model of spinal cord injury where there is currently no effective dose recommendation. Pain can lead to a decrease in baroreceptor reflex [1] and to a global change in gene expression [2] and can activate neuro-inflammation [3]. Therefore we hypothesized that pain stimulus might increase Substance P expression [4] and that such an increase could lead to organ fibrosis [5]. In this study, a systematic behavior analysis on mice, already scheduled for studies on spinal cord injury, was performed with the aim to describe and quantify the specific pain and stress suffered by animals during the procedure. 3 weeks of video observations (1 a week) were performed. The studies were performed to test a new analgesic strategy to decrease suffering in mice undergoing experimental Spinal Cord Injury and were designed as follows: 1 - one preliminary experiment with 12 controls; 2 - two successive studies with 6 controls and 6 treated each. Total number of animals therefore includes 24 controls receiving BUPRENORPHINE (0.15 mg/kg subcutaneously 15 minutes prior to surgery and again 24 and 48 hours after surgery) and 12 treated receiving 5 mg/kg CARPROFEN (subcutaneously 30 minutes prior to surgery) plus BUPRENORPHINE (0.15 mg/kg subcutaneously immediately after surgery and two further buprenorphine injections after 24 hours and 48 hours). All analyses used SPSS Statistics version 21 (IBM Co, Armonk, NY). Continuous variables are presented as means ± standard deviation (SD). At the end of the experiments a series of behaviour analysis and histological stainings were performed, in order to correlate possible pain relief to possible changes in organ histo-morphometry. We verified that an early multi-modal treatment with 5 mg/kg carprofen and 0.15 mg/kg buprenorphine (BUP+CAR) subcutaneously was more effective than buprenorphine alone (BUP) in decreasing peri-operative acute pain. Nevertheless, this particular model of chronic pain did not lead to clear increase in organ fibrosis. A non-significant decrease in inflammatory infiltration was observed in CAR+BUP treated mice with respect to BUP alone treated animals in spleen and heart. An unexpected presence of megakaryocytes in the spleen and of an hypo-perfused zone in heart papillary muscles, might indicate an initial process of organ functions decline. Further experiments are needed to better understand the possible correlation between chronic pain and neuro-inflammation in spinal cord injury. All experimental procedures were reviewed and approved by the Ethics Commitee of the Istituto di Ricerche Farmacologiche Mario Negri, in compliance with national Auth. no. 62/2014-PR Nov. 26, 2014 by Ministry of Health and EU Dir. 2010/63/EU.

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POST TRAUMATIC ANTRAL STENOSIS IN A CAT

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Gastric stenosis can be caused by cicatricial processes, tumors or hyperplastic inflammation [1]. Stenosis localized in the gastric antrum has never been described in cats, either because of congenital abnormalities or acquired diseases. This study aims to report a case of post-traumatic antral stenosis in a cat. A 9 months domestic shorthaired cat was referred because of persistent and refractory vomiting, anorexia and loss of weight. The patient was previously and successfully treated for a left pneumothorax after a presumptive road traffic accident, and an abdominal ultrasound (US) performed on that occasion detected gaseous infiltration of the wall of the stomach. Vomiting and anorexia occurred after resolution of the pneumothorax and persisted despite several days of supportive therapy with gastro-protective, prokinetic and antiemetic therapy. The cat was hospitalized and abdominal US was performed with an Esaote MyLab 70 XVG machine; the gastric fundus appeared mildly distended by food, its wall was mildly thickened (5 mm) with severe serosal irregularities, and a constriction between fundus and body was detected. To better characterize the abnormalities, plain computed tomography (CT) was performed, followed by upper G-I CT with Iopamidol (Iopamiro®, Bracco Imaging S.p.A., Milan, Italy), administered via gastric tube at a dose of 10 ml/kg, diluted 50% with water, and finally post contrast CT with the same contrast medium, 600 mg/kg, intravenously. A severe gastric stenosis between body and antrum with minimum contrast transit was detected, and confirmed by flexible endoscopic examination with a Stortz 60814 PKL machine. The antral localization of the stenosis was revealed after a cranial celiotomy and Y-U plastic was necessary to correct the defect. The patient completely recovered after surgery with significant body weight gain and remained in remission at a 2-years follow-up. We hypothesize that the antral stenosis was traumatic in nature because of the absence of clinical signs prior to the traumatic event. Different imaging modalities were necessary to achieve the correct diagnosis and define an adequate treatment plan, with post-contrast CT being the most useful in diagnosing this unusual condition, later confirmed by endoscopy. In human medicine, antral stenosis is described as a possible complication after endoscopic submucosal dissection for gastric epithelial neoplasm [2], but no information is available regarding a similar condition in the cat. To the best of our knowledge, this is the first case report describing a post-traumatic antral stenosis in a cat.

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COMPARISON OF TWO ANAESTHETIC PROTOCOLS FOR FIELD CASTRATION IN EQUIDS

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In equine field practice surgical procedures, such as orchiectomy, require a short term anesthesia. The most common anestethic protocol used in equine practice consist of an association of alpha2-agonist and a dissociative agent with a benzodiazepine [1]. The aim of the study is to assess the effectiveness of two intravenous anaesthetic protocols for castration surgery in equids. Fifteen male equids were randomly assigned to two groups. In group 1 (7 equids) general anesthesia was induced through intravenous administration of xilazine followed by a combination of ketamine and diazepam and were maintained with an IV combination of guaifenesin, ketamine and xilazine at constant rate infusion (triple drip).
In group 2 (8 equids) patients were sedated by intravenous administration of xilazine, followed by anesthesia induction through a combination of Tiletamine and Zolazepam IV and were eventually maintained with boluses of 1/3 of the initial dose.
In both groups a local anesthesia of the testis was performed with 2% lidocaine solution to produce local analgesia 10 min before surgery. Heart rate, movements during anesthesia, duration of anesthesia and duration of recovery phase were measured and recorded. Recovery phases were recorded with a video-camera and quality of recovery was evaluated by two blinded expert anesthetists.
Both protocols were able to induce and maintain anesthesia for the planned surgical procedure. No case presented unwanted side effects. There was no statistically significative difference between the two groups for heart rate, surgery duration or duration of the recovery phases. No statistically significant difference was found for quality of recovery. A statistically significant negative correlation was found between duration of the anesthesia and mean anesthetic score.
In conclusion both protocols resulted safe and effective in inducing and maintaining general anesthesia for the intended procedure. Recovery resulted smooth in both protocols. The combination of tiletamine-zolazepam is as effective as triple drip when used as an intravenous anesthesia protocol, but without the hazards and legal implications of ketamine-related use and transport.

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COMPARISON OF TWO TECHNIQUES FOR OBLITERATION THE NEPHROSPLENIC SPACE IN HORSES

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The closure of nephrosplenic space has been recommended for left dorsal displacement of large colon in the horses [1]. Closure can be performed in the standing horse using a minimally invasive laparoscopic technique [2]. The objective of this study was to compared two different techniques of laparoscopic closure of the nephrosplenic space in horses.

Ten horses were included in the study and divided in two groups. In all animals the nephrosplenic space were closed in standing with a left flank laparoscopic approach.

In group 1 (n=5) a continuous suture was placed in a craniocaudal direction between the renal and splenic capsules with unidirectional barbed suture material. In group 2 (n=5) the polypropylene mesh was secured to the nephrosplenic space with titanium helical coils using laparoscopic technique. All horses undergoing surgery survived to discharge. In all horses, transrectal palpation was performed 2 months after surgery; at this time, closure of the caudal part of the nephrosplenic space was evident. Postoperative data were obtained from the medical record and by telephone follow up. In group 1 the total surgery time was less than group 2.

The results of our study show continuous barbed suture to be an effective means of closing the nephrosplenic space, faster than mesh placement and easier to perform.

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THE EFFECT OF TOPICAL F.A.G.®, TOBRAMYCIN AND 0.15% HYALURONATE EYE DROPS ON KERATOCONJUNCTIVITIS SICCA IN DOGS: AN EXPLORATORY STUDY

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Keratoconjunctivitis sicca (KCS) is a common ocular disease in the dog and is the most commonly recognized form of dry eye disease (DED) in this species. Topical antinflammatory and immunomodulatory therapy has become the significant way of treating KCS in dogs. However, as the long-term use of routine anti-inflammatory medications is restricted from their side effects, it is inevitable to explore safer and more effective alternatives. Essential fatty acids have proven to be anti-inflammatory systemically, which makes it possible to treat dry eye. Clinical trials have demonstrated that supplementation with either ω-3 or ω-6 essential fatty acids or both has multifactorial efficacies including improvement of subjective symptoms, alleviation of inflammation of ocular surface and eyelid margin, prolongation of tear break-up time and increase of tear flow secretion [1-3]. The aim of this study was to evaluate the efficacy of a periophtalmic cream of a pool of Fatty Acid Group (F.A.G.®) in association with topical tobramycin and 0.15% hyaluronate eye drops in alleviating the clinical symptoms of canine KCS. The clinical study was conducted on 10 dogs diagnosed with idiopathic KCS. The dogs were of various breeds and had been pretreated unsuccessfully only with topical antibiotics. The affected eyes were treated with two applications daily of a periophtalmic cream of a pool of fatty Acid Group (FAG®), one drop three times a day of tobramycin, and one drop three times a day of 0.15% of hyaluronate eye drops. Ophthalmic examination included direct and indirect ophthalmoscopy, slit lamp examination, Schirmer tear test I (STT I) and measurement of intraocular pressure (IOP) with the applanation tonometer. Diagnosis and severity of KCS were evaluated by STT and by assessment of ocular discharge, conjunctival inflammation, corneal inflammatory cell infiltrate and scarring, and degree of ocular discomfort. Clinical and ophthalmologic examinations were performed prior to the treatment as well as after two and eight weeks of therapy. The effect of treatment was pronounced (increase in STT values to higher than 4 mm/min, no signs of inflammation) in 8/18 eyes; moderate (increase in STT values of 3–4 mm/min, mild signs of corneal/conjunctival inflammation) in 3/18 eyes; unsatisfactory in 7 of 18 eyes. No side effects were observed in any case. Results of this exploratory study suggest that association with topical FAG, tobramycin and 0.15% hyaluronate eye drops may be a safe and effective treatment for keratoconjunctivitis sicca in dogs; however, in moderate and advanced stages, efficacy in obtaining reduction of neovascularization or corneal pigmentation was not observed across the treatment period.

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LOCAL ANALGESIC EFFECT OF BUTORFANOL COMPARED TO TRAMADOL IN RATS

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The study was approved by the health ministry with silent consent. Experiments were performed following Italian law (D.M. 116192), Europe law (O.J. of E.C. L 358/1 12/18/1986), and USA laws (Animal Welfare Assurance No A5594-01, Department of Health and Human Services, USA).

Pain control is important because it allows to work in the best way in the respect of animal's welfare.

Although modern protocols of local analgesia are considered effective and safe for most clinical practices, there are some patients who need more care.

The aim of this study was to evaluate the analgesic efficacy of tramadol vs butorphanol, local administered in rats [1].

Thirty adult male Wistar rats were used. The heart rate, respiratory rate, and plantar withdrawal latency being were measured prior and following intraplantar injection of saline solution (group S), butorphanol (group B) and tramadol (group T) [2].

Group B showed lower FC and RR than the group S and the group T, almost at all monitoring times.

The plantar test values were consistently higher in group B respect all other groups. The plantar test values in group T were comparable at the group S if not even lower (time 10 and time 20).

The results obtained from this research indicate that in rats butorphanol compared to tramadol, administered locally, determines a good analgesia [3].

The use of butorphanol is therefore desirable as adjuvant for local analgesia in minor surgery procedures in rats.

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SCIENZE CLINICHE: SIRA

FIRST DATA OF MULTIPLE OVULATION AND EMBRYO TRANSFER IN MODICANA COWS

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The Modicana is one of the minor Italian cattle breeds of limited diffusion, is autochthonous of Sicily and has a prevalent dairy aptitude. Multiple ovulation and embryo transfer (MOET) is an important tool for the genetic improvement and preservation of endangered breeds. Superovulation protocols use FSH extracted from porcine or ovine pituitaries and the two commercial products approved in Italy give comparable results despite different FSH/LH ratio [1]. Despite manufacturers' recommendations, the final dose of the drug is empirically adjusted in relation to aptitude, breed and age. Aim of the study was the application of MOET in Modicana cows using a protocol based on FSH/LH (Pluset, Bio 98). A total of 9 flushings in 9 donors were performed in a Modicana cattle farm placed in Ragusa, Italy. The criteria of selection of the donors were: age of 4-5 years, 90-200 days post partum, body condition score around 3.5, free from diseases with special regard to the genital tract. The study was performed from September 2016 to June 2017. The donors were visited 10 days after the onset of standing estrus to count the antral follicles of 2-3 mm and, eventually, to aspirate dominant follicles. Superovulation was induced by 10 intramuscular injections of Pluset, at 12-hour intervals over 5 days. A total of 500 UI of FSH were administered in declining doses after 6 unsuccessful experiences in 6 different donors with the manufacturer's dose (1000 UI). On day 4, cloprostenol (Estrumate, MSD Animal Health) was given. On day 6 and 7, each cow was inseminated with frozen semen 3 times at 8 hours interval, starting 12 hours after the onset of the standing estrus. Embryos were collected 7 days after inseminations by transcervical uterine flushing using a standard protocol and commercially media. Embryo morphology was assessed under a stereomicroscope according to the International embryo transfer Society classification guidelines [2]. Embryo were transferred in Modicana heifer recipients fresh or after freezing with a standard curve for bovine embryos (from -7°C to -35°C with a rate -0.5°C/min). All the data were presented as mean and standard deviation. Fisher's exact text was used to analyze results. The estimated count of antral follicles in the selected donors was 14±6 resulting in 12±3 corpora lutea in the study group (n=9) versus 2±1 corpora lutea in the manufacturer's dose group (n=6) (p=0.04). A total of 70 embryos were recovered, evaluated and 53 were transferred as fresh (n=18) or frozen (n=35). The number of recovered and transferable embryos for cow was: 10±1 and 8±1. First grade blastocysts (6.1) and morulae (4.1) were the most represented embryos (30 and 32%). Non-transferable embryos (24%) were mainly represented by unfertilized ova (70%). Pregnancy rates (42 day) of recipients were 50% for fresh and 48% for frozen transferred embryos (p=1). Our data suggest that a low dose of FSH is effective for MOET schedules in Modicana breed. Despite individual differences, Modicana breed is candidate to be a good embryo producer among dairy cattle breeds.

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SHORT-TIME PROTOCOL WITH FLUROGESTONE ACETATE TO OESTRUS SYNCRONIZATION IN CHAMOIS COLOURED GOATS

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Intravaginal sponges impregnated of progestins are classically used to synchronize oestrus in small ruminants. Considering the 21-day oestrus cycle of the goat, first protocols scheduled long-time (17-21 days) permanence of the sponges. A shorter and more efficient 11-day protocol was proposed combining progesterone treatment with a single PGF2α dose administration on day 9 or 11 in order to remove an eventual corpus luteum and promote ovulation [1]. Considering follicular dynamics, 5-7-day protocols have been tested in goats with various devices [2,3]. Aim of this study was the comparison of a short lasting protocol (6 days) and a medium lasting protocol (11 days) for the oestrus synchronization of goats. After ethical approval by the Department of Messina (reference number 014/17), 50 Chamois Coloured goats from a farm of Cosenza, Italy (39 degrees N, 16 degrees E, altitude 926 m) were enrolled in the study and randomly assigned to group A and B. The criteria of selection were: age (2-5 years), parity (≥1), days open (more than 5 months from the last kidding), body condition score (from 2.5 to 3.0), health status (lack of evidence of mastitis, abscesses, lameness, ultrasound abnormality of the internal reproductive tract). This study was performed in September (breeding season). In group A (25 goats), the sponge containing 20 mg flurogestone acetate was inserted on day 0 and removed on day 6; 0.05 mg of cloprostenol (Estrumate, MSD Animal Health) and 400 UI of equine chorionic gonadotropin (Folligon, MSD Animal Health) were given on day 6. After 36 hours (day 8), 3 bucks (ratio 1:8) with good libido and of proven fertility were introduced in the group until day 11. In group B, the same sponges were maintained for 11 days whereas cloprostenol and equine chorionic gonadotropin were administered on day 9. Thirty days after the removal of bucks, the goats were evaluated by ultrasound for pregnancy diagnosis. All the data were analysed with Fisher-Yates test, and statistical significance was set at 0.05. Only one sponge (4%, $p>0.05$) was lost in group B and the goat was excluded from the study. At the removal of the sponge, vaginal contamination (fluid and odour) was observed in 10 and 90% of the goats in group A and B ($p<0.01$), respectively. Oestrus and mating was registered in 96 and 100% ($p>0.05$) of goats while pregnancy rate was 79 and 76% ($p>0.05$), in group A and B. No pseudopregnancy was detected. In conclusion, both protocols worked well under the study conditions, allowing a massive synchronization of fertile oestruses from 36 to 72 hours after the sponge removal. The short lasting protocol may give some benefits: easier scheduling, less visits by vets and less costs for the farmer, less hormones in the farm and less residues in the milk, lower exposition time to intravaginal sponges with lower grade of vaginal contamination and improved animal welfare.

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WELFARE ASSESSMENT FROM PERINATAL PHASE TO WEANING IN SIAMANG GIBBON (*Symphalangus syndactylus*): ENDOCRINE AND BEHAVIOURAL MONITORING

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Zoos can play an important role in *ex situ* conservation when they maintain and breed endangered animals and, relating training, education and research on animal biology. Among the topics studied, behaviour and reproduction, closely connected and influenced to each other (1,2,5) represent the main lines of research (4).

The present study's aim was to evaluate, through a non-invasive approach, the endocrine (salivary cortisol) and behavioural response of a siamang gibbons' family hosted in Zoom Torino, a zoological garden in the north of Italy, related to two main reproductive phases: lactation and weaning related to daily temperatures. For the evaluation of animal welfare, a behavioural and physiological approach are the main methods used for a wide and complete view (3). About ethological aspect, behavioural observation of subjects has been carried out for 18 months, recorded through a focal continuous sampling, with a mean of 2 observations for month lasting one hour. Using a software for behavioural analysis (BORIS), an ethogram with behaviours of interest was set up and data were obtained. About physiological aspect, saliva samples were taken through non-invasive methods, in correlation with ethological observations, analysed later to quantify the cortisol levels. Hormonal and behavioural results have been subjected to statistical analysis to highlight differences between three phases of study and variations linked to seasonal temperatures (<15° and >15°C). Only one difference emerged between two phases considered about a possible maladaptive behaviour (self-clasping), more expressed in both subjects in weaning phase. No difference of cortisol concentration between phases was found. Only one statistically significative difference resulted between two temperature classes: during lactation, cortisol in the female is higher at T>15°C. The correlation between cortisol and temperatures resulted positive in both subjects, either in lactation phase or regardless of phases. Observation methods and chosen approaches allowed an adequate welfare condition evaluation: therefore, there aren't significant differences in welfare conditions in both subjects between different considered reproductive phases (lactation and weaning). Observed behaviours of studied subjects follow those recorded in nature among siamang gibbons' couples; only one behavioural pattern is related to a possible maladaptive condition, not supported by endocrine response, but comparable to literature about wild species.

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THE EFFECT OF RESVERATROL TREATMENT DURING MATURATION ON DEVELOPMENTAL COMPETENCE OF PREPUBERTAL OOCYTES SELECTED BY THE BRILLIANT CRESYL BLUE TEST

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Juvenile in vitro embryo production increases rates of genetic gain through a reduction of the generation gap. However, the production of embryos from oocytes of prepubertal females is lower in comparison to those of their adult counterparts [1].
This study was addressed to investigate the effect of the supplementation of resveratrol (resv), a natural antioxidant, to the *in vitro* maturation (IVM) medium combined with oocytes selection using the brilliant cresyl blue (BCB) test on developmental ability of prepubertal goat oocytes. Cumulus-oocytes complexes were recovered from ovaries of slaughtered juvenile goats (30-45 days old) and exposed to 13 µM BCB for 45 min. After staining, oocytes were classified on the basis of their glucose-6phosphate dehydrogenase activity in BCB+ (blue cytoplasm, grown oocytes) and BCB– (colorless cytoplasm, growing oocytes) and matured *in vitro* with (BCB+R+; BCB-R+) or without (BCB+R-; BCB-R-) 1µM resveratrol for 24 h. Intracellular ROS and GSH levels were measured in MII oocytes [2]. In vitro fertilization (IVF) was performed in BO-IVF medium with fresh semen; presumptive zygotes were cultured in BO-IVC medium for 8 days. STATA\IC 11.0 software package was used to data analysis, Kruskal-Wallis test was employed for ROS and GSH levels and Chi-square test to analyze cleavage and blastocyst rates. Resveratrol supplementation significantly ($P<0.05$) increased intracellular level of GSH in both BCB groups (36554.6±3049.2 and 34946.8±1877.8 pixel\oocyte, BCB+ R+ and BCB- R+ respectively) compared to their respective controls (27624±1513.7 and 27665.4± 1489.7 pixel\oocyte, BCB+ R- and BCB- R-respectively). No significant difference in the GSH content was found between control (BCB+R-, BCB-R-) and resv (BCB+R+, BCB-R+) groups. Oocyte intracellular level of ROS did not statistically differ between groups. Resveratrol supplementation during IVM induced a significant increase of cleavage and blastocyst rates (blastocyst/cleaved) in BCB+ R+ group (n=103/116, 88.8% and n=32/103, 31.1% respectively) compared with BCB+ R-(n=87/110, 79.1% and n=14/87, 16.1%), BCB- R-(n=67/95, 70.5% and n=4/67, 6%) and BCB- R+ (n=67/95,76.1% and n=8/67, 12%) groups. No significant differences were found among BCB+R-, BCB- R-and BCB- R+. The results of the present study proved that supplementation of resv during IVM of prepubertal goat oocytes positive to BCB staining improved embryo development. The increase of GSH levels could be one of the mechanisms underlying resveratrol effect on oocyte quality. The selection of oocytes using the BCB test in combination with resveratrol treatment during IVM can be a useful strategy to enhance in vitro embryo production in prepubertal goat oocytes.

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USE OF NIR SPECTROSCOPY AS AN INNOVATIVE METHOD FOR BOVINE OVARIES EVALUATION

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Near Infra Red Spectroscopy (NIRS) explores organic vibrations arising from the IR region (2500-25000 nm) as overtones and combinations which rebound in the NIR (714-2500 nm) region. The first aim, was to understand if NIRS was able to discriminate the corpus luteum (CL) from the rest of ovarian structures (NO-CL); on a second aim, NIRS was tested for its precision in defining the stage of CL, a trait that ultrasound scanning systems cannot provide. For 142 ovaries sampled from Piedmontese cows, examinations were performed with a Perkin Elmer IdentiCheckTM instrument, giving 336 full spectra (714-3333 nm, FS). In addition, a short spectrum analysis (714-1070 nm, SS) was performed to simulate a modern NIRS-miniature. Chemometrics was performed by splitting the dataset in two, then a PLS model on dummy variables calculated the optimal equations. Histology was used as a cross-checking technique, to evaluate the findings observed in NIRS. CLs (n=30) were fixed in formaline, embedded in paraffin and stained with hematoxylin and eosin. CL (%) cells composition (10 fields captured at 400x magnification) was evaluated: small and large luteal cells, non luteal cells and fibrosis (1). The score of any types cells was: 0: 0-10%; 1: 10-20%; 2: 20-30%; 3:30-50%; 4: ≥50%. Finally, the stage of CL based on histology characteristics, was scored in: 1=early; 2=mid; 3=late; 4= regression. The R2 reciprocal validation coefficients were 0.78, 0.76 with the FS, and 0.56, 0.58 with the SS. It has to be noticed that an R2 0.50 is not a 50% hazard, which correspond to R2 0.00, but it is much more. In fact, calculation of the odds ratio revealed a very high specificity for the NO-CL: 99.2, 98.4%, with FS and 98.4, 96.8 with the CTS indicating that the first part of the spectrum contains the information useful for a parenchyma discernment. But, sensitivity of the CL was also high with FS (93.2, 95.5%, P<0.0001) while the short spectra did not provide a useful information (68.2, 68.2% P 0.006). The most noticeable ovarian parameter predictable by NIRS was the counting of small cells, which show a high value of the R2 cross-validated coefficient of 0.74 and 0.46, with FS and SS, respectively. Also, the maturity rank of the CL showed a good correlation with the NIRS full spectrum (0.52) while nothing with the short one. Large luteal cells, P4 concentration, fibrosis and not-luteal cells did not show correlations with NIRS. NIRS of removed ovaries resulted able to discern with very high accuracy the absence of CL in the explored field of the tissue, in both the spectra ranges. Moreover, an interesting rapid and easy NIRS analysis could predict the incidence of the small cells inside the CL that are positively linked to the maturation, together with the fibrousness, opposed, in negative to the large cells count. Availability of new cheap smart-instruments, suggests exciting experiments both in-vivo and on laboratory activities as a technology integrated with the in vitro oocyte maturation technique with by NIR metabolomic profiling (2).

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COMPUTER ASSISTED SEMEN ANALYSIS OF EPIDIDYMAL SEMEN COLLECTED THROUGHOUT THE YEAR SUGGEST A SEASONALITY IN FERAL MALE DOMESTIC CAT

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Seasonal variation in reproduction is common in mammals as an adaptation to annual changes in the habitat. Adult male domestic cats are known to produce sperm throughout the year, although sexual activity is influenced by geographical location. In the northern hemisphere, feral domestic cats reproduce usually between January and July. Thus, seasonality in testicular activity might be suggested [1]. The aim of the present study was to investigate variations of cat semen parameters by Computer Assisted Semen Analysis (CASA) throughout the year. Testes and epididymis of free-roaming cats were collected after castration during a trap-neuter-release program managed by University of Bari Aldo Moro and Ente Nazionale di Protezione Animale (ENPA). Feral cats lived in several colonies in the urban area of the city of Bari, Italy. The study included a total of 40 adult male cats (10 each season) of ages between around 12 months and 2 years and weights between 2.5 and 4 kg. All cats showed male secondary sexual characteristics. For recovering the epididymal sperms three steps were followed: epididymis were first subjected to washing, then minced by the "sliding" technique, at the end epididymis were sectioned by a scalpel and sperms were collected by the blade. Sperm suspension was centrifuged and diluted. Concentration, the Percentage of motile spermatozoa, average path velocity (VAP), straight line velocity (VLS), beat cross frequency (BCF), straightness (STR), linearity (LIN), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), were assessed by CASA (Ivos 12, Hamilton Thorne Biosciences, USA). The most evident seasonal alterations were found for motility, BCF and ALH. The percentage of motile sperm changed during the annual progression with a maximum in winter if compared with autumn (74.17 vs 41.33; $P \leq 0.01$) and in spring if compared with summer (73.33 vs 39.33; $P \leq 0.01$). BCF showed high levels in winter if compared with autumn (30.67 vs 20.37; $P \leq 0.05$) and a reduction in summer if compared with spring (26.40 vs 29.47; $P \leq 0.05$). ALH was at highest levels in autumn with progressively decreasing in winter, spring and summer (9.15 vs 7.25 vs 7.12 vs 7; $P \leq 0.05$). Concentration, VAP, VLS, STR, LIN, VCL had no statistically relevant variation. Results show that sperms are produced throughout the year with seasonal changes in quality due to a higher motility in winter and spring. This finding collimates with previous studies on colony cats indicating peaks in production of litters during spring and summer [2]. The increased percentage of motile sperms as a functional parameter, indicated a real seasonal dependence. A similar tendency was observed by Spindler and Wildt [3]. Moreover, the functional competence of sperm is a critical parameter for fertilizing capability of males [4]. In conclusion, the study suggests seasonal changes in quality of sperm in domestic cat.

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XY DISORDER OF SEXUAL DEVELOPMENT (DSD)/HYPOSPADIAS IN A EUROPEAN CAT

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An intersex is an individual in whom it is difficult to attribute sex showing one or more anatomical features of both sexes. In literature, there are different nomenclatures and classifications but they have historically been improved when sex chromosomes structure started to be analyzed. The first important approach in pets was to categorize the defects based on a chronological sequence of events that lead to sexual development [1]. The intersexes were divided into 3 categories of disorders: chromosomal sex, gonadal sex, phenotypic sex. A new classification has been proposed in humans and recently adapted to the dog and the cat [2], even to create comparative models. All conditions are enclosed in the acronym DSD (Disorders of Sexual Development). Each anomaly is cataloged for the arrangement of the sex chromosomes, therefore for the type of abnormality. The aim of the study is to contribute to these cases with the description of a feline XY DSD. In a 2-year-old European cat a rare urogenital anomaly was diagnosed. There was a cloacal structure with two outlets: the rectum dorsally and the urethra ventrally on the floor of a pseudovagina. Near the ventral commissure of the cloacal structure a peniform structure with spicules was recognized without relationships with the urethral meatus. The testicles were in place but the scrotum was splitted by the cloacal fissure. The cat showed no disorders of urination and defecation; it was destined to castration, being a stray cat. After general anesthesia, prior castration, an exploratory laparotomy was performed to investigate the presence of other possible anomalies of the enteric and genitourinary tract, like megacolon and Müller duct residues, which were excluded. No plastic surgery in the genital area was performed because it was thought to be not necessary. Histologically, the testicles showed normal spermatogenesis. The karyotype was XY. A diagnosis of XY DSD/hypospadias was made. Hypospadias is the ventral displacement of the urethral meatus which, due to a defect of formation, is not at the apex of the glans penis but in a backward position up to a pseudofemale place. A few cases of XY DSD/hypospadias have been described in the cat [2-5], with a comparable anatomical presentaion. Defects and consequent disorders of urinary and enteric tracts are frequently associated and may require corrective surgery, with the exception of the presented case.

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C-REACTIVE PROTEIN AND PROGESTERONE SERUM PROFILE IN THE PERIPARTUM PERIOD OF THE BITCH

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Serum progesterone (P4) concentration towards the end of pregnancy can help clinicians to assess impending canine parturition. Other easy-to measure serum parameters may be useful to increase the accuracy of the diagnosis. C-reactive protein (CRP) is an acute phase protein that rapidly increases in response to an inflammatory stimulus [1]. The aim of this study was to measure P_4 and CRP concentrations in order to verify whether they may represent a marker of end of pregnancy in the bitch.

Seventy serum samples from thirty pregnant bitches were included in the study. All the bitches had been presented to the veterinary teaching hospitals of the University of Padova and Torino for pregnancy monitoring, parturition assistance and C-section. Blood was collected for routine P4 assay or biochemistry evaluation: serum remnants were stored frozen until analyzed. P_4 was measured by CLIA (Immulite 2000®; Siemens Diagnostics, Flanders, NJ, USA), while CRP by a turbidimetric method (BT1500®, Biotecnica instruments SpA, Roma, Italy). Data were analyzed with a multifactorial repeated ANOVA model (SAS Intitute Inc., Cary, NC). Days from parturition (-5 to +2), parity (primiparous vs multiparous), parturition type (eutocic vs C-section), and number of puppies (<4 vs 4-8 vs >8) were considered as fixed effects and dog was considered as random repeated effect. Day 0 was the day of parturition. Significance was set at $P<0.05$. P4 concentration was associated with 'day from parturition': the value of day 0 was significantly different from day -1 to -5, while it was similar to day +1 and +2. The day before parturition, P4 concentration was significantly different only from the values of day -5 and -4. CRP concentration was associated with 'day from parturition' and 'type of parturition': it increased from day 0 and remained high until day +2. Since the bitches subjected to C-section had been sampled only until day 0, this could have biased the analysis, so their samples were excluded. The analysis of data from spontaneous deliveries confirmed the significant effect of 'day from parturition' ($P=0.0086$). In both analyses, mean CRP concentrations were above the normal range from day 0 onwards, beginning to increase at day -1. CRP concentration was found to increase from the day of parturition until day 7 in sows [2] and from two days before parturition in the mare [3]. Our data suggest that parturition, lactation, and endometrium remodeling influence CRP concentration, irrespective of placental type and of uniparity or multiparity of the species. P4 and CRP concentrations may be a marker of end of pregnancy in the bitch; however, CRP does not appear of great help to assess impending parturition.

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AIPVET

LETHAL FIELD-TIP ARROW WOUND TO THE CHEST IN A DOG

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Arrow wounds can be classified under low velocity missile injuries. Arrow injuries have been rarely reported in veterinary medicine [1,2,3]. Features of the entrance wound depend on the shape of the arrowhead [4]. Broadheads used for hunting have triangular blades that create a stellate entrance wound. Field tips used for sports archery are conical-tipped arrows that produce circular or slit-like incisions hard to distinguish from a bullet hole. We report on a lethal field-tip arrow wound in a dog. In March 2018 a mixed-breed adult dog was found dead in a courtyard. Owners witnessed a suspected shooter 'approaching the carcass and retrieving a bullet'. The dog was sent to IZSLT for forensic necropsy to identify the cause of death and to recover any evidence. At necropsy a penetrating skin wound was found at the xyphoid region. The wound was D-shaped with broad axis oriented in a caudocranial sense. An asymmetrical rim of abraded tissue and a bruise surrounding the wound were observed. A muscle stump of the underlying rectus abdominis was everted from the point of penetration. Fracture of the 8th and 9th left ribs at the sternal junction was observed. A bone fragment from the 8th rib fracture was everted from the wound. In the thoracic cavity a bilateral hemothorax was found. Right heart ventricle wall was interested by a full-thickness linear laceration. Lung apical lobes were lacerated. Abundant blood was observed in the trachea and larynx. A comminuted fracture was found at the body of the 1st thoracic vertebra. Cause of death was hemorrhagic shock due to chest penetrating injury. No exit wounds were observed. No evidence (i.e. metallic shreds) was retrieved from the body. X-ray on the remains did not detect radio-opaque fragments. The skin lesion in the xyphoid region is compatible with the penetration of a projectile which has occurred tangentially to the abdominal wall with a caudocranial direction. The projectile has passed through the thoracic cavity, ending up against the ventral aspect of vertebral column. An arrow wound was suspected due to appearance of the entrance wound, lack of cavitation effect, lack of metallic fragments later confirmed by X-ray. The arrow was likely retrieved causing some fracture fragments and torn muscle stumps to protrude from the entrance wound. Authorities were notified in accordance with the Penal Code art 365.Bows and crossbows are popular sports items and can be purchased without restrictions, therefore arrow wounds should be considered when examining penetrating wounds in pets. Conical field tips mimic gunshot wounds and this is a problem in cases where the arrow has been retrieved. Nonetheless internal wounds will lack cavitation effect due to low velocity and research for bullets, fragments or lead residues will be negative [5]. The long and rigid shape of the arrows provides evidence on the trajectory to assist in localizing the relative position of the shooter [5].

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DOMESTIC CAT AS AN ABERRANT HOST OF CYSTIC ECHINOCOCCOSIS

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Cystic Echinococcosis (CE) is a parasitic zoonotic disease of global importance caused by the larval stage *of Echinococcus granulosus*. Life cycle involves dogs and wild carnivores as definitive hosts and ungulates as intermediate hosts in which the infection is characterized by long term growth of hydatid cysts (metacestode). Accidentally, other animals, including humans, could ingest eggs and contract CE acting as aberrant hosts [1]. Herein we described the anatomo-histopathological findings, and molecular analysis of a case of CE involving a domestic cat. A 3 years old female neutered cat with painful and distended abdomen was submitted to ultrasound scan of the abdominal cavity and exploratory laparotomy. Some peritoneal hydatids and spleen collected during laparotomy procedure were promptly fixed in 10% neutral formalin, treated with histological routinary methods and stained using hematoxylin and eosin (H&E) and modified Period-Acid Shiff (PAS) stain. DNA extracted from ethanol fixed protoscolices was analyzed using PCR method, based on the amplification of calreticulin (cal) gene for detection of *E. granulosus* s.s. genotypes G1-G3 [2]. Ultrasound imaging reported a voluminous splenic mass with a multiloculated structure and multiple intraperitoneal vesicles of different dimension characterized by anechoic content and delimited by a hyperechoic rim. Grossly, numerous hydatids ranging 5 to 40 mm in diameter were adherent to omentum or free in the whole abdominal cavity. The hydatids were thin walled and contained semitransparent watery fluid. We also observed a distinct encapsulated cystic mass of 65 mm in diameter protruding at one extremity of the spleen. On the cut section it appeared sponge-like for the presence of multiloculated cysts, which were filled of yellow-grey fluid material. Among the cysts fibrous and residual spleen tissues were observed. Microscopically, the wall of the peritoneal hydatids was formed by an acellular PAS-positive laminated membrane with an inner cellular nucleated germinal layer. In the spleen mass, the lamellated wall of the cysts was also PAS-positive and bordered by a chronic granulomatous inflammation with epithelioid cells and numerous multinucleated giant cells. Occasionally, amorphic necrotic material were present within some cysts. This is the first case report of CE in a domestic cat from Italy. The report of a clinical case of CE in a domestic cat points out the problems dealing with environmental contamination in urban contexts and the implied risk for Public Health.

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LEPTOSPIROSIS IN WILD BOARS AND RURAL PIGS: PATHOLOGICAL AND BACTERIOLOGICAL ASPECTS

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Leptospirosis is a geographically widespread disease of humans and animals, including domestic and wild pigs. In the host, *Leptospira* spp. localizes in the kidney after a transient bacteriemic phase, causing a chronic reactive inflammatory process related to its shedding in the environment [1]. Herein, we study the histopathological lesions and the bacteriological aspects of *Leptospira* spp. infection in wild boar and rural pigs in a defined geographical area of Sardinia (Italy). Our study was carried out in kidneys of hunted wild boar (n=430) and slaughtered rural pigs from no-professional holding (n=60). In addition, we also examined the kidneys of clinically healthy rural pigs (n=11) which were culled after the 2 farm's owners were found affected by *Leptospira* spp.. These kidneys were examined by 16S rRNA gene Taqman real time PCR and specific bacteriological assay. Finally, from a number of these wild boars (n. 214) and from the all 71 rural pigs (n=60 slaughtered pigs and n=11 pigs from the Leptospirosis outbreak) we also collected kidney samples for histopathology as well as for immunohistochemical examinations. Positive real time PCR was found in 37 (8.6%) while *Leptospira Pomona* was isolated in 9 (2%) of the 430 kidneys collected from wild boar. The kidneys from the slaughtered rural pigs (n=60) resulted negative by real time PCR and bacteriological analysis. No macroscopic lesions were detected in all the examined kidneys, although by immuno-histopathological analysis, multifocal chronic interstitial nephritis were detected both in 20 of the 214 wild boar (9.3%) and in 3 of the 60 slaughtered rural pigs (5%). These nephritis were characterized by a prevalent CD3+ T lymphocytes infiltrate. In the wild boar kidneys, *Leptospira* spp. was immunohistochemically observed in 3 cases (1.4%) adhering to luminal surface and within the epithelial cells of the renal tubules. All the 60 slaughtered rural pigs resulted immunohistochemically negative for *Leptospira* spp. In the 11 rural pigs belonging to the farm in which the owners were affected by *Leptospira* spp., real time PCR resulted positive in 8 kidneys, while *Leptospira* spp. was isolated from 4 kidneys by bacteriological culture examination. In-deep analysis, by MLST and MLVA genotyping, showed that all these isolated matched with serovar *Pomona*, the same identified in the 2 infected farmers. Histopathological changes were found in 6 of 11 pigs. Four out of these 6 pigs were immunohistochemically positive for *Leptospira* spp. Our study shows that Sardinia wild boar are highly exposed to *Leptospira* spp. and can potentially act as shedder of this pathogen in the environment more than the rural pigs. Nevertheless, pigs can result the infection source in the leptospirosis outbreaks involving farm workers. Finally, the frequent detection of *Leptospira* spp. in wild boar highlights the potential risk of its transmission to hunters.

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CORRELATION OF PDGFRs WITH HISTOTYPE AND CLINICAL OUTCOME IN CANINE THYROID CARCINOMA

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As in humans, thyroid cancer (TC) is the most common endocrine malignancy in dogs. Most canine thyroid tumours are well to moderately differentiated. PDGFRα and PDGFRβ are tyrosine kinases receptors implicated in the pathogenesis of human TC. In canine tumours these receptors are involved in osteosarcomas, melanomas, mammary carcinomas while no data about their expression and role in canine TC. The aim of the present work is to evaluate the expression of PDGFRα and PDGFRβ in canine thyroid tumours and compare their expression with histological features and clinical follow-up. Thirty-four cases of dogs affected by thyroid carcinoma were surgically treated and clinical parameters such as dimension of the tumors, presence of lymph node involvement (TNM staging), recurrence and metastasis have been recorded. Tumours were classified as follicular or medullar using thyreoglobulin and calcitonin immunostaining. Further histological parameters evaluated included mitotic count (MC, mitoses per 10 high-power fields) and assessment of vascular invasion. IHC results and clinicopathologic findings were grouped into contingency tables and analyzed using Fisher's exact test or Chi2 test. Survival curves were computed using the Kaplan–Meier method and tests for differences in survival, considering all known prognostic factors for TC, were performed using the log-rank test. OS was defined as the number of days between surgery and death, while the DFI was defined as the number of days between surgery and tumor recurrence and/or evidence of metastasis. Twenty-two (74.7%) tumours were of follicular cell origin (FTC) and were well-differentiated, 12 (35.3%) were of medullar origin (MTC). The mean and median value of the MC was 9.76 and 7.5, respectively (range 1-36) while invasion into peritumoural blood vessels was detected in 28/34 cases (82.3%). No statistical association was found between peritumoural blood vessel and MC with FTC and MTC histotypes. Twenty tumours (58.8%) were positive to PDGFR alpha, while sixteen (47%) were positive to PDGFR beta. Significant association was found between medullary carcinoma and PDGFR alpha expression (P=0.003). No significant association was found between PDGFRs expression and MC, vascular invasion, DFI and OS. These preliminary data suggest that the expression of PDGFRs seems do not change the clinical outcome of the patients. Moreover PDGFR alpha should be considered a histological marker for medullar histoptype and seems to be involved in the origin of thyroid medullar carcinoma.

AIR SACS TREMATODIASIS AND RELATED PATHOLOGY IN TWO COMMON BLACKBIRDS (*Turdus merula*)

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Air sacs trematodiasis is rarely reported in birds. Necropsy of two free-ranging common blackbirds (*Turdus merula*), found dead in central Italy, revealed the presence of a large number of flukes in the coelomatic cavity. Histologically air sac walls were covered with a mild fibrinous exudate containing degenerate heterophils, fibrin, some bacterial colonies (gram-positive cocci), and trematodes. The superficial bronchi and parabronchi were markedly distended with mucoid material containing bacterial colonies, and the adjacent pulmonary parenchyma was congested and collapsed. Large numbers of trematodes, surrounded by a mild to moderate suppurative to pyogranulomatous inflammatory reaction, were observed on the intestinal, pericardial, and hepatic serosal surfaces. The parasite number and bacterial bronchopneumonia, aerosacculitis, and serositis were of sufficient intensity to have resulted in death of these birds. At microscopical examination, flukes showed a tongue-shaped elongate body of 2,088-2,314 µm in with and 8,268 -11,830 µm in length, tapered anteriorly and rounded at the posterior end. The mouth was slightly oval and sub-terminal with a weakly developed oral sucker. The oval and well developed pharynx measured 250-309 µm and the two caeca joined posteriorly. Two large (550-702 µm x 450-520 µm), globular testes were situated obliquely to each other, while the intertesticular ovalar (250 x 300 µm) or round (about 334 µm) ovary was placed in a longitudinal straight line with the testes. The ootype was about 110 µm in diameter, while the brown-yellow eggs measured 131.52 x 73.86 µm in mean. The genital pore was post-pharyngeal, while the vitelline glands were arranged symmetrically and were not confluent posteriorly. For morphology and dimensions, the species here examined was identified with *Morishitium (Cyclocoelum) polonicum* as described by Machalska (1980) in *T. merula* (1,2). This fluke species typically inhabits the air sacs of blackbirds and in its life cycle terrestrial snails are intermediate hosts. In Italy, this species was previously reported in *T. merula* from an area neighboring that where the blackbird here examined lived (3). Dimensions and morphology of the species reported by Visconti (1988) are similar to that of the species here examined. In our knowledge, this is the first description of pathological lesions caused by *M. polonicum* in *Turdus merula*.

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BOVINE ATRIAL ANEURYSMS: SURVEY IN BRITISH SLAUGHTERED ANIMALS ON INCIDENCE, LOCALIZATION AND SEX DISTRIBUTION

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Cardiac aneurysms or diverticula are persistent and well circumscribed dilations of cardiac walls, valves or vessels. In human medicine, they represent rare congenital or acquired anomalies identified in adult with equal sex distribution [1]. In veterinary medicine, atrial aneurysms have been reported in several animal species including bovine [2], but a systematic study about incidence, localization and sex distribution in adult cattle has never been performed. Aim of this survey was to systematically describe atrial aneurysms in regularly slaughtered cattle, focusing on their localization, morphology and sex distribution. A total of 3320 hearts from cattle of both sexes (2045 males and 1275 females), regularly slaughtered in southwest United Kingdom abattoirs, were examined between June 2017 and March 2018. The animals, partially housed in tie-stalls with a concentrated diet and partially raised on grass, ranged from 13 to 221 months-old and belonged to different beef and dairy breeds or crossbreeds. At gross examination, the aneurysms involved the atrial appendage and appeared as isolated or multiple, saccular and round structures with a thin and transparent wall, either empty or filled with blood. Aneurysms involved 4.45% (91/2045) of right atria in males and 0.70% (9/1275) of right atria in females. Only 0.097% (2/2045) of left atria were involved in males, while no left atria showed significant alterations in females. Diverticula were isolated, multifocal or organized in clusters in both atria independently on sex. About the size, the prevalence of the right atrial aneurisms in males were the following: 1.8% (37/2045) was small, 1.7% (35/2045) medium and 0.9% (19/2045) large. On the contrary, the females showed 0.15% (2/1275) small, 0.23% (3/1275) medium and 0.31% (4/1275) large diverticula. The aneurisms involving the left atrium of males were always single and large. No other cardiac alterations were observed. In veterinary medicine aneurysms involving the right atrium have been frequently reported in swine, horses and cattle [2,3,4]. They can be congenital or acquired. This study identified the right atrium as more affected, thus confirming the data of the literature and the recently published observations in beef cattle slaughtered in Piedmont region (Italy) [2,3,4]. On the contrary, the prevalence of aneurysms involving the left atrium is significantly lower when compared to that previously reported by Colombo [4] in 2-3 months old calves (0.097% vs 2.3%). The higher prevalence observed in males is in agreement with the literature [4], thus suggesting a genetic predisposition. However, further investigations are needed to confirm this hypothesis.

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C-KIT PROTEIN EXPRESSION IN CANINE UTERINE AND VAGINAL WALL MESENCHYMAL TUMOURS: ARE THEM EXTRA GISTS?

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Gastrointestinal stromal tumors (GISTs) are rare mesenchymal tumors of the gastrointestinal tract that typically express the tyrosine kinase receptor KIT protein (CD117). Albeit rare, GISTs are well documented in man and dog [1,2,3]. Mesenchymal tumors with histological, immunohistochemical and molecular features similar to conventional GISTs are occasionally found in extra-gastrointestinal locations, engendering the subset extra-GIST (EGIST) [4]. The majority of EGISTs have been reported to originate from the mesentery or the omentum, and less commonly from retroperitoneal tissues, vaginal wall, uterus, urinary bladder and prostate [6]. Like conventional GISTs, EGISTs display KIT exon 11 mutations in 40-50% of cases, and a potential good response to tyrosine kinase inhibitors as imatinib is expected [5]. To the best of our knowledge, the presence of EGISTs has never been investigated in canine uterine and vaginal wall. In order to investigate the possible existence of canine EGISTs, a histological and immunohistochemical study was carried out on a series of 20 mesenchymal tumors of the uterine and vaginal wall. The applied immunohistochemical panel included vimentin (VIM), smooth-muscle-actin (SMA), desmin (DES), CD117 and Ki67. According to histology and immunohistochemistry, tumors have been classified as 8 leiomyomas (100% VIM and SMA+, 50% DES+, CD117-), 4 fibromas (100% VIM+, SMA and DES-, CD117-), 2 leiomyosarcomas (100% VIM and SMA+, 50% DES+ and CD117-) and 1 fibrosarcoma (VIM +, SMA, DES and CD117-). Five of them (25%), previously diagnosed as one leiomyosarcoma and 4 fibromas from the vaginal wall, disclosed diffuse cytoplasmic immunopositivity to CD117 (100% VIM+, SMA and DES-) and were provisionally classified as EGISTs. One of these latter cases showed an intense and diffuse cytoplasmatic expression of CD117 and morphologically resembled typical intestinal GISTs (high cellularity, scant extracellular matrix, moderate atypia). The other 4 cases presented a moderate CD117 positivity, but their histological appearance was totally consistent with the former diagnoses of fibroma or leiomyoma. These results confirm that leiomyomas and fibromas are the most frequent mesenchymal tumors of the canine genitourinary tract, but also disclosed that about a quarter of them show an unexpected immunopositivity to CD117. The remark of the presence in the canine species of CD117-positive tumors in the genital tract suggests their potential reclassification as extra-GISTs, and leads to important prognostic and therapeutic implications, such as the possible efficacy of molecular targeted therapies with specific tyrosine kinase inhibitors.

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SYSTEMIC MUCORMYCOSIS IN TWO LOVEBIRDS (*Agapornis fischeri* AND *Agapornis roseicollis*)

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Mucormycosis are rarely described in parrots (1). One year old Fischer's lovebird (*Agapornis fischeri*) and a two year old female Peach-faced lovebirds (*Agapornis roseicollis*) from two different aviaries, died after some days of lethargy and ruffled feathers, were submitted for necropsy. To investigate the cause of death, gross necropsy, histopathological exams (H&E and Groccot stain) and biomolecular analysis were carried out. No gross lesions were appreciated at necropsy, while histopathology evidenced a systemic mycosis. In the Fischer's Lovebird the presence of fungal hyphae and microgranulomatous lesions was evident in liver and spleen. In the Peach-faced lovebirds, kidneys are involved. Lungs of both birds are affected by severe chronic pneumonia with strong interalveolar fibrosis of septa and parenchymal hepatization, with extensive pleuritis and severe mycotic colonization. In histologic sections, pleuritis varied considerably in extent. Generally lesions were diffuses and characterized by a few predominantly mononuclear leucocytes within and around a pleural arteriole and its adjacent capillaries. The surrounding pleural stroma was mildly oedematous and contained a few proliferating fibroblasts. The overlying mesothelial cells were often slightly hyperplastic. The inflammation involving an estimated 25-30% of the pleura in the histologic section. Mononuclear leucocytes predominated in these infiltrates. The interstitial lung disease was also randomly distributed and multifocal in the same bird. In some microscopic fields, lesions were confined to prealveolar arterioles and adjacent alveolar septal capillaries or extended for short distances into the surrounding alveolar septae plus the overlying pleura. Severity of septal fibrosis or lysis could not be correlated with the degree of leucocytic infiltration. Large interstitial tissue leucocytic infiltrative lesions often displayed foamy macrophage aggregates, discrete multinucleated giant cells, patchy fibrosis, and neovascularization. In such exuberant lesions the lung architecture could no longer be recognized. Air sacs were characterized by widespread and evident form of inflammation and degeneration of mesothelial cells. In affected organs, Grocott stain showed characteristic broad and non-septated hyphae suggestive of Zygomycoses. For each bird, DNA was extracted in double from two tissue sections (5-8 µm), by using ReliaPREPtmFFPE gDNA Miniprep System (Promega®). Two different genetic targets - the internal transcribed spacer 1 region rDNA (ITS1) and the extended 28S region of rDNA - were amplified by using universal primers ITS5/ITS2 and 12F/13R, respectively (2). Sequences of PCR products identified *Mucor racemosus* (Fischer's lovebird) and *Mucor circinelloides* (Peach-faced lovebirds) when blasted in Genebank database. Sequences of ITS1 and 26S amplicons confirmed *Mucor* species identity with a similarity of 100% and 97% respectively. To our knowledge, this report is the first description of *Mucor racemosus* and *M. circinelloides* infection and related pathology in Lovebirds.

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Bartonella SPP. IN RATS (*Rattus rattus*) AND MICE (*Apodemus* SPP.) OF PIANOSA ISLAND, ITALY

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Bartonellae are Gram-negative, vector-borne bacteria that colonize the endothelial and red blood cells of numerous mammalian hosts [1]. About 30% of *Bartonella* spp. known is recognized as zoonotic or having zoonotic potential [2] and most of these are associated with rodents [3]. Isolated ecosystems, as small islands, are privileged settings to investigate transmission of infection. Pianosa island (Italy) was selected to study a population of rats and mice that are known to be among the worst invaders. In particular, invasive Black rats *Rattus rattus* (n=15) and mice *Apodemus* spp. (n=16) were captured (project: RESTO CON LIFE - "Island conservation in Tuscany, restoring habitat not only for birds" LIFE13 NAT/IT/000471) and tested by PCR to detect *Bartonella* spp. infection. An end point PCR was performed on DNA extracted from spleen, and *Bartonella* spp. was detected targeting three different regions: genes codifying for the citrate synthetase (gltA) [4, 5 modified] and for the RNA polymerase beta-subunit (rpoB) [6 modified], and the 16S-23S rDNA interspacer transcribed region (ITS) short and long fragment [7 modified]. In order to investigate the possible characterization of the genus *Bartonella* detected in each extracted DNA, amplicons relative to gltA, rpoB, and ITS genes were sequenced. Bartonella spp. was identified in 50% (8/16) of *Apodemus* and in 75% (12/15) of rats investigated. No association of sex or rodents species with the infection status was detected. In conclusion, the present investigation shows a broad distribution of *Bartonella* spp. in an isolated population of rodents. This study is important to evaluate the role of rodents as reservoirs for zoonotic *Bartonella* species, since often humans and rodents share the same habitats in many parts of the world.

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COMPARISON OF DIFFERENT TISSUE PRESERVATION METHODS FOR HISTOLOGICAL AND MOLECULAR ANALYSIS

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The preservation of samples from the time of collection to the laboratory is critical for the success of the analysis. Multiple factors must be considered during a sampling under sub-optimal conditions, when the use of cold storage or specific fixative may not be possible. During this time enzymatic or microbial degradation of molecules and structure is a primary concern [1]. Moreover, the portability and cost of storage equipment, their ease of use and toxicity play a role in their choice. The aim of this study was the identification of a reliable and economic method for tissue preservation suitable for both histological and molecular analysis to be adopted in “in field sampling”. Punch biopsies from cattle liver were collected at slaughterhouse and preserved in RNAlater®, with silica beads or vacuum-sealed, whereas control received no preservation treatment. For each method 5 samples were stored at 4°C and 5 at 24°C. At fixed times (4, 10, 24, 48 and 72 h) punch biopsies from each group and temperature were formalin-fixed and stored at -80°C for analysis. The sampling was repeated on 6 different livers. The integrity of nucleus, cytoplasm, preservation of liver structure and section borders were evaluated by histological analysis and graded 1 to 5. The integrity of the extracted DNA and RNA was evaluated though PCR and by means of an automated electrophoresis station, respectively. Statistical differences were determined by a two-tails ANOVA for repeated measures, followed by the proper post test. RNAlater® and silica beads poorly preserved the histological parameters evaluated, causing a marked vacuolization of the tissue, independently from temperature. Conversely, the vacuum-sealed samples and the controls showed a good grade of preservation until 48 h. There was a significant effect of the preservation method on the considered parameters (4°C: $P<0.0001$ for nucleus, cytoplasm and structure, and $P<0.001$ for borders; 24°C: $P<0.0001$ for all) as well as a significant effect of time (4°C: $P<0.01$ for nucleus and structure, and $P<0.05$ for cytoplasm; 24°C: $P<0.0001$ for nucleus and borders, and $P<0.05$ for cytoplasm). Regarding the DNA analysis, the expected band was obtained for each considered preservation method, temperature and time. The analysis of the RNA integrity showed acceptable result for samples preserved with silica beads, whereas the RNA integrity was not well-maintained in vacuum-sealed samples and controls. Indeed, there was a significant effect of the preservation method ($P<0.0001$ for 4°C and 24°C) and time (4°C: $P<0.01$; 24°C: $P<0.05$) on RNA integrity. The obtained results do not allow the identification of a unique preservation method suitable for both histological and molecular investigations. Interestingly, vacuum packing showed acceptable results for histology and RNA integrity, but only for short times of storage. However, this method has been widely tested in food industry, but not sufficiently studied for preservation of tissues for research purpose [2]. The preservation method appears to be more important than the time of storage and this finding should be taken into account for the selection of the sampling conservation.

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DETECTION OF BOVINE ASTROVIRUS INFECTION FROM ITALIAN CASES WITH NON-SUPPURATIVE MENINGOENCEPHALITIS

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The International Committee on Taxonomy of Viruses (ICTV) divides the family *Astroviridae* in the Avastrovirus and Mamastrovirus genera, affecting birds and mammals respectively. Astroviruses, small positive sense single-stranded RNA viruses, are considered responsible, in humans as in some animal species, of a self-limiting diarrhea and in few cases also associated to brain infection both in children and immunocomprimised patients [1]. Viral metagenomics, bioinformatic studies and the RNA In Situ Hybridization (ISH) techniques have allowed to discover novel Astrovirus in various species, as well as the further development of immunohistochemical methods (IHC) has facilitated the identification of the virus. Recently, two different neurotropic astrovirus genotype (BoAstV-CH13/NeuroS1 and BoAstV-CH15/BH89) were found in brain tissues of bovine with non-suppurative meningoencephalitis of unknown etiology in USA, Switzerland and Germany [2,3,4,5]. The aim of our study was to investigate, retrospectively, the presence of astroviruses in the brain tissues of cattle submitted to the Neuropathology Laboratory of the Italian Reference Centre for Transmissible Spongiform Encephalopathies. Twenty brains collected from 2003 to 2017, have been selected on the basis of the histological diagnosis of bovine non-suppurative meningoencephalitis. Tissues were tested by performing IHC, using two rabbit polyclonal antibodies aimed at structural proteins (ORF2 region), for BoAstV-CH13/NeuroS1 and BoAstV-CH15/BH89 [6]. The IHC screening study was conducted firstly on two brain areas (Brainstem and Hippocampus) of each animal and four animals tested positive for BoAstV-CH13/Neuro S1 and none for BoAstV-CH15/BH89. Subsequently, additional brain regions (Cortex and Cerebellum) from the positive animals were examined and one animal resulted positive in all areas while the three others only in two. IHC positivity was eventually confirmed by ISH [3] testing only one region that showed the better IHC signal; three animals were effectively positives while one was considered doubtful. The neuropathological lesions were widespread in all brain areas with different grade of severity. Our study has showed, for the first time in Italy, the presence and previous circulation of the BoAstV-CH13/NeuroS1 in cows with non-suppurative meningoencephalitis. Further investigations are necessary to better understand the link between astrovirus detection and disease, and to determine the possible correlation of virus replication with neuropathological lesions.

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A CASE OF RETROBULBAR LYMPHOMA IN A CAT

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Lymphoma is the most frequent malignant tumor in cats [1]. It is differentiated on the basis of anatomical site (alimentary, nodal, mediastinal, extranodal) and histological criteria according to WHO classification [2]. A neutered male, 13-year old, domestic short hair cat was presented for exophthalmos and pain at the right eye. At physical examination and magnetic resonance imaging scan an infiltrating mass occupying the retro and lateral area of the eye was detected. The mass was surgically excised and samples were prepared for histology and immunohistochemistry (IHC). The mass was composed of a heterogeneous population of large round atypical lymphoid cells admixed with small hyperchromatic lymphocytes and large histiocytic cells with bizarre and multiple nuclei. IHC showed the presence of small CD3+ and CD5+ T-cells admixed with an atypical population of large CD20+ B-cells. The histiocytic cells were Iba-1+. Morphological diagnosis was Diffuse large B-cell lymphoma with inflammatory component. After treatment with lomustine, the tumor relapsed three months later and the cat was euthanized. In the group of extranodal lymphomas with an inflammatory component the WHO classification recognizes the Extranodal/peripheral T-cell lymphoma (PTCL) and few subtypes of Diffuse large B-cell lymphoma, which includes Lymphomatoid granulomatosis (LG) and T-cell rich B-cell lymphoma (TCRBCL). Due to the B-cell phenotype of the atypical population, PTCL has been excluded from the differentials. Lacking the characteristic angiocentricity and angioinvasion, even LG was less likely. TCRBCL is one of the most described lymphoma subtype in cats. Its microscopical findings are composed predominantly of small reactive T lymphocytes and large neoplastic B lymphocytes. Lately, those TCRBCL with even a consistent component of histiocytic cells has been reported in humans, horses, and dogs as T-cell/Histiocyte-rich B-cell lymphoma (TCHRBCL) [3]. Therefore, according to histological and immunohistochemical characteristics observed in the present case, our final diagnosis was retrobulbar TCHRBCL. The literature refers, despite the low number of TCRBCL reports, a good clinical outcome [4]. Our case demonstrated instead a rapid progression. In conclusion, we report a case of an aggressive retrobulbar TCHRBCL. We consider that the distinction between TCRBCL and TCHRBCL would be useful to collect clinical data in order to add more knowledge about possible different clinical behavior of these two morphological and still under-discovered feline lymphomas subtypes.

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CYCLOPIA IN AN EQUINE FOETUS

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Malformations of the central nervous system are a common observation in domestic animals, resulting from heredity or in utero exposure to teratogenic chemicals or infectious agents [1]. In this respect, the recent epidemic caused by the Schmallenberg virus in ruminants is paradigmatic [2]. We describe herein the main pathological features of cyclopia, which has been recently observed in an equine foetus.

A 9-year-old, Italian Heavy Draft breed mare aborted during the 8th month of gestation. The mare was apparently healthy and did not show any “warning” symptoms. At the external inspection, the foetus appeared of normal size, considering the stage of pregnancy and the standard of the breed. However, an evident anomaly of the head was immediately noted. The skull was globoid, with severe facial malformation and prominent maxillary brachygnathism. Remarkably, a single large median eye was present. After opening the skull, the cranial cavity appeared filled with abundant, yellowish-to-reddish fluid. The brain hemispheres were totally absent, while a sketch of the cerebellum was still evident. Caudally, the gross appearance of the medulla oblongata and of the spinal cord were normal. The remaining part of the skeleton and all of the internal organs were normally developed. The above described congenital defects typically characterize cyclopia, in this case associated with hydrocephalus and cerebral aplasia.

Cyclopia is a very complicated, congenital disease condition, in which striking skeletal malformations are always associated with severe anomalies of the central nervous system. Cyclopia is quite commonly seen in pigs and occasionally detected in other animal species. Epidemic outbreaks of cyclopia can occur in lambs, as a result of *Veratrum californicum* poisoning of pregnant sheep on day 14 of gestation. On the contrary, the aetiology of sporadic cases of cyclopia remains often obscure [1]. To the best of our knowledge, a single case of cyclopia has been so far described in the horse [3], no data being currently available about the aetiology in this species. Nonetheless, the exceptionally rare nature of this pathological condition in horses leads us to believe that an infectious aetiology is unlikely.

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APPEARANCES OF *Chlamydia abortus* IN INFERTILE MARES

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Chlamydia abortus, the etiological agent of Ovine Enzootic Abortion, is an obligate intracellular gram-negative bacteria that infects a large number of mammalian species including *Equidae* causing reproductive and respiratory signs (abortion, pneumonia, conjunctivitis, polyarthritis). Genital infection (occasional abortion and infertility) and conjunctivitis have been reported in mares but relationships between abortion and chlamydial infection is still under discussion [1]. In this study, 50 mares housed in farms located in the area of Turin (Italy) of various ages (between 4 and 20 years, mean and median value = 12) and races with previous history of hypofertility, embryonal or fetal reabsorption, abortions or dystocia were subjected to cytobrush, uterine swab, uterine biopsy and PCR. Small groups of goats and sheep were present on the examined farms.

C. abortus DNA was detected in 6 subjects, by use of a diagnostic and highly sensitive nested-PCR based on ompA gene [2], followed by DNA sequencing, performed by a commercial resource. On microbiological examination one out of all samples, one resulted infected with Enterococcus and one with *S. epidermidis* (both *Chlamydia* positive). In *Chlamydia* infected animals cytobrush showed mucus, debris and >5 PMN/field at 400x and biopsies highlighted different degrees of mononuclear infiltrate and slight desquamation of epithelia. In one PCR+ case, mononuclear infiltrate was mainly localized to submucosa and a moderate periglandular fibrosis was present. All chlamydia-positive mares have been treated with oxytetracycline (6 g intrauterine for 3 days) and 3 of them remained pregnant in the same breeding session. This early phase of our study provide the evidence that the presence of *C. abortus* in mare is underestimated and that its presence can probably play an important role in infertility causing an endometrial, although mild, chronic damage. Moreover, in case of detection of *C. abortus* in infertile mare, intrauterine oxytetracycline therapy may represent a preventive valid treatment in avoiding failure in mare pregnancy.

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PRIMARY RENAL CARCINOMA IN A TIGER (*Panthera tigris*)

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The prevalence of primary renal tumours in domestic animals is less than 1% [1]. In the present case report a metastatic renal carcinoma in a tiger (*Panthera tigris*) is described.
A 12-year-old male tiger, hosted in a zoo, was submitted for post-mortem examination. Referred clinical signs were lethargy, vomiting, constipation and anorexia during the previous week. The animal was sedated for a complete physical examination and diagnostic work-up. Complete blood cell count and chemistry panel were performed: moderate anaemia and increased urea and creatinine concentration were observed. Abdominal ultrasound revealed hydronephrosis of the left kidney. Due to the poor condition, the animal was humanly euthanized. A complete necropsy was performed. On examination, the left kidney was markedly enlarged with a dilated pelvis and a thickened capsule. Cauliflower-like lesions were found adhering to the renal pelvis mucosa. Smaller lardaceous nodules were found scattered throughout the lungs surrounded by sero-haematic fluid.
Samples from kidneys, lungs, and all other organs were fixed in 10% neutral-buffered formalin, processed and stained with haematoxylin & eosin. Further sections were subjected to immunohistochemical staining (IHC) whit cytokeratin AE1/AE3 and vimentin.
Histologically the left kidney mass showed the presence of multiple nodules characterized by papillary proliferation of epithelial cells resembling renal tubular epithelium. Cellular atypia and mitotic figures were also evident. Fibrosis, necrosis, and haemorrhages were consistently encountered in all sections. Multiple metastatic lesions were observed in the lungs, right kidney, left adrenocortical gland and mesenteric lymph node. IHC revealed strong positivity for cytokeratin and moderate positivity for vimentin. The lungs showed also multifocal pyogranulomatous inflammation and alveolar oedema.
Several samples were submitted to bacteriological investigations. *Escherichia coli* was isolated from lungs and left renal fluid. Routine parasitological exam and molecular analyses resulted negative. A metastatic renal carcinoma was diagnosed but further IHC evaluations will be necessary to better characterize the tumour.
Primary renal tumours are rare in domestic cats, with carcinoma being the most recurrent diagnosis [4]. No report about their occurrence in tigers or other wild felids exists. In retrospective studies, neoplasms are the second most frequent necropsy findings, however the primary tumour was rarely localized in the kidney [2,3,5]. It has been recognised that the paucity of descriptions of primary renal neoplasms in the literature makes their diagnosis more difficult [4]. This remarks the necessity of collaboration between institutions, such as IZSTO and Safari Ravenna, to collect uniform post-mortem data to better characterise rare diseases, particularly in captive wild animals.

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IMMUNOFENOTIPICAL EVALUATION OF APOPTOSIS IN LYMPHNODES OF PIGS EXPERIMENTALLY INFECTED WITH HIGH AND NORMAL PATHOGENICITY STRAINS OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRS)

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“Porcine reproductive and respiratory syndrome" syndrome virus (PRRSV) affects sows and piglets causing reproductive failure, respiratory problems and increased mortality in growing subjects. The disease causes huge economic losses in pig farms all over the world [1]. A single-stranded *Nidovirales* order, *Arteriviridae* family, *Arterivirus* genus RNA virus is responsible for this syndrome. In recent years, episodes of disease associated with high pathogenic PPRSV (HP) strains characterized by higher morbidity and mortality, than in the normal pathogenicity strain (NP), in subjects of all ages have been described (4). Transmission occurs mainly through direct contact between subjects, inanimate, animated or aerosol vectors, vertically, trans-placentally, causing fetal death or stillbirth of infected pigs. The virus infects pulmonary alveolar macrophages (PAMs) and intravasal macrophages (PIMPs) where it replicates and subsequently spreads in lymphoid organs. The immune response is delayed and shows impaired efficiency, often leading to secondary infections (*Salmonella, Mycoplasma hyopneumoniae* and others). Some important pathogenetic aspects are the apoptosis of infected cells and adjacent healthy cells, the release of inflammatory cytokines (TFN-α, IL-1 and IL-6) by infected macrophages, the activation of B lymphocytes and a low phagocytic activity. Overall, the immune response to PRRSV is highly dysregulated, probably due to a reduced, or totally absent, production of INF I or by the rapid destruction of PAMs with a related weak innate immunity and a delayed acquired immune response. Therefore, a persistent infection (3) occurs in the lymphoid organs, where a strong depletion of the germinal centers is observed, probably induced by apoptosis. Aim of the study is to define whether apoptosis is actually induced by the presence of the virus and a possible different degree of apoptosis in infection caused by the two different strains. We immunohistochemically evaluated the expression of caspase-3, activated, fundamental enzyme for the initiation of the apoptotic process. Thirteen pigs were enrolled; 3 were used as control, 5 were infected with a highly pathogenic (HP) strain, while 5, with a normal pathogenic strain (NP). Samples were paraffin embedded. Five µm thick serial sections were immunohistochemically evaluated with ABC technique, using an antibody anti Human/Mouse-caspase 3 (active) (R&D System). The number of immune-positive cells and cell fragments in each slide were counted in 5 microscopic fields at 20x magnification. The results obtained revealed that lymph nodes of PRRSV NP infected showed a higher immune-positivity to AB anti Caspase 3 (active), than PRRSV HP. This might be caused by a direct severe pro-apoptotic effect of NP strain and a lower thymic apoptosis [2]. Conversely in HP strain, the observed more severe lymphocytopenia is likely caused by a dramatically thymus cell depletion [2].

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SYSTEMIC INFECTION WITH ENDOCARDITIS DUE TO *Streptococcus dysgalactiae* SUBSPECIES *dysgalactiae* IN AN ADULT DAIRY CATTLE: A CASE REPORT

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Streptococcus dysgalactiae is divided into two subspecies: *S. dysgalactiae* subsp. *equisimilis* (SDSE) for human isolates and *Streptococcus dysgalactiae* subsp. *dysgalactiae* (SDSD) for strains of animal origin. SDSE is a commensal microorganism; however, virulence factors similar to those of *Streptococcus pyogenes* isolates have been found and invasive infections are increasingly reported worldwide. [1,2] SDSD is a component of the vaginal and tonsillar microflora of carrier animals and, in cattle, it is mainly associated with subclinical/clinical mastitis. [3] Sporadic cases of human SDSD infection and presence of virulence genes have been recently reported; therefore, this microorganism is now considered an emerging zoonotic pathogen. [4] The aim of the present report is to describe a case of systemic infection with endocarditis due to SDSD in an adult dairy cow. The animal has been referred for the necropsy and selected samples from liver and right ventricle were collected for histopathological examination. Moreover, bacteriological investigations were performed on different sites, and biochemical identification was obtained following the methods reported in the literature [1]. Macroscopically, the main findings were thoracoabdominal serous effusion, severe hepatomegaly with multifocal areas of necrosis and severe, vegetative, endocarditis involving the tricuspid valve. Microscopically, there were moderate, multifocal, centrilobular, necrotizing hepatitis and severe, neutrophilic, valvular endocarditis characterized by granulation tissue formation, abundant fibrin deposition, and intralesional bacterial colonies. Presumptive identification of all the isolates was compatible with a pure culture of group C Streptococcus. The biochemical reactions identified the strains as SDSD. To the best of our knowledge, cases of bovine SDSD generalized infections have never been previously recorded except from an episode of perinatal septicaemia due to *S. dysgalactiae* in calves [3]. Moreover, SDSD has been isolated in a case of cellulitis followed by toxic shock-like syndrome in a Brown Swiss cow and in a human patient with bacteremia and endocarditis [5,6]. Therefore, further studies towards the virulence factors and zoonotic potential of this pathogen are suggested.

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METABOLIC PLASTICITY IN CANINE MAMMARY TUMORS. PRELIMINARY INVESTIGATION

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Cancer cells must maintain metabolic homeostasis in a wide range of adverse condition linked to a harsh microenvironment, in which they have to survive and proliferate. Therefore the metabolic plasticity is an indispensable requisite for cancer cells allowing the latter to activate different metabolic pathways, lipid and/or glycolytic, in order to respond to high energetic demand (1). Several studies demonstrate that alterations of energy metabolism could represent a "hallmark" of cancer, indeed several neoplasms can be linked to mitochondrial dysfunctions and carnitine could play an important role as a marker of these dysfunctions. This because it acts as a "shuttle" since its main function in the organism is to facilitate the transport of long chain fatty acids from the cytosol to the mitochondria and the energy production, through the Krebs cycle and oxidative phosphorylation. Components of the carnitine systems (CS) are enzymes involved in the bi-directional transport of acyl moieties from cytosol to mitochondria and vice-versa, thus playing a fundamental role in tuning the switch between the glucose and fatty acid metabolism. Carnitine O-acetyltransferase (CrAT) closes the carnitine cycle, catalyzing the addition or removal of carnitine from medium and short-chain Acyl-CoA and allowing the acetyl-carnitine passage from mitochondrial matrix (2). The aim of this study was to assess the expression of CrAT in 5 samples of normal mammary gland tissue, in 5 benign and 10 malignant spontaneous canine mammary tumors by immunohistochemistry and Western blot analysis. Neoplastic samples were classified according to Goldshmidt criteria and divided into grades I to III, applying parameters proposed by Pena. A semiquantitative count of immunostained cells was performed and results were expressed by percentage. In normal mammary glands, strong cytoplasmic expression of CrAT was evidenced in epithelial ductal cells. In G1 canine mammary tumors the intensity of immunostaining was strong and the number of positive cells was higher than 50%. A progressive decrease of immunostained cell percentage and a reduction of the immunostaining intensity were observed from G2 to G3 carcinomas. Western blot analysis confirmed the cross-reactivity of the anti-human CrAT antibody in canine mammary gland. Our results appeared to be superimposable to those obtained in a previous evaluation of CPT1A suggesting that both proteins can play a role in more differentiated tumours (G1) in which the metabolic flexibility can make neoplastic cells able to adapt their metabolism to enable a rapid proliferation and a continuous growth. The lower CPT1A and CrAT amount determined in less differentiated carcinomas, points out that the mitochondrial damage or an often hypoxic microenvironment can impair the Carnitine cycle, and the glycolytic could be the only metabolic pathway. The observation by western blot analysis of a higher expression of Hexokinase 1, a key enzyme involved in the glycolytic process, in tumors with respect to normal mammary gland could confirm these conclusions.

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MAMMARY CARCINOMA WITH OCULAR METASTASIS IN A MARE

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Mammary neoplasias in mares are considered a rare event, usually documented via case reports [1,2,3] or in small case series [4], with a carcinoma prevalence rate of 0.11% [3]. An endo-ocular mass from a 24 years old nonpregnant Sella Italiano mare was conservatively removed and histologically and immunohistochemically diagnosed as a neuroepithelial benign tumor (adenoma of the ciliary body). A second eye mass was then surgically excised with a second histological outcome consistent with a solid carcinoma with squamous differentiation. Due to poor clinical conditions, the animal was euthanized and sent to the necropsy service of the Department of Comparative Biomedicine and Food Science. Necropsy findings showed a primary solid firm mass with a diameter of 15 cm, involving the right mammary gland, with a yellow-whitish cut surface with a central portion of necrosis. One to 6 cm in size, similar nodular masses were found in several tissues and organs including skin, lungs, lymph nodes at different sites, heart, liver, pancreas, parotid gland, intestinal wall (rectum and colon), adrenal glands, muscles and brain. Samples for histological and immunohistochemical examination were collected and routinely processed from all the grossly affected tissues. H&E sections from the primary mass showed multifocal necrosis and a primary neoplastic population of closely packed malignant epithelial cells organized in solid sheets, with foci of squamous differentiation and occasional tubule formation, admixed in abundant fibrovascular stroma and associated with a second population of benign myoepithelial cells. The latter were not detectable in most of the metastases analyzed. Selected sections of the primary tumors and metastases were then immunohistochemically stained for panCK, CK14, CK5-6, CK8-18, vimentin, p63 and calponin (avidin-biotin complex method with a BenchMark automatic immunostainer). As positive control, a dog mammary gland comprehensive of healthy skin and mammary gland was used. The antibody-panel showed: i) strong or mild positive staining in both the primary tumor and metastases for panCK (showing epithelial differentiation) and CK5-6 and CK14, consistent with a proliferation either of basal epithelial cells or myoepithelial cells; ii) occasional positivity for vimentin and p63, and positivity only in the metastases for calponin, consistent with a mild and focal myoepithelial proliferation; iii) inconsistent results for CK8-18, consistent with a low or insignificant proliferation of the luminal epithelium. The findings are compatible with an infiltrative solid carcinoma associated with hyperplasia of the myoepithelial cells. Our findings suggest that the second endo-ocular mass clinically detected was not a relapse of the adenoma of the ciliary body, but a metastasis of the undiagnosed mammary tumor. Despite the reported tendency to metastasize to multiple organs [1,2,4], this is (at the best of our knowledge) the first confirmed ocular metastasis of a mammary carcinoma in a mare.

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SUDDEN DEATH IN ADULT HORSES: A CASE SERIES

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In human medicine a concept of sudden death has been defined by the World Health Organization (WHO) as a rapid death during the first 24 hours after the onset of symptoms. In contrast, in veterinary medicine a universal definition is missing. However, some authors defined sudden death in animals as death that occurs in few minutes or several hours to pre-existing disease or functional disorder [1-3]. The aims of this study were: 1) estimate the incidence of sudden death in adult horses; 2) determine the contribution of post-mortem examination in establishing a cause of death; 3) estimate the proportional mortality in adult horses in correlation with age and breed. An observational retrospective study of 700 dead adult horses presented by veterinary surgeons, owners or law enforcements to the University of Naples “Federico II” or University of Liverpool was carried out over a 10-year period (2005-2017). The submission forms were reviewed to obtain information about medical history, breed and age of the animals. On the basis of the medical history, the animals were divided in the two categories: 1) horses with clinical history of rapid and unexpected death and 2) horses with clinical diagnosis of sudden unexpected death. Sudden death was considered as non-violent and unexpected death occurred less than 6 hours from the onset of the symptoms. On the basis of age, the available data were categorized as following: (1) 1-4 years; (2) 5-15 years; (3) 15+ years. Each horse included in the study was subjected to a complete necropsy and bacteriological analysis; microbiological results and necropsy reports were both extracted from the informatics systems of the two departments. Each cause of death was classified by OS (organ system) and by PP (pathophysiological process). Frequency of each organ injury and pathophysiological process was calculated for each categorical variable included in the study (age and breed). The results were subjected to statistical analysis with x2 test performed with Spss (ver. 21). Of the 700 necropsy reports examined, 50 cases of sudden death were considered (50/700;7.14%). In the OS category, the proportion of causes of death were: 50% respiratory system; 30% gastrointestinal system; 20% cardiocirculatory system. In the PP category, the proportion of causes of death were: vascular injury 60%; inflammation 22%; paratopy 12%; infection 4%; intoxication 2%. No differences were detected in the frequency of causes of death between variables examined. In particular, in the Respiratory and cardiocirculatory system, the most common PP was vascular injury (exercise–induced pulmonary hemorrhages and cardiocirculatory arrest); in contrast in the gastrointestinal system was inflammation associated to acute gastric wall rupture. Our study demonstrated that sudden death is an uncommon diagnosis in adult horses and it is not correlated with age or breed. However, the post mortem examination appears to be a reliable technique to establish the cause of sudden and unexpected death in adult horses.

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DUODENAL GANGLIONITIS, LEIOMYOSITIS AND GASTRIC DILATION IN A DOG – A CASE DESCRIPTION

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Enteric ganglionitis (EG) and leiomyositis (EL) are regarded as relevant topics in the field of gastroenterology. EG and EL can occur primarily or can be secondary to other disease conditions, their clinical picture depending on the affected portion of the gastrointestinal tract [1,2]. The present report aims to describe the peculiar clinic-pathological findings observed in a dog affected by acute gastric dilation.

A 1-year-old, male Weimaraner dog was submitted for clinical evaluation because of anorexia and vomiting during the previous 12 hours, hypothetically due to the ingestion of foreign bodies. The dog received regular vaccination against canine infectious diseases and preventive anti-helminthic treatment. At presentation, the dog showed tachypnoea, tachycardia and abdominal distention. Abdominal radiographs and ultrasonography demonstrated the dilation of the stomach, which was filled with gas, the increase of intestinal peristalsis and the thickening of the duodenal wall. Haematological and blood biochemical parameters were normal. Considering the severity of the clinical picture, an exploratory laparotomy was elected and promptly performed. The careful inspection of the affected segment of the duodenum ruled out the presence of foreign bodies. During laparotomy, a full-thickness biopsy was collected, fixed in 10% neutral buffered formalin and routinely processed for histopathology.

Microscopically, scattered infiltration of neutrophils between the smooth muscle layers was the most important observation. Aggregates of neutrophils were also seen within the enteric nervous system, covering like a coif some submucosal and myenteric ganglia and/or filling the blood vessels surrounding the enteric plexuses. The intestinal epithelium contained a high number of goblet cells, while the lamina propria was infiltrated with mononuclear inflammatory cells and neutrophils. Based on histopathological findings, duodenal EG and EL, lymphoplasmacytic and catarrhal duodenitis were morphologically diagnosed. The dog was treated with anti-inflammatory and prokinetic drugs for 10 days, with progressive remission of symptoms. To date, about 6 months after treatment, the dog is apparently healthy, although it has lost about 10% of its body weight.

The aetiology and pathogenesis of EG/EL are largely unknown and usually attributed to undetermined autoimmune mechanisms. In domestic animals, EG and EL are rarely reported and regarded as responsible for severe, chronic intestinal pseudo-obstruction, unresponsive to any pharmacological therapy [3,4]. It is difficult, if not impossible, to establish the exact role of EG and EL in the development and evolution of the present clinical case. Further studies are highly desirable to clarify the real incidence of EG and EL, their aetio-pathogenesis, as well as their role, if any, in the development of life-threatening disease conditions, such as canine gastric dilation.

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POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD) IN A GILT

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One of the 18 pigs enrolled in a study aimed to identify early biomarkers of renal rejection (Ministerial Authorization n.279/2013-B) developed a Post-Transplant Lymphoproliferative Disorder (PTLD) and the anatomopathological features are here presented. To develop a model of renal transplantation a 4-month old commercial pig underwent monolateral nephrectomy and a subsequent renal transplantation, receiving tacrolimus as immunosuppressive therapy. Thirty days after transplantation euthanasia and necropsy followed. Samples of tissues were formalin fixed paraffin embedded, stained with haematoxylin-eosin and by immunohistochemistry to CD79 and CD3 markers. Paraffin embedded tissues from lymph nodes was used to assess the presence of porcine lymphotrophic herpesvirus-1 (PLHV-1) and porcine endogenous retroviruses (PERVs) genomes by PCR (1,2). Blood sampled before euthanasia showed cell counts within normal range. During necropsy several lymph nodes (superficial inguinal, splenic, gastric, mesenteric, aortic, tracheobronchial and mediastinal) appeared markedly enlarged (up to 5 cm), edematous and pale. The cut surfaces were white, homogeneous and firm. The spleen was moderately enlarged and on cut surface white pulp appeared as multiple round prominent and bulging nodules, 0.5 to 1.2 cm in diameter. Histologically, in the lymph node, the architecture was effaced by neoplastic tissue, composed of densely cellular sheets of round cells, supported by scant fibrovascular stroma. Neoplastic cells were 40-50 µm, with distinct cell borders, high N/C ratio and scant eosinophilic cytoplasm. Nuclei were round and paracentral with finely stippled chromatin and 2-3 basophilic nucleoli. Anisokaryosis and anisocytosis were severe. Mitosis were 10 per HPF. Intermixed there were numerous, 10 µm in diameter, round cells with high N/C ratio, consistent with small lymphocytes. Splenic architecture was replaced by neoplastic lymphocytes and remnants of red pulp were visible at the periphery. Neoplastic cells in both lymph nodes and spleen were CD79a positive and CD3 negative. PCR allowed the identification of the genome of PERVs and failed to identify the genome of PLHV-1. PTLD is an abnormal lymphoid proliferation that affects both humans and animals (3,4). In humans PTLD is strictly linked with Epstein-Barr virus. PTLD in swine is mainly described in miniature pigs and PLHV-1 seems to be involved as causative agent (3, 4) but few PTLD cases are negative for PHLV-1 genome and some non-PTLD cases are positive (3,4). Both PHLV and PERVs are common infections in conventional pig herds and their precise role on the pathogenesis of tumours in the specie has still to be elucidated. The present case of PTLD is, to the best of our knowledge, the first reported in a non-miniature breed of swine, and shares the following features with the model known in the pig: time of appearance after the transplantation (30 days in this case, from 21 to 45 days from literature); a prevalent involvement of lymphoid organs rather than invasion in non lymphoid tissues; a B cell penothype. PTLD should be take into account in the management of swine during transplantation and its management can be a useful model for the human counterpart of the disease.

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POST-MORTEM FINDINGS IN THREE UNDER HUMAN CARE GIRAFFES (*Giraffa camelopardalis*)

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Despite giraffes (*Giraffa camelopardalis*) are one of the most common animals kept under human care in most of the European zoological parks, scarce literature about their diseases and pathological findings could be found [1]. This could be related to difficulties in performing detailed physical and/or post-mortem examinations of animals belonging to this species due to their size and features.

Over the last five years, five adult male giraffes died in zoological parks in Northern Italy.

In one case neurological signs and ataxia were reported, one animal presented chronic articular inflammation (more severe in the knees), while the other three giraffes were found dead with no previous clinical signs.

All the animals were in good preservation status and necropsies were carried out within 32 hours, routinely collecting samples for histopathological, virological (BVDV), microbiological and parasitological examinations.

All the examined animals were in moderate nutritional status. Four of them showed severe meteorism in the post-mortem examination but only in two cases it was deemed to be the likely cause of death. In another case acute hemolytic anemia was suspected due to splenic and renal hemosiderosis; while the fourth animal died for multiple skull fractures and cerebral haemorrhage. The last giraffe presented a chronical mild to severe diffuse abomasitis and its death appeared to be connected with a dysfunction of the gastro-enteric apparatus with possible dismicrobism.

Common findings in the gastrointestinal tract were mild to severe, diffuse, acute abomasitis (4/5) with ulcers and enteritis (3/5). Two of the animals with enteritis grew E. coli, and one had severe suppurative typhlitis (1/3). Other findings were marked, diffuse red-pulp depletion (4/5) and chronic passive hepatic congestion (2/5). No viral and parasitic infections were identified.

Even if different causes of death were found after post-mortem examination, the gastro-intestinal tract presented similar lesions in all five giraffes, possibly leading to meteorism and dysbiosis condition.

Therefore, it is possible to assert that in these animals no specific infectious diseases were detected, but the digestive tract is the most tricky district [2,3]. Further studies should be aimed at ameliorating their feeding and diet composition to reduce gastro-intestinal dysbiosis and potential associated diseases.

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AN EPISODE OF MYCOBACTERIOSIS IN MBUNA AFRICAN CICHLIDS

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Piscine Mycobacteriosis is a chronic progressive infectious disease caused by microorganisms belonging to the genus *Mycobacterium*. Clinical signs often appear at advanced stages of the disease and are non-specific such as emaciation, abdominal swelling, haemorrhagic and dermal lesions [1]. *M. fortuitum, M. marinum, M. chelonae* are the main etiological agents of fish mycobacteriosis, but in recent years other species were also associated with this disease: *M. abscessus* and *M. peregrinum*. These species are also the most frequently associated with human infections. The family *Cichlidae* includes hundreds of species that play an important role in the ornamental fish industry and their international trade from all over the world is increasing. The aim of the present study was to describe a case of mycobacterial infection in African cichlids from Lake Malawi termed mbuna, held in a private aquarium in Italy. During fish health monitoring activities, performed in 2017 by Fish Diseases Laboratory of Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, 40 cichlids were examined. Fish presented non-specific signs that cannot be easily differentiated from the other fish disease. The animals were euthanatized by overexposure to Tricaine Methanesulfonate (MS-222) and subjected to necropsy. Samples from liver, spleen and gut were collected and partly fixed in 10% neutral-buffered formalin for the histological examination, while another portion, not fixed, were used for parasitological, virological and bacteriological investigations including mycobacterial culture. The tissues for histopathology were processed by standard paraffin wax techniques, cut in 4±2µm sections and stained with haematoxylin-eosin (HE) and Ziehl–Neelsen stain (ZN) to detect acid-fast bacilli. For mycobacterial culture the liver was homogenized, decontaminated and inoculated on 2 Stonebrink's tubes and 2 Löwenstein-Jensen medium tubes. One tube from each medium was incubated at 28±1°C and 37±1°C respectively. All suspected colonies were microscopically examined using ZN; DNA from positive samples was extracted and subjected to amplification as indicated in literature [2]. PCR amplified products were purified, sequenced and compared to those submitted in NCBI database for mycobacterial species identification. At necropsy, no visible lesions in the visceral organs were observed. Microscopically, nine fish revealed single or multiple granulomas in late evolution stage, predominantly located in the gut and only 5 were positive at ZN. Fifteen fish (prevalence 37.5%) resulted positive at culture and the isolates were identified as *M. peregrinum* (7), *M. fortuitum* (2), *M. abscessus* (1), *Mycobacterium* spp (1). Two co-infections (*M. peregrinum - M. gordonae*; *M. gordonae - M. chelonae*) are also been identified. Piscine mycobacteriosis is often underestimated, monitoring activities are an important tool to preserve the welfare of ornamental fish and to safeguard public health. This study showed that ornamental fish may be source of infection for risk groups, such as fishery professionals or ornamental fish hobbyists.

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PERITONEAL MESOTHELIOMA IN A WILD BOAR: AN ENVIRONMENTAL SENTINEL FOR HUMAN HEALTH HAZARDS

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Mesotheliomas are tumours arising from serous cells limiting pericardial, pleural and peritoneal cavities. They occur sometimes in cattle and dogs, but occasionally are seen in different species including horses, lambs, cats and rats [1]. Especially in humans the incidence of mesothelioma has been associated with exposure to asbestos [2]. The author describe the pathological findings of a peritoneal mesothelioma observed in a wild boar knocked down during the last hunting season in Caulonia, a small village in Calabria region (south of Italy). A 3 years old, female, wild boar, 70 kg of weight, was regularly admitted to post-mortem inspection. During the opening of the abdomen, multiple, isolated or coalescing firm and white nodules, varying in size from 2mm to 3cm in diameter, were observed attached to the peritoneum throughout the abdominal cavity. Diaphragm, intestinal serosa, urinary bladder, liver and kidneys were covered with the same nodules. No lesions were detected in thoracic cavity. A diagnosis of tuberculosis was suspected. Samples from the peritoneal masses, liver, kidney and diaphragm were collected for microbiological and histological investigations. Culture test from *Mycobacteria* spp. was negative. Histologically nodules were composed of polygonal, oval, cuboidal or spindle cells organized sometimes in a papillary pattern sometimes in solid structures (nests, cord or sheets) supported by a variable quantity of connective tissue. Necrosis and non suppurative inflammation were frequently observed. Based on the morphological pattern a diagnosis of biphasic mesothelioma was made. Primary tumours of the peritoneum are rare and must be differentiated from tuberculosis, parasites or metastatic tumors. In the present case asbestos fibers were not detected and the association between the exposure to asbestos and the tumor was not demostrated. This wild boar lived in an area rich in abandoned mineral sites. Healthy lung tissues from two boars from another geographic area of Calabria (Monte Reventino area) showed tremolite fibers confirming the spread of asbestos fibers in the environment [3]. The role of wild boar as environmental sentinel for human health hazard must be considered. An active surveillance on regularly slaughtered domestic and wild animals is suggested to quantify the potential human risk of exposure.

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PARATHION POISONING IN BEEF HERD IN SICILY

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Organophosphorus (OP) and carbamate (C) insecticides are used commonly in agriculture to control pests of crops and animals [1]. Aim of this paper was to describe an outbreak of poisoning due to parathion in a beef herd in Sicily. In February 2018, veterinary surgeons were asked for an acute outbreak of suspected poisoning in a herd of about 300 crossbreeds beef calves. Anamnestic data were collected, and clinical, hematological and biochemical exams were performed. Anatomopathological, microbiological and toxicological investigations on selected organs of died animals were made. The animals, 6-24 months-old, were kept in several pens and fed with two types of unifeed made in farm using straw (same for growing and finishing groups) and feedingstuff (different for the two groups of animals). Water ad libitum was the same for all the boxes. Only the finishing beef (95 animals), showed marked or slight clinical signs with tremors, seizures, hypersalivation, recumbency, profuse sweating, lowing, depression and gastrointestinal atony with few feces covered with mucus. Within 7 days, 28 finishing beef calves died. Morbidity and mortality were 100% and 29% respectively. Increase of CPK, LDH, AST, ALT and K was detected in both markedly symptomatic finishing animals (G1) and slightly symptomatic finishing animals (G2). Increased urea values were found in G1 only. Growing beef groups showed no symptoms or alterations of hematological and biochemical profiles. Necropsies, performed on 6 calves, showed diffuse petechial hemorrhages on subcutis, mesentery, epicardium and heart, edema of mesentery, hemorrhagic enteritis and sometimes diarrheal material in rectum, renal and hepatic congestion, pulmonary congestion and edema with froth in the airways, lungs and nostrils. Main histological findings were marked congestion of lungs and spleens and marked/moderate pulmonary edema. Toxicological exams performed in a calf samples detected parathion in liver and spleen at 0.4 mg/Kg and 0.64 mg/kg respectively. OP and C pesticides are often implicated in poisoning in mammals, fishes and birds. The poison inhibits the enzyme acetylcholinesterase at the muscarinic, nicotinic and central nervous system synapses [2]. Acute toxicity develops within minutes to a few hours and includes signs of muscarinic (salivation, excessive lacrimation, frequent urination, and diarrhea), nicotinic (weakness and tremors) and central nervous (depression and seizures) toxicity. Tremors and seizures can cause muscle damage and increase of muscle enzymes and potassium [1]. In fatal cases, death is often caused by respiratory failure and/or bradycardia and heart block. Furthermore, hemorrhagic lesions may be caused by prothrombin deficiency due to the hepatic toxicity of parathion derivatives [3]. The use of parathion is forbidden in Italy, but illicit purchase is not excluded. In this case a malicious poisoning was suspected.

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ANIV
MALATTIE PARASSITARIE E SANITÀ PUBBLICA

ANTIBIOTIC SUSCEPTIBILITY AND VIRULENCE FACTORS IN *ESCHERICHIA COLI* FROM SYMPATRIC WILDLIFE OF APUAN ALPS REGIONAL PARK (TUSCANY, ITALY)

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In the last years, the number of studies concerning antibiotic resistance (AMR) constantly raised as well as that of studies considering the role of wildlife [1]. Currently, few researches has been carried out in Italy concerning AMR in free ranging animals, especially mammals [2; 3]. The aim of this study was to isolate and identify *E. coli* from faeces of wild animals living in the Apuan Alps Regional Park (Tuscany, Italy) and to evaluate some of their antibiotic-resistance and pathogenicity traits. From April 2017 to October 2017, 85 fecal samples were collected from different animals, with different diets (carnivorous, omnivorous and herbivorous). Sampled species were wolf (42), badger (6), moufflon (9), fox (13), wild boar (3), wild goat (5), red deer (3), roe deer (2) and hare (5). *E. coli* isolates were obtained employing TBX agar plates, identified by MALDI-TOF MS analysis, subjected to antibiograms and PCR for the detection of antibiotic resistance genes and pathogenicity factors. Seventy-one isolates were obtained and correctly identified as *E. coli*. The highest resistance rates were found against cephalotin (39.4%) and ampicillin (33.8%), followed by amoxicillin-clavulanic acid (15.5%), streptomycin (12.7%), tetracycline (5.6%), trimethoprim-sulfomethoxazole (2.8%), gentamicin (1.4%), cefotaxime (1.4%) and enrofloxacin (1.4%). No resistance was found against cefoxitin, chloramphenicol, imipenem and aztreonam. As concerns the detection of resistance genes, 28/71 isolates were negative for all genes; among β-lactams-resistant isolates, 55.3% harboured blaCMY-2 gene, but not blaTEM gene, whereas 44.7% showed the absence of both genes; among trimethoprim-sulfamethoxazole-resistant isolates, 100% showed positivity to sul2 gene, whereas no positivity was found to sul1 and sul3 genes; among streptomycin-resistant isolates, 44.4% harboured strA-strB, whereas the aadA1 gene was found in an intermediate and in one susceptible isolate; lastly, among tetracycline-resistant isolates, 100% showed positivity to tet(B) and 25% to tet(A), whereas no positivity was found to tet(G). As concerns genes encoding virulence factors, 45 out of 71 isolates were negative for all genes; 21.1% of the isolates carried only astA gene (EAEC), whereas 9.9% of them carried both escV gene and eaeA gene (aEPEC); single isolates (1.4%) harboured escV (aEPEC), escV with astA and eaeA (EAEC-aEPEC), astA with stx2 and hlyA (EAEC-EHEC) or astA with stx1, stx2 and hlyA (EAEC-EHEC). Obtained results show how even free-ranging wildlife from a non anthropized environment, such a natural park, could resent of antimicrobial resistance phenomena, carrying not only virulence factors, but also antibiotic resistance determinants.

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RISK ASSESSMENT OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* (MAP) IN PIEDMONT: THE ROLE OF CAMELIDS

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Paratuberculosis, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is increasingly important, and competent authorities designed a control programme in bovine farms, in Piedmont region [1]. MAP can infect camelids, such as llamas and alpacas [2][3], which are not considered in control programmes. In this study, we evaluated the occurence of MAP, and assessed the potential role of camelids in the maintenance and spread of the infection. In a risk assessment approach, we framed two risk questions: 1) What is the risk of introduction and maintenance of the infection in a MAP-free camelid farm? 2) What is the risk of spread of MAP from an infected camelid farm to other animals? We collected a total of 51 fecal pool samples from 20 camelids farms, which were analyzed by isolation, hemi-nested PCR and VersaTREK [4]. Prevalence of MAP was 15% (IC 95% 3.2-37.9%), which is lower than prevalence in other ruminant species [5]. We analyzed an outbreak in a farm, where a llama died of MAP, and where all animals had been individually analyzed months before our study. We analyzed new individual animal samples, and gathered information on animal movements. We used scientific literature and expert opinions to identify possible MAP introduction routes, including infected llamas or goats. We concluded that the deceased llama acted as a super spreader. On the other hand, the other positive animals were classified as passive shedders, since the prevalence of MAP in these individuals was decreasing between two subsequent samplings. Based upon our results, the risk of MAP in a camelid farm in Piedmont and its subsequent transmission is low, but it should not be completely disregarded in paratuberculosis control programmes. Moreover, we suggest that more accurate control of movement of camelids, including individual identification, should be implemented, and MAP testing should be periodically carried out on these animal species.

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ANTIBODY TITERS AGAINST PANLEUKOPENIA, HERPESVIRUS AND CALICIVIRUS INFECTIONS IN STRAY CATS OF MILAN

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In many Italian cities, stray cats can live together in freedom in small or big groups, called "colonies": they are protected by National and Regional laws, which regulate their correct management. The purpose of our study was to test the antibody protection against feline panleucopenia virus (FPV), feline herpesvirus type 1 (FHV-1) and feline calicivirus (FCV) infections in 120 stray cats living in colonies in Milan city in order to obtain an indication of the diffusion of these three pathogens. Blood samples were collected (upon authorization of University Ethical Committee) by the veterinary service of the ATS (Agenzia di Tutela della Salute) of Milan during mandatory sterilization procedures for the control of births in stray cats population (Lombardy Regional Law n. 2009/33, [1]), and then tested with Vaccicheck Feline (Biogal/Agrolabo), an in-practice kit based on a solid-phase ELISA test that detects the presence of protective antibodies specific for core vaccine antigens (FPV, FHV-1 and FCV). Cats were divided in different categories: age (10 kittens, 56 young adults, 42 adults, 12 seniors), sex (49 males and 71 females), neuter status (92 sexually intact and 28 neutered) and health status (90 healthy and 30 not healthy). According to the indications of the kit, a cat was considered protected with an antibody titer ≥1:80 for FPV, ≥1:16 for FHV-1 and ≥1:32 for FCV. The mean antibody titer for the whole feline population was highly protective (FPV ≥1:150, FHV-1 ≥1:16, FCV ≥1:100) but the percentages of protected cats were different: less than 50% for FPV and FHV-1 (47.5% and 37.5% respectively) and higher for FCV (85.8%). As the age increases, also the titers increase: kittens FPV ≥1:160, FHV-1 ≤1:4, FCV ≥1:70; young adults FPV ≥1:180, FHV-1 ≥1:10, FCV ≥1:100; adults FPV ≥1:120, FHV-1 ≥1:20, FCV ≥1:120; seniors FPV ≥1:180, FHV-1 ≥1:40, FCV ≥1:100. Both females and males had protective mean antibody titers for FPV and FCV (F=FPV ≥1:150 vs M=≥1:170, F=FCV ≥1:110 vs M=≥1:100), but only females resulted protected against FHV-1 (F=≥1:20 vs M=≥1:10). The mean titers of sexually intact and neutered cats against FPV and FCV were protective (I=FPV ≥1:150 vs N=≥1:170, I=FCV ≥1:110 vs N=≥1:100) but only neutered cats were protected against FHV-1 (I= ≥1:10 vs N=≥1:30). No main differences between healthy (FPV ≥1:170, FHV-1 ≥1:16, FCV ≥1:100) and not healthy (FPV ≥1:120, FHV-1 ≥1:20, FCV ≥1:110) cats were noted and all the mean titers were protective. Our results suggest that the tested feline population is generally protected against FPV, FHV-1 and FCV, and that feline calicivirus represents the most common pathogen. In conclusion we decided to compare our results to a similar study conducted in Florida (USA): of 347 cats entering in an animal shelter, only low percentages were protected against all the diseases (FPV=39.8%, FHV-1=11%, FCV= 36.6%) and the most common pathogen was feline parvovirus and not calicivirus; on the other hand, all the other variables were superimposable to our results [2].

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BOVINE HERPESVIRUS-4 BASED VECTOR DELIVERING PESTE DES PETITS RUMINANTS VIRUS HEMAGGLUTININ ORF INDUCES BOTH NEUTRALIZING ANTIBODIES AND CYTOTOXIC T CELL RESPONSES

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Peste des Petits Ruminants Virus (PPRV) is an extremely infectious morbillivirus that primarily affects goats and sheep. In underdeveloped countries where livestock are the main economical resource, PPRV causes considerable economic losses [1]. Protective live attenuated vaccines are currently available but they induce antibody responses similar to those produced in PPRV naturally infected animals. Effective vaccines able to distinguish between vaccinated and naturally infected animals are required to PPRV control and eradication programs. Hemagglutinin (H) is a highly immunogenic PPRV envelope glycoprotein displaying both hemagglutinin and neuraminidase activities, playing a crucial role in virus attachment and penetration [2]. The aim of this study was to generate and characterize a recombinant Bovine Herpesvirus-4 (BoHV-4)-based vector delivering an optimized PPRV-Hemagglutinin expression cassette, BoHV-4-A-PPRV-H-ΔTK, and to assess its immunogenic properties in mice. Since BoHV-4-based vector has been successfully employed to immunize several animal species as mice, sheep and goats [3], in the present work, a preliminary immunization study for PPRV in mice, before applying BoHV-4-based vector in sheep and goats, was performed. A recombinant BoHV-4 expressing the PPRV Hemagglutinin gene, BoHV-4-A-PPRV-H-ΔTK, was generated through BAC recombineering homologous recombination and inoculated in immunocompetent C57BL/6 mice. Animals were bled at scheduled days post first immunization; five animals per group were also sacrificed at day 7 post-boost to perform T cell response experiments. BoHV-4-A-PPRV-H-ΔTK-immunization elicited both cellular and humoral immune responses with specific T cell and cytotoxic T lymphocyte activation, as demonstrated measuring intracellular IFN-gamma production and through cytotoxicity assay and sero-neutralizing antibody production against PPRV.

Since these data suggest that recombinant BoHV-4-A-PPRV-H-ΔTK provides a strong specific immune response against PPRV in mice it could be an effective vaccine candidate in small ruminants against PPRV herd infection, potentially applicable for eradication programs thereby distinguishing between infected and vaccinated animals.

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A LARGE-SCALE INVESTIGATION ON THE RELATIONSHIP OF CATHELICIDIN ELISA WITH SOMATIC CELL COUNTS IN COW MILK

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Mastitis is one of the most economically relevant diseases in dairy cows, causing reductions in milk quality and yield. Currently, mastitis monitoring is based on the milk somatic cell count (SCC), but inflammation-specific protein markers might provide sensitive and reliable alternatives and enable immunoassay-based screening strategies. Cathelicidin is an inflammatory protein released in milk with good diagnostic performances in cows [1] and ewes [2], and promising results also in goats and in water buffaloes. Adding to potential improvements in terms of sensitivity and specificity, an immunoassay tool such as an ELISA would provide additional advantages, including the ability of testing stored frozen samples in an automated fashion. Since SCC is the most consolidated, reliable and widespread mastitis marker, in this work we assessed the relationship of the two markers on a large and heterogeneous milk sample set, evaluated the performance of different ELISA thresholds and estimated the intra and inter-assay variability. A total of 1690 quarter milk samples from 18 Holstein-Friesian herds were available. Of these, 1494 samples from 17 herds were obtained as a result of laboratory routine testing at the University of Milan, and 196 samples were from cows experimentally infected with different *Streptococcus uberis* strains. All cows were in full lactation. SCC was evaluated with automated cell counting instruments and expressed as linear score (LS). ELISA was carried out as described previously and results were expressed as normalized OD450 (NOD450) against six negative control reference samples [1,2]. According to ROC curve analysis against SCC>200,000 cells/mL as the gold standard, the AUC of cathelicidin was 0.971 (95% CI 0.962-0.979). Concerning sensitivity vs specificity relationships, the intersection of the two curves was at NOD450 0.05, while the Youden's index was at NOD450 0.1. Cohen's kappa for these two NOD450 values were 0.860 and 0.869, respectively. Result distributions and frequency classes did also support a very high level of correlation of the two markers and a similar class distribution, with the ability of the two thresholds to reliably separate low from high LS sample classes. With a threshold of NOD 0.05, 750 out of 766 (97.9%) cathelicidin-negative samples had LS<2, while 595 out of 601 (99.0%) cathelicidin-positive samples had LS>6. These two classes included the majority of samples (1367 out of 1690, 80.9%). Class inversion was seen at LS 4. With a threshold of NOD450 0.1, 759 out of 766 (99.1%) cathelicidin-negative samples had LS<2, while 589 out of 601 (98.0%) cathelicidin-positive samples had LS>6. Class inversion was seen at LS 5. Intra-assay variation CV% was <11.5% on 40 replicates of 2 samples. Inter-assay variation CV% was <15.1% on 3 replicates of 9 samples in 3 experiments. In conclusion, the cathelicidin ELISA showed a very high correlation with SCC and very promising diagnostic performances in terms of sensitivity, specificity, and robustness for cow mastitis detection.

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BACTERIA ASSOCIATED WITH SKIN FOLDS IN HEALTHY AND DISEASED BRACHYCEPHALIC DOGS

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Brachycephalic breeds (for example, Boxer, Bulldog) are very appreciated but the particular conformation of cranium (width wider than length) could cause the folding of exceeding skin. The formation of skin folds mainly at nasal level could induce an alteration of the cutaneous microenvironment with bacterial and fungal growth and the appearance of dermatologic diseases (pyoderma) [1]. Generally skin folds are infected by commensal microorganisms with *Staphylococcus pseudintermedius* the main etiological agent [2]. Furthermore, the use and abuse of antimicrobial molecules for treatment of bacterial skin diseases led to the emerging problem of multidrug resistant bacteria [3]. The aim of this study was the evaluation of bacteria associated to nasal skin folds in brachycephalic healthy and pathologic dogs. Bacterial isolation and characterization, both in 32 healthy and 16 diseased dogs (selected by clinical examination), was performed using microbiological standard methods. All the swabs were streaked on blood agar plates (Tryptic Soy Agar with 5% of sheep blood, Microbiol, Italy) and incubated for 24 h at 37°C under aerobic conditions. Isolates were identified phenotypically by colony morphology, haemolysis, Gram-stain, some biochemical tests (catalase, oxidase) and growth on selective media [4]. Bacteria isolated from diseased dogs were tested for susceptibility to different antimicrobial molecules by Kirby-Bauer sensitivity test and the results were interpreted using the CLSI criteria [5]. According to the literature [1] 32% of our swabs from healthy dogs was positive for *S. pseudintermedius* and 16% for other species belonging to *Staphylococcus* genus. In pathologic skin folds *S. pseudintermedius* was the prevalent species (44%), followed by *Pseudomonas aeruginosa* (12%) and *Proteus mirabilis* in association with other bacteria (38%). *S. pseudintermedius* strains were sensitive to amoxycillin + clavulanic acid and fluoroquinolones, while microbial associations showed high resistance levels (MDR). So, our results confirmed the double role of *S. pseudintermedius* as a skin commensal and a real pathogen in some dermatological diseases (e.g. pyoderma). Moreover, microbial associations showed higher rate of antimicrobial resistance than single bacterial species.

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GENETIC CHARACTERISATION OF CANINE ADENOVIRUS TYPE 1 DETECTED IN THE TONGUE OF A WOLF

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Evidence of exposure to Canine adenovirus type 1 (CAdV-1), the aetiologic agent of the infectious canine hepatitis in dogs, has long been reported in wolves in several geographic areas. In this study, the genetic characterization of CAdV-1 detected in the tongue sample of a male pure Italian wolf (*Canis lupus*) found dead in Tuscany, Italy, in December 2014, is described. A complete post mortem examination was carried out and samples of mesenteric lymph node and tongue were collected. CAdV-1 DNA detection was carried out using a real-time PCR able to simultaneously detect and differentiate the CAdV-1 and the CAdV type 2 [1]. Amplification, nucleotide sequencing and assembling of hexon and fiber CAdV genes were done as previously reported [2]. The obtained nucleotide sequences were aligned with reference sequences of canine adenoviruses from GenBank and translated into amino acid sequences using BioEdit 7.2.5. Phylogenetic relationships were evaluated for the concatenated hexon and fiber genes sequences using MEGA version 6.0.6. At necropsy, the wolf appeared to have been in good general health. The subject was killed by a motor vehicle collision. Wolf age was estimated to be a second class (12-24 months) according to tooth development. CAdV DNA was detected in the lymph node ($6.7x10^4$ DNA copies/g) and in the tongue epithelium ($1.6x10^6$ DNA copies/g), with a melting temperature specific for the type 1. The CAdV-1 hexon and fiber genes sequences differed from all the CAdV-1 reference sequences both at the nucleotide and the amino acid levels. The position 388 of the predicted CAdV-1 hexon protein differentiated the Italian sequences from the other reference strains by having serine instead of asparagine. Several nucleotide and amino acid mutations in the hexon and fiber genes allowed to distinguish all the italian strains submitted to GenBank until now in two groups. Phylogenetic tree confirmed the clusterisation of the Italian CAdV-1 sequences. This is the first detection of CAdV-1 DNA in a tongue sample of wolf and a high amount of the target CAdV-1 DNA was detected in this sample. Hence, it is possible to speculate that the tongue epithelium represents an important site for viral replication. This tissue could be used as a complementary sampling site for CAdV-1 testing in wildlife, especially in deceased animals undergoing postmortem changes, as previously reported for parvovirus [3]. Sequence alignment and phylogeny confirmed that several genetic variants of CAdV-1 circulate in Italy [2], and allowed to distinguish Italian CAdV-1 in two subgroups, each of which infects various animal species, suggesting that the transmission of the virus from wild animals to the dog, and vice versa, can frequently occur.

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CHRONIC MASTITIS BY *LISTERIA MONOCYTOGENES* IN A CLINICALLY HEALTHY GOAT

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Listeria monocytogenes is an ubiquitous gram positive bacterium responsible for a severe foodborne disease. The genus *Listeria* includes 6 facultative intracellular species, but only *L. monocytogenes* is pathogenic for humans, while *Listeria ivanovii* is a strict animal pathogen. The clinical manifestations of listeria infection in goats are encephalitis, septicemia, abortion, and also diarrhea. Goats, like sheep, may be asymptomatic carriers, shedding *L. monocytogenes* in feces and milk. Aims of the study were: 1) isolation and molecular identification of the pathogen, 2) cell localization in the udder parenchyma of a clinically healthy animal. The herd consisted of 200 lactating goats, housed in a free stall and milked using a machine equipped with automatic take-off device. The farmer produces cheese, therefore bacteriological analysis for foodborne pathogens is compulsory three times a year. At the control in June, bulk milk tested positive for *L. monocytogenes*, but no goat showed clinical signs of listeriosis. Then, all goats ware sampled in pools of 20 animals each; the goats of the positive pool were sampled once more, to identify the shedding one. Just before culling, milk samples were taken from each half gland; the udder was transported to the laboratory, where tissue samples were collected for bacteriological analysis and formalin fixed for histological examination. Molecular characterization of isolates was performed using 3 set of specie-specific primers, as already described [1]. Tissue injury was evaluated on haematoxylin-eosin stained slides. IF was performed to observe the presence of *Listeria*, using the following antibodies: *L. monocytogenes* (LMZ), MAC 387, Ly6B (clone 7/4), and Cytokeratin peptide 18 to target the bacterium, macrophages, neutrophils and epithelial cells, respectively. Colonies of growth resembling *L. monocytogenes* were isolated from the milk, the cistern and the parenchyma of the right half udder. The presumptive identification was confirmed at genus level using Api ID32 Strep (Biomerieux, F). The PCRs results showed amplicons of the right size for all gene tested and confirmed the belonging to the species *L. monocytogenes*. Histological examination highlighted an interstitial mastitis, with high infiltration of macrophages and PMN cells. In some areas of the tissue, atrophy of the alveoli and corpora amylacea could be observed. IF showed a low presence of *L. monocytogenes*, localized mainly in macrophages and neutrophils. Some bacteria were evidenced also in epithelial cells and in alveolar lumen. The results of the study demonstrate that the pathogen is able to invade the mammary tissue of the goat, establishing a chronic but asymptomatic infection. This represents a serious problem for the management of the infection in bred, with enhanced risk of infection of the newborns, and raises concern regarding the health of goat cheese consumers.

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COMPARATIVE ANALYSIS OF THE ANTIBIOTIC RESISTANCE IN METHICILLIN-RESISTANT AND METHICILLIN-SUSCEPTIBLE *Staphylococcus pseudintermedius* STRAINS OF CANINE ORIGIN

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Staphylococcus pseudintermedius is involved in a wide variety of infections, mainly in dogs. In the past, this strain was generally susceptible to beta-lactam antibiotics, but already since 2006 methicillin-resistant *S. pseudintermedius* (MRSP) strains have been isolated becoming a relevant animal health problem in veterinary medicine [1,2]. MRSP has also been proved to be resistant to most of the antimicrobial agents approved for veterinary applications. However, variability of phenotypic and genotypic features, including virulence, molecular epidemiology, and biological characteristics, have not been fully explored in methicillin-susceptible *S. pseudintermedius* (MSSP) isolates.

The present study aimed to determine the antibiotic resistance patterns and frequencies of some genes of resistance among MRSP and MSSP isolates recovered from dogs with otitis externa or pyoderma, which attended, during the years 2015-2017, the Veterinary Teaching Hospital of the Department of Veterinary Medicine and Animal Production of University of Naples "Federico II".

We isolated, on Mannitol Salt agar plates and then identified by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS), a total of 129 *S. pseudintermedius* strains. We detected by PCR for *mec*A gene 21% of MRSP and 79% of MSSP. The antibiotic resistance profiles were evaluated by disk diffusion method on Mueller Hinton agar plates, according to the Clinical and Laboratory Standards Institute guidelines [3]. The resistance rates to penicillin and ampicillin were of 100% and 75% for MRSP and MSSP, respectively. None of the MSSP isolates showed resistance to oxacillin and cefoxitin disk diffusion tests, confirmed also by the absence of mecA gene; and 12% of these isolates were susceptible to the twenty-one tested antibiotics.

MRSP strains showed high resistances to amoxicillin-clavulanate (96%), erythromycin (89%), kanamycin and streptomycin (81%), sulfamethoxazole-trimethoprim and tetracycline (74%), whereas MSSP isolates showed lower 50% resistance to the same antibiotics.

However, it is worth noting that more than 25% of MSSP strains showed resistance to almost 10 antibiotics. Therefore, the spread of multidrug-resistant MSSP should be monitored and their pathogenic role, related to antibiotic resistance, deserves further studies.

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THE ITALIAN SURVEILLANCE PLAN FOR INFECTIOUS BOVINE RHINOTRCHEITIS: TWO YEARS OF ACTIVITY

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Infectious Bovine Rhinotracheitis (IBR) is a disease that affects domestic and wild ungulates, caused by the Bovine herpesvirus-1 (BoHV-1; Herpesviridae family) [1]. BoHV-1 may cause production losses, its effect on animal health and welfare manifests as respiratory and genital disease, reduced fertility and abortion [2]. The virus is distributed world-wide, but has been eradicated in some European countries such as Austria, Denmark, Finland, Sweden, Switzerland, Norway, Germany, United Kingdom (Jersey), and Italy (Bolzano Province, Valle d'Aosta Region) [3,4]. The italian Ministry of Agriculture, Food and Forestry, in June 2015, approved the first National Surveillance Plan for Eradication of BoHV-1, applied to beef breeds recorded in the National Herd Book (maintained by the "Associazione Nazionale Allevatori Bovini Italiani da Carne - ANABIC"). The aim of this study was to evaluate the results of the first two years of the Plan. Objective of the Plan was to eradicate IBR in herds recorded in the National Herd Book for Italian beef cattle breeds (Marchigiana, Chianina, Romagnola, Maremmana and Podolica breeds), over six years. Monetary incentives were planned to encourage breeders to reach the expected annual seroprevalence limits. Participation in the plan was on voluntary basis. Serum samples were tested for presence of the antibody to glycoprotein E of BoHV-1 using commercially available enzyme-linked immunosorbent assays. If a single cow (aged more than 12 months), is found positive for IBR, then the whole herd is considered infected. IBR surveillance data for 2015 and 2016 were obtained from the National reference laboratory for IBR at the Istituto Zooprofilattico Sperimentale Umbria-Marche "Togo Rosati" and the ANABIC. The cattle herds registered in the the National Herd Book, were 5148 in 2015 and 5298 in 2016. The cattle farms joined to the Plan were 1424 in 2015 and 1127 in 2016. The farms tested with at least one seropositive animal were 820 in the first year and 718 in the second; a decrease in seropositive farms of the 12.5% were recorded. The farms enrolled in the plan in both years were 498. In addition, 322 farms were tested in the first year but not in the second year, while 220 farms were tested in 2016 but not in 2015. Although in both years more than 50% of farms turned out positive in 2015 the seroprevalence was 61% and in 2016 was 69%. About 97% of farms reached the goals set by the Plan. Seroprevalence was highest in Podolica cattle (2015: 55.14%, 95% CI 54.07–56.21; 2016: 54%, 95% CI 53-55), lowest in Maremmana cattle (2015: 9.95%, 95% CI 7.99–12.31; 2016: 11%, 95% CI 8-14), and intermediate in Chianina (2015: 22.01%, 95% CI 21.03–23.01; 2016: 19%, 95% CI 18-20), Marchigiana (2015: 24.85%, 95% CI 23.52–26.23; 2016: 27%, 95% CI 26-28), and Romagnola (2015: 15.60%, 95% CI 14.62–16.64; 2016: 14%, 95% CI 13-15) cattle. A greater involvement of breeders in the Plan for Eradication of BoHV-1 is required in order to ensure an effective control of the disease in the Country.

[1] OIE Terrestrial Manual, 2017 Chapter 2.4.12 Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis. [2] EFSA Journal 2017; 15(7): 4947. [3] Raaperi K., Orro T., Viltrop A. Epidemiology and control of bovine herpesvirus 1 infection in Europe. The Veterinary Journal 201 (2014) 249–256. [4] Commission Implementing Decision (UE) 2017/888 of 22 May 2017.

PRELIMINARY STUDY OF A POST-DIPPING PRODUCT CONTAINING BACTERIOCINS FROM *LAC. LACTIS* SUBSP. *CREMORIS*

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Mastitis is one of the most diffused and costly diseases of the dairy herd, causing huge economic losses worldwide. Among the prevention tools, the use of post-dipping products is fundamental to protect the teat from the entry of pathogens after the end of milking. Various formulations, containing different chemicals, are on the market, while a few products are lactic acid-based. Due to the restrictions imposed by European legislation to the use of biocides [1], the search for new antibacterial products is of great interest. Accordingly, a post-dipping formulation was developed, using a culture of *Lac. lactis* subsp. *cremoris* isolated from a goat cheese and characterized by a high activity against the most diffused mammary pathogens. Emollients and humectants have been supplied by Allegrini S.p.A. A preliminary field study was performed in a dairy farm located in Lombardy: it is a free stall with cubicles and has a one-side herringbone milking parlor, the herd consists of 22 lactating cows. Quarter milk samples were taken from the animals for bacteriological analysis and count of somatic cells, following Hogan et al [2]. Then, the cows were split in 2 groups: the experimental one, treated with the product under study, and the control, treated with a highly effective commercial iodophor. Four cows were not included, because infected by *S. aureus*. For the following 8 weeks, quarter milk samples of all animals were taken and analyzed and the hygiene score was recorded at legs and mammary level. The efficacy of the post-dipping was evaluated using GLM. The frequency of mastitogen bacteria did not show significant differences between the two groups, remaining at very low levels. Also, new infections by *S. aureus* were not diagnosed. Regarding SCC, statistically lower values were recorded in the treated group in 3 observations ($P<0.05$). The hygiene score showed similar values in both groups, indicating a good uniformity of the herd. In conclusion, even if the number of cows included was very small, the preliminary results of the field trial showed that the experimental formulation had an efficacy similar to that of a highly effective iodine-based commercial product. Such results would suggest to further investigate the possible application of chemical-free post-dipping products.

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PREVALENCE OF HAEMOSPORIDIAN PARASITES IN RURAL POULTRY FARMS OF TURIN PROVINCE

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Haemosporidian parasites causing avian malaria (*Plasmodium* spp., *Haemoproteus* spp. and *Leucocytozoon* spp.) are cosmopolitan in birds. Several species can cause severe disease in wild and domestic birds [1], contributing strongly to the economic loss in the poultry industry in Southeast Asia. Conversely, in Europe and Italy the prevalence in poultry is poorly studied. The aim of this study was to investigate the prevalence of haemosporidian parasites in rural poultry farms of the Turin province. Blood samples were collected from 147 chickens (*Gallus gallus domesticus*) divided as follows: 100 laying hens (96 animals > 1 year), 9 male chickens (> 1 year) and 38 broilers (undetermined sex; < 1 year). Farms (n=6) were set in mountain (n=3) and flat land (n=3). Analyses were performed on blood smears (n=142) and on the DNA extracted from blood clots (n=147) following a nested-PCR protocol [2]. DNA sequencing was performed on tested positive samples and cytochrome b gene haplotypes were identified by comparison with obtained sequences from GenBank and MalAvi. The association between the infection status and sex, age, poultry breed or altimetric position of the farms was evaluated using the Fisher's exact test. A $p<0.05$ was considered statistically significant.

Eleven out of 147 (7.5%) animals tested positive by nested-PCR for *Haemoproteus/Plasmodium* spp. and 4 (2.7%) for *Leucocytozoon* spp. DNA sequencing allowed identifying only the *Leucocytozoon* lineage L-CORNIX02. Seven out of 142 (4.9%) blood smears tested positive for *Haemoproteus/Plasmodium* spp. and 1 (0.7%) for *Leucocytozoon* spp. No association between the infection status detected by PCR or light microscopy and the considered parameters was revealed for both *Haemoproteus/Plasmodium* spp. and *Leucocytozoon* spp.

These results demonstrated the presence of haemoparasites in poultry of the Turin province, also in mountain farms, with a greater prevalence for *Haemoproteus/Plasmodium* spp. In literature, *Haemoproteus* spp. is the most frequently observed blood parasite in birds [3]. The absence of statistical association between infection and age was previously reported [2], but other authors observed a greater prevalence in juveniles [4] or in adults [5]. Usually, females are more parasitized because the reduced movement during the nesting period increases the probability of their infection [1]. The close proximity between males and females in farm maybe determines a similar likelihood of infection. The identified lineage L-CORNIX02 was previously reported in feral pigeons [2] and hooded crows [6] in the same area. Then, a cross infection of chickens with haemoparasites typical of migratory or nonmigratory bird species is possible and should be further investigated and monitored.

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ANTIMICROBIAL RESISTANCE TRENDS OF ESCHERICHIA COLI: 2012-2017 RETROSPECTIVE STUDY IN CENTRAL ITALY

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The WHO has classified *Escherichia coli* (EC) resistant to third-generation Cephalosporins, extended spectrum β-Lactamases, and Fluoroquinolones as one of seven bacteria of international concern [1]. Surveillance actions became important and necessary to monitor epidemiological trends in both human and veterinary medicine [2]. To improve the knowledge of multiple drug resistance spread of EC in Central Italy, a retrospective study (2012-2017) on animal and human EC strains was carried out. A total of 364 EC isolated from dogs/cats (50%), cattle/sheep/goats (15%), horses/pigs (14%), poultry/rabbits/reptiles (20%), and humans (1%), were isolated and characterized. Susceptibility testing to both veterinary and human antibiotics was evaluated by disk diffusion method [3] following the CLSI and EUCAST guidelines. Chi squared or Fisher exact tests, as appropriate, was used (Software STATA 13.0). To examine temporal changes in antimicrobial resistance, logistic regression was used to test for a linear trends throughout the six-year study period. $P<0.05$ was considered statistically significant. Overall, the EC strains were cultured from respiratory/ocular (24%), enteric (25%), genital/urinary (26%), skin/breast (10%), ear (4%), joint/bones (1%) sites and systemic infections (10%). The 91% of isolates were resistant to >1 antibiotic showing a higher number of resistance in animal (81%) vs human strains (40%, $P=0.025$), especially in dogs/cats and poultry ($P<0.05$). The most common resistance phenotypes to older classes, such as Tetracyclines (average 79%: range 40-78%), Penicillins (66%: range 32-78%), Sulphonamides (65%: range 24-97%), and Quinolones (48%: range 20-60%), were observed. Nevertheless, from 2012 to 2017, significant decreasing resistance trends for Aminoglycosides ($P<0.001$), Quinolones ($P<0.001$), Sulphonamides ($P<0.001$), Penicillins ($P=0.0004$), first and fourth generation Cephalosporins ($P=0.0001$; $P=0.0007$), were recorded. A not significant increase of resistance trend was observed for Carbapenems ($P=0.346$) in dogs/cats, Tetracyclines ($P=0.330$) and second-generation Cephalosporins ($P=0.088$) in cattle/sheep/goats, Tetracyclines ($P=0.523$) in poultry/lagomorphs, first and second generation Cephalosporins ($P=0.371$) and Sulphonamides ($P=0.408$) in horses/pigs. The study confirms the high average resistance rates for EC strains. The decreasing resistance trends observed during 2012-2017 for some classes of antibiotic is encouraging. The steady and incisive veterinary awareness has proved to be an important strategy for the proper use of antimicrobials. Our results could help the medical choice in case of empirical therapy considering the pathogen resistance profile in the geographic area concerned. When coupled with previous surveillance data, these results could provide a wider picture of evolution of resistance and lay the groundwork for understanding genetic mechanisms of resistance development.

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FIRST DETECTION OF REPLICATIVE DWV GENOME IN *V. CRABRO* AND *V. VELUTINA* SPECIMENS

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Deformed wing virus (DWV), a ssRNA(+) virus belonging to the *Picornaviridae* family within Iflavirus genus is a honeybee's pathogen distributed worldwide and it is transmitted mainly by the bite of the ectoparasitic mite *Varroa* destructor which is its main biological vector (1,2). Within a bee family/beehive, horizontal transmission between infected and non-infected bees could also occur. Recent studies have proven the presence of DWV on flower pollen, pollen load and in other bee products supporting additional horizontal transmission routes (3). The possibility of other transmission routes due to honeybee predatory insects such as those belonging to the *Vespa* genus should be considered. Among those, *Vespa crabro* (European hornet) which is widely distributed in Italy and in particular the alien *Vespa velutina* (Asian hornet), which has been introduced only recently (2014) can cause intense honeybees loss and concern among beekeepers. The aim of our investigation was to evaluate the presence of DWV in *V. crabro* and in *V. velutina* specimens collected in Italy during 2016 and 2017, to reveal a possible virus spill over from *A. mellifera* to the predatory hornets. Total RNA was extracted from specimens and the presence of DWV genome evaluated and quantified by real time RT-PCR. In addition, a strand specific RT-PCR was used to confirm the presence of replicative form of the genome. Sequence analysis performed on positive samples confirmed the RT-PCR results and indicated that the virus belongs to the worldwide diffused and less virulent genetic variant DWV type A. As *V. crabro* and *V. velutina* are neither pollinators nor susceptible to *Varroa* destructor we can exclude transmission by exposition to flower pollen and parasite bite. By finding the replicative form of DWV genome in newly emerged *V. velutina* samples we can assume that wasps could have been exposed to the virus by eating DWV infected honeybees during their imago larval state. These results indicate the establishment of a possible new ecological equilibrium between honeybee prey and alien *V. velutina* predator.

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COINFECTION BY DOLPHIN MORBILLIVIRUS, HERPESVIRUS, *Toxoplasma gondii*, MONOPHASIC VARIANT OF *Salmonella* Typhimurium 1,4,[5],12:i:-, IN A STRIPED DOLPHIN

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Cetaceans are protected by international agreements since the conservation status of several species is regarded as vulnerable or endangered (IUCN, 2016). Results of post-mortem investigations represent a source of valuable scientific information, useful for the conservation and the management of these *taxa.*

This study aims at describing a case of coinfection involving four agents identified in an adult female striped dolphin (*Stenella coeruleoalba)* stranded on the coast of Savona, in the Pelagos Sanctuary, in September 2017.

The animal, submitted for post-mortem investigations at the National Reference Center for Diagnostic activities on dead stranded cetaceans (C.Re.Di.Ma), was well-preserved (post mortem condition code 2) and in poor nutritional status. There was no evidence of interaction with fishing activities, and the stomach chambers contained undigested seagrass.

Organ and tissue samples were collected at necropsy and divided into two aliquots: one kept frozen for microbiological and biomolecular investigations for the main cetacean pathogens, and the other preserved in 10% buffered formalin for histopathological and IHC analyses [1]. A sample of frozen blubber was also submitted to the University of Siena for ecotoxicological analysis [2].

The animal was highly infected by endoparasites in blubber, muscle, peritoneum and lungs. The gross, histopathological and ancillary investigations allowed to identify a subacute systemic Dolphin morbillivirus (DMV) infection associated to *Toxoplasma gondii* and *α Herpesvirus* (HV) infections. In addition the animal showed a septicaemia due to monophasic variant of *Salmonella* Typhimurium 1,4,[5],12:i:-, ST 34 characterized by a multidrug resistant phenotype (ASSuT). Finally, the animal resulted immunocompromised because of hazardous levels of PCBs. Besides the cetacean-specific agents confirmed in this specimen (HV and DMV), *T. gondii* and *Salmonella* are of greater concern because consist of faecal biological contaminants with a zoonotic potential. While *T. gondii* is a common cause of stranding in striped dolphins [3], *Salmonella* 1,4,[5],12:i:- has not been previously described in marine mammals, but it is one of the most common serovar in human clinical cases and food products worldwide and it's characterized by the highest rate of Ab resistance in Europe [4]. So far, little is known about its transmission pathways and likely sources of exposure.

Our results confirm the role of cetaceans as sentinel species of the marine environment health *status* and suggest a high level of seawater contamination in the Pelagos Sanctuary.

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THE ONE HEALTH APPROACH FOR TICK-BORNE DISEASES SURVEILLANCE IN PIEDMONT REGION (2012-2017)

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Approximately 60% of pathogens with a potential to harm humankind have their origin in animals as well as 75% of emerging diseases. Among these, vector-borne diseases, spreading worldwide, are considered of big concern. The present study describes the surveillance system enforced in Piedmont region (Northwestern Italy), since 2012 on tick borne diseases, according to the One Health concept. An information network was activated between veterinary services and local human health authorities in order to obtain ticks collected from bitten humans for identification and pathogens screening (*Borrelia burgdorferi* sl, *Rickettsia* spp. and *Anaplasma* spp. [1,2,3]). Human health authorities were informed about results to carry out further diagnostic tests on patients and to start therapy if necessary. Furthermore, a communication campaign was carried out to raise citizen awareness about problems associated with tick bites and to provide practical prevention advices. A total of 1286 ticks from 1146 bitten humans was submitted and identified to species level. Most tick bites were collected between May and September (N=1111; 83,4%), with a peack reached in June. The tick most commonly retrieved belonged to the *Ixodes* genus (N=1231; 95.7%), mainly *Ixodes ricinus* (N=1004; 78.07%). Moreover, 21 ticks were identified as *Rhipicephalus sanguineus* s.l., 6 as *Dermacentor marginatus*, and 2 belonged to the *Haemaphysalis* genus (1 *Hae. concinna* and 1 *Hae. punctata*). Two hundred-twenty seven ticks (17.6%) were identified only at genus level, since they were damaged. According to the life stage, most frequently collected ticks were nymphs (53%) and females (41%), followed by larvae (2.5%) and males (1%). In 2012 and 2013 all the ticks (n=288) were tested for the detection of pathogens and 61 (21.18%) were positive to any pathogen, with an infection prevalence of 15% for *Rickettsia* spp., 5% for *Borrelia burgdorferi* s.l., and 1.7% for *Anaplasma* spp. Tree ticks were co-infected by more than one pathogen. Since 2014, according to a risk based evaluation, only ticks removed from children (<18 years), seniors (>70 years) or immunocompromised people were analyzed (n=343). The overall positivity of samples to any pathogen was 20.41% (n=70), with an infection prevalence of 14.3% for *Rickettsia* spp., 6.4% for *Borrelia burgdorferi* s.l., and 1.5% for *Anaplasma* spp. Seven ticks were co-infected by more than one pathogen. Tick bites are increasingly reported in Piedmont in the last years and an information campaign has been useful to raise awareness among citizens to adopt preventive measures. This diagnostic approach could provide useful information to physicians addressing rapid diagnoses and treatment decisions and could be considered a good example of sustainable mechanisms for early detection and rapid response to prevent public health.

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CANINE ADENOVIRUS TYPE 1 AND CANINE PARVOVIRUS CO-INFECTION: AN UNUSUAL MULTIPLE VIRAL INFECTION IN PUPPIES

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Canine adenovirus type 1 (CAdV-1) is a member of the genus Mastadenovirus (fam. *Adenoviridae*) and the causative agent of infectious canine hepatitis (ICH), a systemic disease characterized by fever, hemorrahagic diathesis, respiratory distress and, rarely, neurological signs [1]. Despite the vaccination contributed to the reduction of CAdV-1 circulation, occasional evidences of infections were reported. More rarely, co-infections with other viruses of dogs, such as canine parvovirus type 2 (CPV-2), were reported [2]. The present study reports a puppies mortality caused by CAdV-1 and CPV-2 co-infection. Six puppies were admitted from stray at childbirth, without receiving maternal colostrum. Few days after the first vaccine administration puppies died and were submitted to the Istituto Zooprofilattico Sperimentale (IZS) della Sicilia "A.Mirri" for diagnostic purposes. At necropsy, serosanguineous fluids, ecchymoses and petechiae were observed. Liver appeared enlarged and congested. Pulmonary oedema and catarrhal exudates were also evidenced. Fluid and tissue samples were collected for histopathological and virological assays. Histology demonstrated features of serous hepatitis, glomerulonephritis, lymphocytic infiltrates in lungs, brain and intestine, lymphocytic depletion in spleen were evidenced. Using different molecular assays (PCR, RFLP-PCR), samples tested positive for CAdV-1 and CPV. Sequence analyses of E3, Hexon and Fiber genes of CAdV-1 and of VP2 gene of CPV were assessed, evidencing a high identity rates with circulating CAdV-1 and CPV-2c strains but with specific molecular divergences. Observed CPV variant (CPV-2c) was different to the administered CPV vaccine strain. Viral isolation of both viruses was carried out on MDCK and A72 cell monolayers. Samples tested negative for other viral pathogens (CAdV-2, CDV, CCoV, CHV and CRoV). To date, most of the studies on CAdV-1 have been carried out by a biomolecular approach, with limited information on related pathological evidences, particularly in cases associated with a multiple viral infection. Despite ICH mortality rate is low, co-infections with other canine viruses can exacerbate the disease and increase the mortality rates [1]. Findings described in the present study, compatible with a CAdV-1/CPV co-infection, contributed to add more information on these rarely reported events. Viral isolation evidenced the active viral replication in tissues, related to the pathological findings. Moreover, sequence analysis excluded any relation with the CPV modified live virus contained in the vaccine, rather than explaining the infection due to a field CPV-2c strain related to those circulating in the same region [3]. This study contributes to elucidate evidences in a rarely reported case of multiple viral infection. Therefore, it underlines the need to monitor the spread of the less reported but still circulating CAdV-1.

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MOLECULAR EPIDEMIOLOGICAL SURVEY OF CANINE PARVOVIRUS (CPV) TYPE 2 IN SICILY

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Canine parvovirus (CPV) type 2 is the etiological agent of one of the most severe and often fatal diseases in domestic and wild carnivores. Clinical signs are characterized by hemorrhagic gastroenteritis and leukopenia. CPV is a member of the Protoparvovirus genus (fam. *Parvoviridae*, subfam. *Parvovirinae*), now included with feline panleukopenia virus (FPLV) in the unique viral specie Carnivore protoparvovirus 1. First evidence was in late 1970s and during years the CPV original type (CPV-2) was totally replaced by three genetic and antigenic variants named CPV-2a, CPV-2b and CPV-2c [1]. Sequence analysis provides to type and to compare the molecular features of circulating CPV strains, adding further information about its evolution. Moreover, the complete genome sequencing could fill the gap of information concerning the less studied CPV non-structural genes. Aim of this work was the genetic typing and characterization of the most recent circulating CPV strains in Sicily. For this purpose, samples were collected from dogs suspected of parvovirosis and analyzed at Istituto Zooprofilattico Sperimentale della Sicilia “A. Mirri” (Palermo, Italy) in 2016/2017. Sequence analysis was conducted on 55 CPV strains, from different geographical origins. A long genome sequence encompassing both ORFs encoding for nonstructural (NS1-NS2) and structural (VP1-VP2) proteins was amplified and analyzed. Sequences were also compared with those obtained from a previous CPV molecular survey in the same region [2] and with related sequences in GenBank. The current study showed that all three CPV types were observed in Sicily, with different distributions among provinces. The most prevalent type remains the type 2c, despite an increased prevalence of type 2a was observed in late 2016. Type 2b was only an occasional finding. Specific and unreported amino acid changes were evidenced in both ORFs (NS1: I60V, N239T, D350N, L397F, Y544F, E545V, P590S, L597P, L630P - VP2: A5G, P13S, V139I, F267Y, Y324I/L, Q370R, A371G, I418T). Since November 2016, a novel CPV-2a mutant [3] has been observed among strains from Trapani and Palermo provinces. Moreover, in February 2017 a CPV-2c strain collected from a dog imported from Asia was evidenced [4]. CPV-2c strains with the VP2 amino acid change V139I, previously collected also from a cat [5], are still circulating. The molecular characterization of CPV strains in this study still evidences the need of a continuous molecular survey, in order to elucidate CPV genetic, providing new useful data for further evolutionary analyses. Determination of complete genome sequences allow to better trace the origin and spread of CPV variants among different regions or countries. Moreover, our data improved the limited information on NS1 sequence of circulating CPV strains.

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SERUM CONCENTRATIONS OF TRACE ELEMENTS IN LEISHMANIOTIC DOGS COMPARED TO HEALTHY CONTROLS

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Canine leishmaniasis, a severe and systemic chronic inflammatory disease, is caused by *Leishmania infantum* and is transmitted by the bite of phlebotomine sand flies. Infected dogs are the main reservoir of the parasites and play a relevant role in the transmission to humans, in which the parasite causes visceral leishmaniasis [1]. Some studies checked a potential link among histopathology and some trace elements in canine leishmaniasis [2]. Trace elements include essential and toxic metals, such as heavy metals; due to the environmental pollution, that has caused the contamination of both soil and irrigation water, they can enter the food chain in a wide range of concentrations [3,4,5]. The risk associated with an excessive exposure to heavy metals (lead, chromium, cadmium) has been shown to cause various diseases [4]. Particularly, for some metals, developmental neurotoxicity, cardiovascular effects and nephrotoxicity in adults have been demonstrated. Certain metals have been reported to seriously affect the immune system resulting in a broad range of harmful health effects. Other metals (cobalt, manganese, zinc, copper) are defined essential, while for other elements useful biological activities have not yet been recognized; in general, for all trace elements, an excessive intake may cause toxic effects. Some studies were conducted on the heavy metal content in serum of dogs to evaluate the degree of exposure in urban or industrial areas [6,7]. In the present study we aimed to determine trace element concentrations in serum of dogs to investigate if impaired levels of some metals are a factor contributing to leishmaniasis vulnerability. Elemental composition was determined by using inductively coupled plasma mass spectrometer in serum of dogs with cutaneous leishmaniasis (n=19) and statistically compared to control group (n=74). The serum samples were from dogs living in different geographic areas of Campania Region, endemic for *Leishmania infantum*. After dilution of aliquots of serum 1:10 (v/v) with HNO3 1%, the analysis of seventeen trace elements (As, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Se, Sr, Tl, V, Zn) was performed using an ICP-MS NexION 350X (Perkin Elmer, USA). Concentrations were calculated by using calibration curves and were expressed as mg/L. Results showed that there were significant differences in the values of Fe, Sr and Mn between two groups ($p<0.05$). Instead no significant differences in level of other trace elements were observed between leishmaniotic dogs and control group ($p>0.05$). Fe, Sr and Mn levels were found out to be (2.76±1.77), (0.008±0.004) and (0.064±0.013) µg/mL respectively in leishmaniasis case and statistically different compared to the controls (4.26±3,02), (0.006±0.002) and (0.057±0.023) µg/mL, respectively. The study provides data on trace elements levels in serum of dogs living in Campania and could be useful to assess possible correlations with leishmaniasis. However, preliminary data from this study require further investigation.

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INVASIVE MOSQUITOES SURVEILLANCE IN THE LIGURIA REGION, NORTH-WESTERN ITALY (2011-2017)

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Invasive mosquito species (IMS) of the genus *Aedes* represent a major threat for human health, given their ability to spread into new territories and transmit important human viral diseases, like dengue, yellow fever, zika and chikungunya. In Italy, the Asian tiger mosquito *Ae. albopictus* was first recorded in Genova in 1990 [1]; since then, the species has spread across the country, being responsible for two chikungunya epidemics, in 2007 and 2017 [2,3]. The present study reports the results of the entomological surveillance on invasive mosquito vectors performed in the Liguria Region by IZSPLV in 2011-2017. Mosquitoes were collected fortnightly from May-June to October-November of each year, by means of CO_2- and lure-baited traps, and hay infusion-baited Gravid traps, in sites at risk for the introduction of alien mosquitoes or for Flavivirus circulation. The no. of trapping sites ranged from 6 to 23, depending on the year, with a particular focus on the city of Genova in 2016-17 (10 traps out of 23). Adult mosquitoes were identified to the species level following morphological keys; female specimens were pooled and screened for flaviviruses by means of a Real-Time RT-PCR distinctive for WNV and USUV, as well as an End-point RT-PCR assay targeting the NS5 region of the Flavivirus genome [4]. Amplicons obtained were subjected to sequencing and sent to the National Reference Centre (CESME) for confirmation. A total amount of 33,244 adult mosquitoes representing 17 species, including 11,947 *Ae. albopictus* and 46 *Ae. koreicus*, were collected. The latter was first detected in Genova in September 2015, representing the first report of the species in NW Italy. While a *Culex pipiens* pool was found positive to WNV Lineage 2 in 2014 and three more pools were USUV-positive (one each year in 2014, 2015 and 2017) [5], no pathogenic flaviviruses were detected in the *Aedes* pools analysed. Three *Ae. albopictus* pools were found positive to Insect-specific flaviviruses, having no zoonotic potential, in 2013 (n=2) [6] and 2016 (n=1). Despite no human patogenic flaviviruses were detected in *Aedes* mosquitoes, the presence of potential mosquito vectors across the whole Region is cause for strong public health concern. This work highlights the importance of monitoring mosquito vector populations, in order to early detect the arrival of alien mosquito species and the circulation of mosquito-borne flaviviruses.

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ANALYSIS OF BLUETONGUE SEROTYPE 3 SPREAD IN TUNISIA AND DISCOVERY OF A NOVEL STRAIN RELATED TO THE BLUETONGUE VIRUS ISOLATED FROM A COMMERCIAL SHEEP POX VACCINE

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Bluetongue (BT), is one of the OIE-listed major diseases of ruminants. Following the official report of BT virus serotype 3 (BTV-3) in a sheep in Cap Bon (Tunisia) [1], blood and serum samples of ruminants were collected from some areas of Tunisia to further investigate the presence of this virus in the country. A quantitative real time RT-PCR has been first developed for the detection and quantitation of BTV-3 RNA from field specimens. Out of 62 collected blood samples, 23 were shown to be positive for BTV-3 RNA. Isolation on cell cultures was also possible from six samples. Genome sequencing revealed the circulation of two unrelated western strains of BTV-3, one circulating in Cap Bon and neighboring areas, and the other circulating nearby the border with Libya. The presence of a putative novel BTV serotype (BTV-Y TUN2017) in sheep introduced from Libya to Tunisia, genomically related to the BTV strain contaminating a commercially-available sheep pox vaccine [2] and to BTV-26, has been also demonstrated. This finding highlights the pressing need for a prompt production and release of a novel inactivated BTV-3 vaccine to be used in case of emergence or proactively in the areas of Southern Europe at major risk of BTV introduction. The assessment of a novel vaccine will certainly exalt the role and importance of surveillance activities and collaboration with Northern African countries.

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EVALUATION OF ANTIMICROBIAL RESISTANCE IN FATTENING PIG HERDS IN PIEDMONT

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Antimicrobial resistance (AMR) is a growing public health concern, causing annually almost 25 thousand deaths in EU. The development and spread of antimicrobial resistance has often been related to the use of antimicrobials in food-producing animals. In swine production, the association of antimicrobials use and antimicrobial resistance has been well reported [1]. The aim of this study was to investigate levels of antimicrobial resistance in fattening pig farms in Piedmont Region. Faecal samples of sows, piglets, weaning and fattening pigs along with water and sewage samples were collected in six fattening pig herds during the period January-December 2017. Bacterial strains were isolated in faeces samples and identified by phenotypical analysis. The antimicrobial susceptibility testing was carried out using the Kirby-Bauer disc diffusion method. 67 wastewater samples and 16 drinking water samples were analysed by liquid chromatography, coupled with DAD and FLD detectors in order to detect antimicrobials. *E. coli* strains with phenotypic β-lactams antimicrobial resistance were analysed for the extended spectrum β-lactamase production (ESBL and AmpC) and of Beta Lactamase Metal (MBL). Enterococci resistant Vancomycin strains were analysed by mass spectrometry for biochemical identification and the VRE phenotype were determined. Out of the 641 bacterial strains, 40 ESBL E. coli and 37 AmpC *E. coli* were detected in faecal samples of sows (12% in either the cases), piglets (18% and 15% respectively) and weaning pigs (11% and 17% respectively), whereas only AmpC resistance was detected in fattening pigs (10%). MRSA strains (n=19) were isolated in 4 farms, in weaning and fattening pigs. VRE strains were not detected. Considering *E. coli*, the most frequent phenotypic resistance was related to lyncomicin, tylosin, and erytromicin (100% of the isolates), followed by tiamphenicol (90%). Tetracycline resistance was detected in over 52%, whereas sulfisoxazole, trimethoprim -sulfamethoxasole, tiamulin and florfenicol varied considerably, between 13% and 100%. Gentamicin, colistin, ceftiofur and enrofloxacin were below 50%. *Staphylococcus aureus* strains were detected in weaning and fattening pigs and were resistant to amoxicillin and ceftiofur (100% in 5 farms, 50% in one farm) and to lincomycin, tetracycline, colistin and cefoxitin in all 6 farms (100% of the isolates). Resistances to tiamulin and enrofloxacin were detected only in one farm. Trimethoprim-sulfamethoxazole and sulfissoxazole resistances varied from 25 to 100% depending on the farm considered. Regarding *Enterococcus* spp. strains, the most relevant phenotypic resistance was related to ceftioufur, lincomycin, sulfisoxazole, erythromycin, colistin, whereas in farm 2 and farm 4 resistance to tiamfenicol and florfenicol (100%) were detected. Drinking water and wastewater samples tested negative for tetracyclines, sulfonamides and quinolones. These findings showed a selective pressure exerted by antimicrobials administered to animals, providing ideal conditions for the emergence and selection of resistant bacterial strains.

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ANTIMICROBIAL CONSUMPTION ANALYSIS IN FOOD-PRODUCING ANIMALS IN THE VAST AREA 3 OF THE MARCHE REGION: THE 2015-2017 TREND

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In November 2011 the European Commission has launched a 5-years action plan against the rising threats from Antimicrobial Resistance [1]. Among the 12 actions proposed from the Commission, there is also that of strengthen surveillance systems of AMR and antimicrobial consumption in animal medicine. The aim of this study is to evaluate the livestock antimicrobial consumption in the 2015-2017 in the Vast Area 3 of the Marche region, in order to define what is the farm with the greater use of antibiotic and the trend of antibiotic consumption during these 3 years. We digitized all the prescriptions for food-producing animals and evaluated indicators for consumption, with a Defined Daily Doses-based approach [2]. We calculated the antimicrobial consumption both per animals (DDD 1000 animals-die) and per farms (DDD 1000 farms-die), starting from the prescribed-DDD. During the 3 years it has occurred an increase of +43.20% in the use of overall antimicrobials, linked most of all to their consumption in pig farms, which have reached almost 30 days of treatment per head in 2017; whereas the use of critical importance antimicrobials is decreased of -28.58%. This drop is mainly due to the recent decision of European Commission [3], concerning the suspension of the marketing authorizations for all veterinary medicinal products containing "colistin" in combination with other antimicrobial substances to be administered orally. Pleuromutilins and zinc oxide have been used as alternatives to polymyxins in the treatment of piglet enteric diseases. The antimicrobial consumption in the other livestock sectors, unlike the pig one, is decreased during the 3 years: -14.41% for ovine and goats, -34.81% for broilers, -29.44% for aquaculture and -23.20% for rabbits. The penicillins were the antibiotic class more used, followed by the tetracyclines and pleuromutilins. The persistent control of antimicrobial consumption in livestock, together with the analysis of antimicrobial resistance factors of germs, will allow to obtaining accurate evaluation of this event with the aim to understand the benefits deriving from the elaboration of action plans in order to reduce the recourse to antibiotic.

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BLACK RATS FROM THE PONZIANE ISLANDS: HEALTH ASPECTS OF AN ERADICATION PROGRAM

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Biological invasions have become a growing issue especially with regard to island habitat, which are especially prone to the detrimental impact of alien invasive species and consequent loss of biodiversity [1]. Rats are among the most loathed alien species worldwide and are hosts of a plethora of pathogens, many of which are zoonotic. In the Ponziane Islands (Italy), the Life Project PonDerat (LIFE NAT/IT/000544) aims at eradicating the black rat *Rattus rattus* from Palmarola and Ventotene, while biosecurity measures have been carried out in Ponza. Among the pathogens present in the Mediterranean basin, vector-borne protozoa and bacteria together with *Toxoplasma gondii* and *Neospora caninum* are especially relevant because of their burden on human and/or animal health. In this context we invesigated presence and prevalence of selected pathogens in the insular populations of black rats.

R. rattus were trapped on the islands of Ponza, Ventotene and Palmarola. Specific PCR protocols were carried out to detect *Leishmania infantum*, *Babesia/Theileria* spp., *Anaplasma/Ehrlichia* spp., *Borrelia burgdorferi* s.l., *T. gondii* and *N. caninum* [2,3,4,5].

T. gondii was detected in 42.11% of the sampled rats (IC95% 27.85%-57.81%), being the rats trapped in Ponza and Palmarola more infected than those from Ventotene. *Babesia/Theileria* spp. were detected with a prevalence of 36.84% (CI 95% 23.38%-52.72%). Significantly higher prevalence was recorded in the rats from Palmarola and Ventotene compared to those from Ponza. Lower prevalences were instead recorded for *L. infantum* which was detected in 2 animals form Ventotene (P=5.26%; CI95% 1.46%-17.29%) and for *B. burgdorferi* s.l. which was detected in one animal. None of the animals tested positive neither for *N. caninum* nor for *Ehrlichia/Anaplasma* spp.

The main goal of eradication programs in insular environments is restoring islands biodiversity. This work showed how alien species, like black rats, play a key role not only for the ecology of insular native species but also from a public health/veterinary point of view. Sanitary implications should be considered as an added value in eradication programs planning and management.

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PRELIMINARY DATA ON ANTIBIOTIC SUSCEPTIBILITY IN STRAINS ISOLATED FROM WILD ANIMALS IN SICILY

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The last few decades have seen the inexorable proliferation of antibiotic-resistant bacteria (AMR) often with multiple resistances in some geographical areas, which have caused the failure of antibiotic therapies both in human and veterinary medicine. As a result, considerable efforts were made to expand the knowledge on the dynamics of AMR in humans and domestic animals. In fact, bacteria may have intrinsic resistance or may acquire them following direct exposure to antibiotics or by exploiting multiple biochemical mechanisms or and extraordinary genetic flexibility. Several studies have shown that resistance genes are everywhere in nature (pathogens, commensals and environmental microorganisms). For this reason, microorganisms from wild environments, where animals represent reservoirs for the spread of antibiotic resistance genes should be monitored [1]. Considering the possibility of transmission of resistance genes between microorganisms, the aim of this study is to evaluate the antibiotic susceptibility of bacteria isolated from wild animals like birds and mammals in Sicily. Organ samples and swabs, collected from these animals, were tested for various zoonotic bacterial agents in the laboratories of Istituto Zooprofilattico Sperimentale della Sicilia. From January to April 2018, 24 bacterial strains were isolated. The isolated species belong to the *Enterobacteriaceae* family (*E. coli, Klebsiella, Citrobacter*) and *Aeromonadaceae* (*Aeromonas hydrophila*). Antibiotic susceptibility of the isolated strains was tested by the agar disc diffusion methods and the diameter of the inhibition zones was compared with CLSI standards [2]. The susceptibility of the isolated strains was tested for 8 different antibiotics: ampicillin (10 µg), ceftiofur (30 µg), chloramphenicol (30 µg), enrofloxacin (5 µg), gentamicin (10 µg), sulfisoxazole (300 µg), sulfamethoxazole / trimethoprim (1.25 µg + 23.75 µg), tetracycline (30 µg). The antibiograms showed the presence of multiple resistances in four strains of *E. coli* isolated from wild birds. Three of these strains were resistant to ampicillin, sulfisoxazole, sulfamethoxazole/trimethoprim and tetracycline, while one showed resistance to all the antibiotics tested excepted to ceftiofur. *E. coli* is one of the microorganisms that can acquire and transfer resistance genes. It is present as commensal of mammal and bird intestinal tract, therefor it can be considered as an indicator of the evolution of antibiotic resistance in wild animals [3]. The isolation of these four multi-resistant strains in wild animal samples analyzed in this study could be related to the transmission of resistance genes. For this purpose, further studies will be conducted both to deepen the knowledge on antibiotic resistance in microorganisms isolated in wild animals and to study their resistance mechanisms.

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FELINE PYODERMA: CHARACTERIZATION AND ANTIMICROBIAL SUSCEPTIBILITY OF SKIN *STAPHYLOCOCCUS* SPP. POPULATION

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Feline pyoderma is a clinically entity that receives little attention as compared with canine pyoderma but may be more prevalent than previously thought. There are limited reports in the literature on this topic with more examining the flora of normal feline skin and oral cavity and only a few evaluating bacteria isolated from clinical lesions [1]. The aims of this study were to identify *Staphylococcus* spp. population associated with feline pyoderma and to assess its antimicrobial drug susceptibility. The animals were examined at the Vet. Hospital of Alfort (France) and evaluated by veterinary dermatologist. Samples were obtained from 41 domestic cats diagnosed with pyoderma. Skin lesions of all cats revealed bacteria in presence of concurrent inflammation, satisfying the definition of pyoderma currently accepted for dogs. All cats were client owned, and aged from 6 months to 10 years old (mean=4 and median value=3). None animal received antimicrobial drugs 3 weeks prior to inclusion in this study. Samples were obtained by swabs from 1-2 skin lesions/cat. Additional samples were collected from 3 sites without evidence of skin infection. All samples were analyzed to microbiological laboratory of Department of Veterinary Sciences of Turin (Italy) for *Staphylococcus* spp. identification. Bacteria were identified with PCR and sequencing [2]. *S. aureus* strains were characterized in order to assess the spa-type (software Ridom Staph Type), and the AST was performed on all isolates using the disk diffusion method (EUCAST) [3]. In total, *Staphylococcus* spp. was identified in 30 out of 41 animals with an occurrence of 37 isolates. The most frequently isolated organism was *S. aureus* (n=13), followed by *S. pseudointermedius* (n=11) and *S. felis* (n=8). Ten different spa-types were identified in *S. aureus*, associated with human clonal complexes. Fourteen staphylococcal isolates showed resistance at least 3 antibiotics and the majority of strains were resistant to beta-lactam drugs used in veterinary practice, included markers for methicillin-resistance (35% of strains). *S. aureus* remains the agent most frequently isolated from feline pyoderma as previously reported [4]. However, in this study *S. pseudointermedius* and *S. felis* were frequently isolated from feline skin infections. The real prevalence of *S. pseudointermedius* and *S. felis* in feline pyoderma may have been underestimated. This could be attributed to the fact that the traditional methods used in clinical microbiology laboratories would be likely to misidentify these isolates. Most data on antimicrobial susceptibility are related to canine infections and show an increase in the drug resistance of staphylococci strains. Our data suggest that feline staphylococci may have an antimicrobial resistance similar to that observed in dogs.

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IDENTIFICATION AND GENETIC CHARACTERIZATION OF BOVINE ENTEROVIRUS BY COMBINATION OF TWO NEXT GENERATION SEQUENCING PLATFORMS

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Prompt and accurate diagnosis is warranted for infectious diseases of domestic animals which may have a significant impact on animal production or clinical practice. In this study, we describe the identification and genetic characterization of a bovine enterovirus (BEV) strain isolated in cell-culture from a calf with diarrhea. This calf tested negative for the most common viral and bacterial pathogens affecting the enteric tract of ruminants. BEV was identified by MinION and confirmed by a specific real time RT-PCR test. To achieve the whole genome of the occurring strain BEV Italy/17DIAPD2208/15/2017, data reads obtained by MinION were coupled with those originating from NextSeq500 (Illumina). Genomic relatedness and phylogeny with extant BEV strains is also performed. Overall, this study highlights the use of the portable MinION sequence technology as a tool for support diagnostics in veterinary practice.

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EVALUATION OF *Coxiella burnetii* PRESENCE IN WESTERN SICILIAN DAIRY CATTLE HERDS COMPARING PCR AND ELISA RESULTS

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Coxiella burnetii is an intracellular zoonotic bacterium able to cause Q fever in humans as well as several animal species: sheep, goats and cattle are the primary animal reservoirs [1]. Q fever in humans is considered an endemic occupational disease in Mediterranean countries. In cattle, abortions are less frequent but reproductive disorders and mastitis can occur. Infected animals excrete the bacteria into the environment mainly during and after parturition or abortion, through vaginal mucus and birth products. The spread of *C. burnetii* from contaminated farms to the environment may occur with soil, animal skin, non-pasteurized milk and wastewater [2]. The most common useful techniques in animal Q fever serological diagnosis are the IFA, the ELISA and the CFT. Q fever diagnosis can also be based on PCR [3].

The aim of this study has been to estimate the presence of *C. burnetii* DNA in mass milk of some herds present in Agrigento, Palermo and Trapani provinces. Moreover, ELISA test and Real Time PCR have been carried out in individual bulk milk and sera samples coming from Sicilian farms in which *C. burnetii* DNA was found.

The study has been run in two times. In the first part, a total of 14 western Sicilian dairy herds has been selected randomly among all western Sicilian dairy farms with less than 200 heads. Mass milk samples coming from 4 farms in Agrigento, 9 in Palermo and one in Trapani have been analysed by Real Time PCR by VetMax *C. burnetii* Absolute Quant Kit (ThermoFischer Scientific) to evaluate the presence of *Coxiella burnetii* DNA. In the second part of the study, 135 individual milk and sera samples were collected in all herds in which the *C. burnetii* DNA was found. All sera have been analysed for the detection of anti-*Coxiella* antibodies by a commercial ELISA (ID SCREEN® Q Fever Indirect Multi-species), while milk samples were processed for DNA extraction and Real Time PCR analysis. The Real Time PCR on mass milk has revealed the presence of *C. burnetii* DNA in one farm of Agrigento, 3 of Palermo and in one herd of Trapani. *C. burnetii* seroprevalence of 9.6% has been found at animal level: 6.6% in Agrigento, 10.3% in Palermo and 7.7% in Trapani. Concerning *C. burnetii* DNA in cattle milk, at the animal level, a presence in 7.4% of the samples was observed. In particular, *C. burnetii* DNA was found in 6.6% of the milk samples collected in Agrigento, 5.6% in Palermo and 23% in Trapani. Eighty percent of the samples showed DNA of *C. burnetii* but no antibodies in the serological survey. Data obtained from the biomolecular investigations have shown an actual elimination of *C. burnetii* DNA in milk also from seronegative heads. These data could represent a first piece of information useful to take preventive measures in an attempt to reduce the disease prevalence in herds, as dairy cattle may transmit *C. burnetii* to other species, including humans. It would be significant to continue monitoring farms in the territory and complete the surveys with cultivation tests in order to assess the pathogenicity of *C. burnetii* agent.

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AIVI

RARE EARTH ELEMENTS IN ANIMAL FEED

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Also called lanthanides, the rare earth elements (REE) are members of Group IIIA in the Periodic Table and share similar physical and chemical properties. REE are also distinguished according to their atomic weight (Z), into light REE (LREE) - from lanthanum to samarium - and heavy REE (HREE) - from europium to lutetium. Scandium and yttrium are often considered HREE because of their similar chemical properties.

In the last 20 years, the REE have been widely utilized, especially in China (which owns the largest mineral deposits) in agriculture - as fertilizers - and in animal husbandry as growth promoters. China still uses REE mixtures as crop fertilizers, and their successful effect in improving crop yields has been documented [1]. Many reports from China, reviewed by He and Rambeck [2], have focused on the application of REE as feed additives for farm animals, such as pigs, cattle and chickens, claiming that small amounts of REE in feed can cause an increase of body weight and a substantial improvement in the production of milk and eggs. REE are actually emerging as contaminants worldwide, following their applications in industry, technology, medicine and agriculture. Moreover, in 2016, the first REE-based feed additive - a zootechnical additive for weaned piglets - was authorized in the EU [3]. Therefore, information about the natural content of REE in animal feed of vegetal origin is required to evaluate the potential use of REE as a growth promoter.

The concentrations of rare earth elements (REE) were determined by ICP-MS in feed for farm animals in three regions of Northwestern Italy. This is the first study aimed at defining the levels and patterns of REE in feed for different animal species.

There was a high variability in the REE content of the animal feed in the three different Italian regions, and REE content varied according to the different animal species for which the feed was intended. Raw materials were shown to have higher ΣREE concentrations (mean concentration 2.4 mg kg^{-1}) than complete or complementary feed. Considering the animal species, mean ΣREE concentrations were as follows: horse feed (2.7 mg kg^{-1}) > poultry feed > bovine feed > swine feed (0.61 mg kg^{-1}). The REE levels we found were in line with the few bibliographic studies relative to REE concentrations in vegetables from Italy; moreover, the lowest REE levels was found in swine feed, the only species for which the use of an REE-based feed additive has been authorized in the EU.

Considering the REE concentrations we found in animal feed, which reflect the levels of REE in the environment, we can suggest that a potential supplementation of REE in feed should take into account the basal levels of these elements in the areas where the raw materials are cultivated.

[1] USEPA, Rare Earth Elements: A Review of Production, Processing, Recycling, and Associated Environmental Issues. United States Environmental Protection Agency, Cincinnati, OH, EPA/600/R-12/572,2012. [2] He, M.L., Rambeck,W.A., Rare earth elements-a new generation of growth promoters for pigs? Arch. Anim. Nutr. 53:323–334, 2000. [3] EFSA, 2016. Safety of Lancer (lanthanide citrate) as a zootechnical additive for weaned piglets. EFSA Journal 14, 4477, 2016.

TRACE ELEMENTS BIOACCUMULATION IN WILD BOAR FROM LIGURIAN REGION

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Industrial activities have resulted in a constant increase in metals environmental levels, potentially affecting wildlife. Natural populations could be utilized as sentinels for environmental contamination, and game species are often utilized in biomonitoring studies cause their tissues are suitable bioindicators for metal pollution [1]. Wild boar (*Sus scrofa*) are omnivorous animals, abundant in agricultural and forest areas of all Europe Regions, then are a reliable bioindicators of the environmental contamination. Essential (chromium, copper, cobalt, indium, iron, magnesium, manganese, molybdenum, nickel, selenium, and zinc) and nonessential trace elements (aluminum, arsenic, beryllium, bismuth, cadmium, gadolinium, lead, rubidium, silver, thallium, tin, uranium and vanadium) were determined in liver and kidney of 4 wild boar (*Sus scrofa*), 3 females and 1 male found dead in Ligurian Region.

Wild boar's liver and kidney were homogenized and subjected to microwave digestion (ETHOS 1, Milestone) with 7 mL of HNO_3 (70% v/v) and 1.5 mL of H_2O_2 (30% v/v). Multi-elemental determination was performed using ICP-MS (ICP-MS Xseries II from Thermo Scientific).

Trace elements (mg kg^{-1}) were found at higher levels in females, with the exception of Mg in liver and Mo in kidney that have shown comparable levels in the two gender; Zn that was higher in males liver. The highest mean values of Fe (1076), Cu (20), Mn (5.5), Rb (2.6), Mo (2.5) and Pb (1.1) were found in females liver, while the highest values of Mg (234), Al (57), Cd (17), Se (2.2), Ni (0.20), Cr (0.17), V (0.089) and As (0.059) in females kidney. Only for Zn the highest concentration was found in male liver.

In our knowledge data about metals levels in wild boar tissues are scarce worldwide; in the analyzed females we found considerable high values of Cd and Pb in comparison to those observed in Northern Italy by Chiari and coauthors [2] and by Amici and coauthors [3] in wild boar from Central Italy. Accordingly, in females we recorded notable values of Fe in liver and of Al and in kidney, suggesting a different pathway of heavy metals bio accumulation than in male. In fact, gender specific differences in the bio accumulation patterns of metals have already been reported in several mammal species and may be due to different feeding habits or different metal toxicokinetics. It should be further investigated whether other factors such as age, diet and season influence heavy metal accumulation in wild boar. Moreover, we suggest that wild boar tissues should be extensively monitored due to the potential role in increasing heavy metals exposure in population groups that consume game species.

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HYGIENIC PROFILE OF HALAL MEAT RETAILED IN TUSCANY

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In recent years, the halal meat market in Italy is growing at a remarkable rate, due to the rise in the number of immigrants of Islamic faith (1). In Tuscany, the ritual slaughtering is possible in 5 authorized slaughterhouses for ungulates and 2 for poultry meat. Despite the increase in halal products variety in ethnic restaurants and fast food, in this region fresh meat is still mostly purchased from independent halal butchers. Significantly, while butcher's shops have been disappearing from the high street retail for some time, the number of halal butcher's shops has continued to grow. The aim of this research is to evaluate the hygienic profile of fresh meat and preparations of beef meat from animals slaughtered according to Islamic ritual and marketed in halal butchers in Tuscany. In order to verify the evolution of post-mortem changes after ritual slaughtering, pH of longissimus dorsi muscle of 84 bovine carcasses at 24 hours from ritual slaughtering was measured at a slaughterhouse in Pistoia. At retail level, a total of 22 butcher's shops from 5 provinces of Tuscany were selected: 8 in Florence, 4 in Prato, 3 in Livorno, 6 in Pisa (3 in the city of Pisa and 3 in the province of Pisa, 2 in Pontedera and 1 in Montopoli Val D 'Arno) and 1 in Pistoia. A total of 96 beef samples were purchased from the 22 selected bazaars and they were chosen basing on availability and diversity of types of fresh and prepared meat (fillet, stew, minced meat and sausage). All meat samples were tested for the main pathogenic microorganisms (*Salmonella* spp., *Yersinia enterocolitica* and *Listeria monocytogenes*) and hygiene indicators (*Enterobacteriaceae*, *Escherichia coli*, *Enterococcus* spp. and positive coagulase staphylococci). Values of pH of bovine carcasses after 24 hours from ritual slaughtering were below 5.8 only in 11.1% of carcasses. *Salmonella* spp. was absent in all specimens, while 10 samples were positive for *Listeria monocytogenes* and 7 for *Yersinia enterocolitica*. High *Enterobacteriaceae* loads (>10^5 cfu/g) were detected in 40.6% of specimens, positive coagulase staphylococci in 24%, enterococci in 3.1% and *E. coli* in 1%. In many butcheries examined, minced meat was the most contaminated prepared meat. The results of pH measurements show that halal meat can be easily contaminated (2). These foods are consumed after cooking, but the presence of pathogens and, in some cases, the high bacterial counts for hygiene indicators and, in particular, for positive coagulase staphylococci, producers of heat-resistant enterotoxins, suggest the need for constant and accurate monitoring in these commercial realities, addressed not only to the products, but also to the hygienic practices adopted.

(1) Priore L. Il consumo di carne *halal* nei paesi europei: caratteristiche e trasformazioni in atto. Polis Working Papers dicembre 2011. (2) Piras et al. Valutazione della *shelf-life* di carni bovine *halal* sezionate e macinate. Ital J Food Saf 2011, 1, 55-60.

MULTIRESIDUE ANALYSIS OF ANTIMICROBIALS IN FEEDINGSTUFFS AT CARRY OVER LEVEL TO PREVENT ANTIBIOTIC RESISTANCE

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In the last years, the European Committee issued some regulations about hystomonostats and coccidiostats in feed for non-target species, as a consequence of incomplete cleaning up of the production pipelines after the formulation of feedstuffs added with these drugs. This possibility is referred to as “unavoidable carry-over in feed for non-target species” and can expose consumers to residues of these drugs in the food. Likely, the European Committee is going to issue a regulation about medicated feedstuffs, to harmonize their safety, production, commercialization and use. The Directive 90/167/EEC [1] will be repealed, and maximum limits will be set for unavoidable carry-over of antimicrobials in feed, on the basis of a risk evaluation for both animals and humans; for antibiotics the limits will be evaluated considering 1% maximum carry-over. To this aim, it is necessary to develop test methods to determine antibiotics at carry-over levels in feed, showing analytical performance fitting for the purposes of official control. In this work, a multi-residue and multi-class test method is described, for determination and qualitative confirmation of different antibiotics in feed (tetracyclines, sulphonamides, quinolones, macrolides, pleuromutilins, diaminopirimidines, streptogramines). The method is based on triple quadrupole mass spectrometry coupled to liquid chromatography, after liquid extraction from feed. Chromatography was carried out by an Agilent 1200 HPLC system, coupled through an ESI source to a QTRAP 4000 mass spectrometer (ABSciex). An Ascentis Express RP-Amide column was used for separation. The method has been developed to determine all antibiotics below the maximum limits that will be set in the regulation to be issued, to control the unavoidable carry-over in feed for non-target species. Up to 34 antibiotics can be determined in a single step. The quantitative confirmatory method was validated according to the Regulation 882/2004/EC [2], evaluating the analytical parameters linearity, specificity, limits of quantifications (LOQs), precision, trueness and ruggedness for slight changes, in the perspective of accreditation UNI EN ISO/IEC 17025:2005. For unambiguous confirmation of antibiotics the identification point system described in the Decision 2002/657/EC [3] was applied. The method is characterized by high sensitivity, and ensure complete identification of the drugs. The innovative approach of this work is the development of a rapid, sensitive, multi-class and multi-drug methods, to substitute those for a single group of antibiotics. We started out by sulphonamides, tetracyclines and quinolones, but then realized that high selectivity of mass spectrometry detector allowed us to introduce also other antibiotics. This makes more effective the control activity.

[1] D.M. 19.10.1999, Official Journal of Italian Republic, 191, 2000. [2] Regulation 882/2004/EC, Official Journal of European Union, L 165: 1-141, 2004. [3] Decision 2002/657/EC, Official Journal of European Union, L 221: 8-36, 2002.

WHOLE GENOME SEQUENCING TYPING TO BUILD OPEN ACCESS *Staphylococcus aureus* DATABASE

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The availability of whole genomic data led to development of a broad sector of microbiology, linked to genomic analysis of bacteria. Among model organisms, *Staphylococcus aureus* strains plays an important role: it is an ubiquitous pathogen responsible for one of the most widespread food borne disease and have direct impact also on animal health, causing clinical and subclinical mastitis.[1] The Italian National Reference Laboratory for Coagulase Positive Staphylococci incl. *S. aureus* (IT-NRL for CPS) carries out official analysis in food borne outbreaks, collects and processes regional and national data on staphylococcal enterotoxins (SE) poisoning. In particular, IT-NRL for CPS performs biotyping, multiplex PCR for genes encoding SE and genotyping by Pulse Field Gel Electroforesis (PFGE). Nowadays, these classic methods should be combined with analytical methods that allow a deeper characterization such as Next Generation Sequencing (NGS). The aim of this study was to perform Whole Genome Sequencing (WGS) typing on strains collected by IT-NRL and therefore constitute an open access database for *S. aureus*. We have included in our strains collection: 40 strains encoding SE isolated from food matrices in Piedmont region, 80 strains previously isolated during food borne outbreaks, 40 strains isolated from bovine mastitis and 33 from oropharyngeal swabs belonging to healthy volunteers. In the present work, we report the preliminary characterization with WGS of 18 strains selected from the first three groups. The NGS was performed on MiSeq platform (Illumina, San Diego, United States), with 200-bp paired-end reads on libraries which were prepared following the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, United States). The reads were first subjected to FastQC: Read Quality reports tool and then were trimmed, assembled and generated contigs were checked for quality. All these tools are accessible through Galaxy public servers. All samples were processed also for Multilocus Sequence Typing (MLST) with MLST 1.8 and for the presence of antimicrobial resistance genes with ResFinder 3.0. A phylogenic comparison was operated with CSI Phylogeny 1.2. The analysis operated with the web tool MLST 1.8 highlighted that ST-133 is the most frequent sequence type (27%), ST-8 is present in 22% strains and ST-398 in 16% and ST-71, ST-3078, ST-5, ST-97, ST-6 were assigned at one strain, respectively. The results of web tool ResFinder 3.0 showed, for all sequenced samples, the presence of norA gene (Fluoroquinolone resistance), the 50% of strains were positive to blaZ gene (Beta-lactam resistance), 38% were positive to tet genes (Tetracycline resistance) and 33% were mecA strain (Methicillin resistance), the 60% of the sequenced strains showed two or more resistance genes. The tree drawn with CSI Phylogeny 1.2 allowed us to represent the phylogenetic relationships between the strains and identify several clusters. Concerning the molecular typing, the WGS yields a greater and more accurate amount of data than the single traditional technique produces. All collected data allow a deep characterization of strains that will be used to build an open access database so as to provide useful information related to diagnostic and surveillance purpose.

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EVALUATION OF THE PRESENCE OF *Sarcocystis* SPP. IN BOVINE MEAT AND MEAT PRODUCTS AT RETAIL LEVEL

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Sarcocystis is an intracellular protozoan parasite which can infect a wide range of animals, including humans. The zoonotic cattle species can be transmitted through ingestion of contaminated raw or undercooked meat. Although considered as a minor zoonosis, it can represent a threat for immunocompromised individuals. Moreover, *Sarcocystis* sp. can occasionally lead to macroscopic lesions on carcasses, causing economic loss in many countries (1).

The present study was carried out to investigate the prevalence of *Sarcocysis* sp. in beef products in Italy.

Fifty-one samples from bovine minced meat and meat preparations were collected from discounts and large supermarkets in Turin's province (Piedmont region, Northwest Italy). The samples were examined by Multiplex PCR, targeting the 18s rRna gene (2).

The results showed that, overall, 86% of the tested samples were infected with *Sarcocystis*. The infection rate in minced meat and meat preparations was 94% and 74%, respectively. Regarding species identification, the prevalence resulted 74 % for *S. cruzi*, 25% for *S. hominis*, and 2% for *S. hirsuta*. Finally, 26% of the samples showed the simultaneous presence of multiple species. The high rate of *Sarcocystis* sp. contamination showed in the present study in raw beef products is consistent with the results of previous studies conducted in our region on cattle carcasses (2).

The relevant prevalence of the zoonotic species, *S. hominis*, needs to be assessed according to the recently revision of the taxonomy of the cattle *Sarcocystis* species: in fact, based on 18S rRNA gene sequences the morphologically is indistinguishable taxa in the two hosts (3). The partial sequencing of Cytochrome C Oxidase I (COI), currently under way, will give a more accurate prevalence of the cattle *Sarcocystis* species, with the possibility to evaluate the exposure of consumers to the zoonotic species, given the large consumption of raw bovine meat in this region.

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RAPID CAPILLARY ELECTROPHORESIS APPROACH FOR DETECTION OF THE ADULTERATION OF WATER BUFFALO MOZZARELLA WITH COW'S MILK

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The dilution of buffalo milk with cow milk is a common fraud in dairy industries. The combination of consumer demand, the limited amount of available water buffalo's milk and the high price obtainable make buffalo mozzarella an interesting target for adulteration. The EU reference method (European Commission, 2008) to detect cow's milk in buffalo mozzarella is based on gel isoelectric focusing of gamma-caseins after plasminolysis. It provides a sensitive detection of a possible adulteration but constitutes a laborious procedure. Capillary electrophoresis (CE) is a protein separation technique. Previously Trimboli et al. (2017) proposed CE as a tool to identify and quantify ewe milk in ovine/bovine milk mixture. In the present study CE separation technique was employed for the first time for the detection of the adulteration of water buffalo mozzarella by cow's milk. Cow mozzarella and buffalo mozzarella, some of which were declared as Mozzarella di Bufala Campana PDO, were purchased at various shops and farm retail in Italy. The mozzarella was cut and the obtained exudate was used for analysis. Samples were centrifugate at 3000 g for 15 min at 4°C to remove lipids and caseins and filtrated throught a syringe filter 0.45 micron. CE analysis was carried out using a Minicap capillary electrophoresis system (Sebia, Lisses, France) equipped with two 17 cm (16 cm to detect point) x 25 micron I.D. coated fused-silica capillaries, and a 200 nm UV detector at the cathode extremity. For calibration purposes mixtures of exudate from buffalo mozzarella, appositely produced for this aim, and exudate from cow mozzarella were prepared in the following volumetric ratios: 100/0, 99/1, 97.5/2.5, 95/5, 90/10, 85/15, 80/20, 75/25, 70/30, 65/35, 60/40, 55/45, 50/50, 40/60, 35/65, 30/70, 25/75 20/80, 15/85, 10/90, 5/95, 2.5/97.5, 1/99 0/100. The samples were divided in two sets, calibration (n=37) and validation set (n=22); the first set was used to established a calibration model that fit with protein fraction area selected to percentage of buffalo milk. The validation test was used to test the predictive ability of estimated regression model. CE profile of buffalo and cow exudate were very different and the overlay of protein profiles identified a specific cow protein fraction corresponding to Lactoglobulin variant A. The determination coefficient (r2) of calibration model was r2=0.9546. When the regression model was applied to validation set the r2 was 0.9478. The CE analysis is very fast and it works in fully automated manner. The approach proposed in this study provides a useful tool to rapid recognize whether buffalo milk is mixed with bovine milk to produce mozzarella cheese and to detect fraud in dairy industries.

[1] European Commission (2008). Commission regulation (EC) No 273/2008 of 5 March2008 laying down detailed rules for the application of council regulation (EC) no1255/1999 as regards methods for the analysis and quality evaluation of milk and milk products. Official Journal of the European Communities, L88, 1–115. [2] Trimboli F. et al. "Rapid capillary electrophoresis approach for the quantification of ewe milk adulteration with cow milk." Journal of Chromatography A 1519 (2017): 131-136.

FRESH OR FROZEN-THAWED FISH? A HISTOLOGICAL TOOL FOR REVEALING FRAUDS

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Seafood fraud is a serious global problem. Selling fish products as "fresh" when they have been frozen-thawed is a common fraudulent commercial practice; moreover, fish intended for raw or almost raw consumption must be previously frozen according to EU Regulations [1,2], in order to protect consumers from parasites and to avoid a potential sanitary fraud. Nevertheless in no case it is possible to automatically exclude possible negative effects on health even for commercial frauds and particular attention must be focused on all frauds. Differentiating between fresh and frozen-thawed fish is not easy; since years we have been evaluating the most performing techniques in distinguishing fresh from frozen fish in order to make available reliable tools. Aim of the work was to set up a valid histological method for the identification of fish as fresh or frozen-thawed. Preliminary investigations on samples frozen at -80°C were conducted even if these data were not included in the validation process. The study has taken into account time/temperature combinations considered by law, i.e.-20°C/24 hours [1], -35°C/15 hours [2], and also a very quick protocol not considered by law, i.e.-40°C/2 hours. In all, during successive experiments, 423 muscle samples of 35 fish species, never subjected before to temperatures below zero, were divided into 2 groups of reference samples: group A (n=273), fresh samples (stored at 0-4°C); group B (n=150), experimentally frozen, then thawed at 0-4°C. After, respectively, refrigeration and freezing/thawing, samples were fixed in 10% neutral buffered formalin and routinely processed. Paraffin embedded blocks were cut on a microtome into 3-5 µm sections and stained with haematoxylin and eosin. Slide preparations were examined by optical microscopy at increasing magnification (x4, x10, x20, x40). Different morphological parameters were evaluated but sensitivity and specificity of the method were estimated only on presence/absence of vacuoles of various dimensions, optically empty or filled with eosinophilic material, caused by ice crystals, in the cytoplasm of muscle cells (the only parameter able in differentiating fresh/thawed samples). Histological method resulted in 94.20% sensitivity (C.I.95%: 90.10-97.00%) and 97.70% specificity (C.I.95%: 94.20-99.40%) in differentiating fresh/frozen-thawed samples and it was validated, irrespective of the fish species analysed, and accredited. For the purpose of the histological interpretation, no significant differences from fish to fish were noticed. The method is now applicable to fresh and to prepared fishery products derived from fish and also to transformed ones, e.g. marinated, smoked fish, "transformed sashimi". Furthermore, a ring test recently performed in the italian network of Istituti Zooprofilattici Sperimentali obtained an optimal K-combined value of 0.94 (C.I.95%: 0.89-0.99). The method represents a valid and cost-effective tool, able to distinguish fresh from frozen-thawed fish and it can be used for revealing frauds regarding the storage conditions of the fish, protecting consumers from frauds. In 2017, 13.2% of 53 batches of samples declared as "fresh" and analysed by histology was actually frozen-thawed. Still, many important challenges are standing in the seafood sector: e.g. fast and reliable methods that would differentiate fresh/thawed cephalopods are being sought and we are working on it.

[1] Regulation (EC) 853/2004. [2] Regulation (EU) 1276/2011.

ARNA E PRODUZIONI ANIMALI

THERMOGRAPHIC TECHNIQUE CONFIGURATION (IRT) FOR THE STUDY OF THERMOREGULATION IN NON-HUMAN PRIMATES (*Pan troglodytes*)

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Thermography is a non-invasive imaging technique which allows to analyze, through thermal images, the emitted temperature of any object. [1] This feature allowed the thermographic technique to find wide use in those fields in which the representation of the temperature distribution is crucial. One of these fields is veterinary medicine, where the “non-invasive” technique might lead a better investigation of thermoregulation processes, inflammatory states, physiological and pathological conditions. [2]. A previous study was carried out to highlight the possibility of using the thermographic technique to study remote thermoregulation, with a non-invasive way, on the same colony of non-human primates. [3]
The goal of this study is to adjust and calibrate the thermographic system composed by a thermal imager and a telephoto. These preliminary operations were needed to improve the performance of the system. The results obtained from the tests were then applied to an experimental trial performed on field. [4]
In the first part of the work, performed in the laboratory, five different tests were done to adjust the thermal imager. This new thermal imager was then compared to a thermal imager already adjusted and calibrated. Two tests were performed to study the temperature of the heated sample materials on a plate, while the other three tests were performed to study the temperature of the same sample materials, this time heated in an oven.
In the second part of the work three field trials were performed, where, at a distance of 20 meters, measurements were taken on a colony of 11 chimps to check the effectiveness of the thermal imager in an uncontrolled external environment. Furthermore, the variations in temperature and thermoregulation of the host specimens based on characteristics such as age, gender, breeding methods and social status were observed.
The results obtained show that the thermal imager produces a systematic error that repeats constantly. The temperatures of some anatomical areas were compared with other different characteristics and a correlation has been observed between temperature and breeding method. The thermographic technique seems to be a valid method for the long-distance study of animals living in semi-wild conditions. However, further studies are needed since this technique seems to be influenced by factors such as the fur of individual, external climatic conditions.

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PRESENTATION OF THE COORDINATED RESEARCH CENTER INFRARED NON-INVASIVE IMAGING OF THE UNIVERSITY OF MILAN

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In recent years the interest in the study of animal welfare has led the various areas of scientific research to coagulate their knowledge for the development of non-invasive diagnostic methods.

Specifically, about the imaging sector, the "Coordinated Research Centre (CRC) for clinical and laboratory applications of non-invasive techniques of multispectral analysis and translational research" is presented, coordinated by the Dept. of Physics applied to Cultural Heritage Biology and Medicine of the University of Milan. The Centre, founded in 2015, relies on the collaboration of the Department of Veterinary Medicine, the Department of Biomedical Sciences for Health and the Department of Pharmacological and Biomolecular Sciences of the Milan University. For several years the University of Milan has been active in the field of non-invasive diagnostic imaging with infrared thermography techniques thanks to a significant system of scientific knowledge acquired over the years through numerous collaborations with highly interdisciplinary characteristics in the field of life sciences. The centre presents itself interdisciplinary characteristics directed also outwards of the research group, representing a uniqueness at a national level for the study and application of non-invasive imaging techniques.

Specifically, the main research objectives of the Centre can be summarized as follows:

a) build and maintain relationships and forms of collaboration with similar subjects and institutions in Italy and abroad; b) to promote and coordinate interdisciplinary studies, projects and research for diagnostic techniques of non-invasive imaging and in particular of infrared thermography in the life sciences; c) to contribute to the formation of an increasingly sensitive public opinion and participates in all the issues of diagnostics using non-invasive methods, promoting the organization of events, conferences and popular initiatives; d) to promote the documentation, the gathering and the dissemination of the results of its activities. In particular, the collaboration between the various Departments regarding imaging techniques used in Veterinary Medicine has led to the publication of the results in the use of thermography in the following topics: equestrian rehabilitation to study the situations of empathy/stress horse-rider, the possibility of studying thermoregulation in mouse models used in scientific research and, as regards the zootechnical field, the presence/absence of animal subjects (small species) present within the breeding structures. Finally, collaborations with Human Medicine have highlighted the possibility of studying the peripheral microcirculation in large obese patients and the physiology of athletes under stress and in rest conditions.

At the end of the present review of the research activity of the CRC the main bibliographic surveys of the three-year period (2015-2018) considered are available by the contact of the corresponding author.

IS PLASMA PROTEOMICS IN LIVESTOCK MATURE? COMPARISON OF TWO DIFFERENT COMMERCIAL KITS FOR SERUM ALBUMIN DEPLETION

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The depletion of high abundance proteins (HAP) is one of the rate-limiting steps in proteomics, especially on biological fluids like plasma, where albumin and immunoglobulins can hamper the detection of low-abundance proteins (LAP) [1]. The detection of LAP requires reliable methods to remove HAP. The available methods act on the depletion of HAP or the enrichment of LAP [2]. Several commercial kits have been developed to analyse human plasma samples, but their effectiveness on different species is not well described yet. Aimed to optimize the proteomic analysis in the bovine plasma, we assessed the depletion efficacy of two kits, AlbuTrial™ Kit AlbuSorb™ (Biotech) (ALBIO) and Albumin Magnetic Beads (Millipore) (ALMIL), in comparison to non-depletion. Overall three plasma samples of lactating dairy cows were analysed under each condition. Each sample was analysed 5 times per method. The efficiency of depletion has been verified by analysing total proteins and albumin by an auto-analyser for biochemistry. The proteomic assessment has been done through a mass spectrometry (nano LC with Q-TOF data-dependent MS/MS). Total protein content was reduced, in comparison with non-depleted samples, by 97% and 95% in ALMIL and ALBIO respectively. A 98% albumin depletion was recorded with both kits, whereas immunoglobulins were removed by 96% and 93% using ALMIL and ALBIO respectively. A total of 160 proteins was detected in non-depleted plasma samples (control), but only 59 of them were present in at least four out of five replicates, whereas 75 proteins were identified in only one out of five replicates. When samples were processed with ALMIL and ALBIO, a total of 93 and 90 proteins could be detected, respectively. The number of proteins identified in each replicate was different across treatments: 47 proteins were detected in at least four out of five replicates following ALMIL depletion, 42 of which (89%) were found also in non-depleted samples. However, 42 proteins were identified in at least four out of five replicates in ALBIO, 36 (86%) of which were found in both depleted and non-depleted samples. The LC-MS/MS analysis showed that albumin remains the most abundant protein also in depleted samples. Our findings also indicate that the relative number of albumin spectra was higher in samples treated with ALMIL compared to non-depleted samples, and lower in ALBIO compared with control samples (respectively 31.4% in ALMIL; 12.5% in ALBIO; and 20,1% in control). Volcano Plot analysis (ANOVA $p<0.05$, fold-change>3) evidenced significant differences in proteins' abundance when compared to control. Using both kits, up- and down-accumulation of plasma proteins could be detected ($p<0.05$) in comparison to whole plasma. The most of proteins passing the Volcano thresholds were shared between ALMIL and ALBIO. Our data show that the choice of different commercially available kit for albumin depletion might affect the results in animal proteomics studies. Despite the different rationale behind their operating principles, both kits altered the proteins quali-quantitative composition during albumin depletion, likely because of co-depletion effects. Therefore, the tested kits provided a limited improvement for LAP detection.

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DIETARY SUPPLEMENTATION WITH INCREASING LEVELS OF ORGANIC ACIDS IN BROILERS: EFFECT ON GROWTH PERFORMANCE, CARCASS TRAITS AND GUT MICROBIOTA

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Antimicrobial growth promoters (AGPs) have been used in animal production for the last few decades to improve the growth and reduce mortality. The use of AGPs in controlling pathogenic microorganisms is successful to a large extent [1]; however, acquired resistance of antimicrobial agents is a major concern [2] and has been phased out by the European Union [3]. As a result of these circumstances, the poultry researchers are focusing on the alternative to AGPs to optimize gut health and improve animal performance. One such alternative possessing growth-promoting characteristics is organic acid, which can maintain gut health and improve animal performance by balancing normal gut flora [4], moreover organic acids and their derivatives are considered safe for consumers. Therefore, the effects of organic acids supplementation in broiler chickens diet on growth performance, carcass traits, and caecal microbiota were investigated in the present feeding trial. A total of 330 day-old male chickens (Ross-308) were randomly allocated to eleven dietary treatments having three replicates with ten chicks each. Broilers received for 42 days a basal-diet without feed additives as control, whereas the other groups fed diets supplemented with different levels of acetic acid (AA; 0.1, 0.2, 0.3 and 0.4%, respectively) or formic acid (FA; 0.5 and 0.8%) and their combinations (n=11 dietary treatments). Based on results feed intake of broilers was not influenced by the addition of organic acid to diet. Conversely, feed efficiency was improved by adding a combination of 0.4% AA and 0.5% FA, in the first growing period (1-21 days), whereas in the second period (22-42 days) by adding 0.4% AA and 0.8% FA. In overall, supplementing both organic acids in broiler diet decreased organs weight and significantly increased meat cuts (breast and drumstick). Gut microbiota as *Escherichia coli* and Coliforms were significantly reduced by adding organic acids in diet, whereas Lactobacilli count was not influenced by dietary treatments. From our findings, it was demonstrated that supplementing organic acids in broiler diet is associated with an improvement of feed utilization and an enhancement of gut microbiota.

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PASSIVE MICRORHEOLOGY APPLIED TO MILK OF DIFFERENT SPECIES

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Passive microrheology consists of using micron sized particles to measure the local deformation of a sample resulting from an applied thermal energy (~kbT). This technology based on Diffusing Wave Spectroscopy consists of analysing the interferential images of light, which is backscattered by the sample. Monitoring the Brownian motion of the particles that scatter the light, different parameters can be obtained directly from the Mean Square Displacement (MSD) curve, including a fluidity index (FI), a solid–liquid balance (SLB), an elasticity index (EI), and macroscopic viscosity index (MVI). The variation of the images as a function of time can be directly correlated to the viscoelastic properties of the sample. Considering the main advantages of microrheology, it is clear that this tool is particularly suitable for the analysis of foods [1]. Considered that MSD curves, as a function of variables (time and temperature), enable the monitoring of curd process the aim of this work was to determine the exact gel point of milk from different species. Therefore, 4 bulk tank milk samples, 1 for cow milk, 1 for buffalo's milk, 1 for goat's milk, 1 for ewe's milk from 4 different farms across Calabria, were examined. Percentage of fat, protein, lactose, casein and freezing point (°C), urea (mg/dl), acetone (mM) and beta-hydroxybutyrate (mM) were determined using Milkoscan FT+. pH of each samples was assessed using a pH meter (Mettler Toledo, Columbus, Ohio, US). Rennet (CHY-MAX Plus 200, CHR Hansen, Hoersholm, Danemark) was added at each sample (20µl) then put in a vial and analyzed. Rheology measurements on milk samples were performed at 37°C for 30±2 minutes with Reolaser® Master (Formulaction, France). The obtained data were calculated by the software Rheosoft Master 1.4.0 and expressed as gel point. Our results show that fat ranged from 2.96 to 8.17%; proteins from 3.42 to 5.87%; lactose from 4.33 to 5.05%; casein from 2.86 to 3.87%. Freezing point ranged from a maximum of -0.522 to a minimum of -0.565°C; urea ranged from 28.4 to 79.2 mg/dl; acetone from 0 to 0.21 mM, whereas beta-hydroxybutyrate ranged from 0 to 0.04 mM. pH values recorded varied from 6.71 to 6.92. Cow milk reached gel point in 24 min and 2 seconds; buffalo in 3 min and 24 seconds; ewe milk 11 min and 2 seconds whereas for goat milk gel point was recorded at 10 min and 29 seconds. This work focuses on the evolution of the viscoelastic properties of milk of different species and shows the advantages of using a non-invasive method to detect initial destabilisation of the microstructure. Gel point time is the moment at which a sample spanning network is formed: our data suggest that milk with a bigger percentage of fat and proteins reaches gel point in less time. Ramsch et al. [2] described as Rheolaser observes syneresis and identified gel point as a flocculation, but no information was give about milk composition neither to different type of milk. Findings of this research are useful for monitoring modifications of milk during curdling. Further developments can be applied in food industry, where raw material with non-homogeneus composition make difficult to achieve the same end product.

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PASSIVE MICRORHEOLOGY APPLIED TO BOVINE COLOSTRUM AND RELATIONSHIP WITH IMMUNOGLOBULIN G

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In ruminants, colostrum, the first mammary secretion after parturition represents an important source of nutrition and passive immunity which ensures protection in early life [1]. Passive rheology is based on Diffusing Wave Spectroscopy that consists of analysing the interferential images of light, which is backscattered by the sample. Monitoring the Brownian motion of the particles that scatter the light, different parameters can be obtained directly from the Mean Square Displacement (MSD) curve, including a solid–liquid balance (SLB), an elasticity index (EI), and a macroscopic viscosity index (MVI). In recent years, the application of the rheology in food is becoming increasingly and allows to characterize rheological properties of different matrices [2]. The aim of our study was to define rheological parameters of bovine colostrum and their relationship with IgG concentration. Colostrum samples (n=31) were collected from eight Simmental dairy cows at different times after parturition (0, 12, 24, 36, and after 48 hours from parturition). Rheology measurements on colostrum samples were performed at 24°C for 30±2 minutes with an automated instrument, Reolaser® Master (Formulaction, France). The obtained data were calculated by the software Rheosoft Master 1.4.0 and expressed as MSD, SLB, EI and MVI. The IgG concentration was assessed by radial immunodiffusion technique using Bovine IgG IDRing® Test (IDBiotech, ImmunoDiffusion Biotechnologies SARL, Issoire, France). In colostrum samples, SLB value ranged from 0.356 to 1.067x10-1; EI value ranged from 4.09 to 11.64x10-4; nm-2s and MVI value ranged from 2.115 to 30.73x10-6. Concentration of IgG assessed by RID ranged from 0.1 to 187.4 g/L. After statistical analysis, SLB EI values and IgG concentration decreased significantly during time ($p<0.005$). In addition our results shown a positive correlation ($r2=0.727$) between EI and IgG concentration. Since in bovine colostrum immunoglobulin G represent the major component of total protein, forming the “gel network”, the IgG content has a direct influence on the elasticity of the sample. For this reason, elastic properties reflect the amount of immunoglobulin inside colostrum. In conclusion the rheological properties of colostrum, and in particular SLB and EI during time, may be a laboratory tool to asses colostrum quality.

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ASSESSMENT OF ANIMAL WELFARE IN DAIRY HERDS WITH IDEAL SOFTWARE: PRELIMINARY RESULTS

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Animal welfare, defined as the respect for the five fundamental freedoms of animals [1], is considered a key aspect of livestock production in developed countries. Thus, ensuring a high welfare level in farm animals is a growing concern among informed consumers. Additionally, maintaining a good level of welfare is essential also for farmers to increase animal performance and profitability. The aim of this study has been to further elucidate the link between welfare status, assessed via qualitative and quantitative indicators, and the performance levels in high yielding dairy herds. This report comprises preliminary data (8 farms) of an ongoing survey in the Po Valley. The level of welfare has been evaluated with the Integrated Diagnostic System of Animal Welfare (IDSW) [2], developed by the DiANA department at the Università Cattolica del Sacro Cuore di Piacenza, and now accessible through the software IDEAL (Integrate diagnostic system for dairy cow welfare). The model includes many direct and indirect indicators of welfare, grouped in the three clusters: Housing and equipment, Feeding and Animal. For each farm, outputs of the model consist of a global IDSW score and partial scores of each component and cluster. The dataset of detected indicators, of ECM (Energy Corrected Milk at 305 days) not included among previous indicators, and the IDSW scores (global and partial) was analyzed using R (version 3.4.4) to calculate the Pearson correlation coefficients. Results have shown adequate ISDW scores (7.41±0.10) for 6 farms, and inadequate for the other 2 (6.30 and 4.40). Global IDSW score, as well as partial scores related to barn and equipment and diet characteristics resulted positively correlated with ECM ($r=0.71$, $P<0.05$; $r=0.77$, $P=0.02$; $r=0.83$, $P<0.01$ respectively). Additionally, relevant correlations are also observed among components "Health, Diseases and disorders" with "Productive performances" ($r=0.67$, $P=0.07$), as well as "Health, Diseases and disorders" with Feeding cluster ($r=0.68$, $P=0.07$). Reproductive performances were not strictly correlated with ECM, components or clusters of the IDSW model. These preliminary results confirm that the simple introduction of new technologies does not guarantee an acceptable level of welfare. Furthermore, they proved the importance of the partition of global welfare score in clusters and components to obtain suggestions on specific traits involved in the reduction of the welfare and their relative relevance. This approach allows to discover incipient issues which can be overshadowed in the global score. Finally, correlations between global welfare with productive performance and health status demonstrates that IDSW constitutes a powerful tool for farmers aiming to improve the profitability of their herds.

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PRELIMINARY SURVEY OF THE WELFARE OF DAIRY DONKEYS IN NORTH-WESTERN ITALY

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There is an increasing interest in donkey farming in Italy, because of the use of donkey milk to feed children affected by Cow's Milk Protein Allergy. Limited information is available about the management characteristics of dairy donkeys' facilities, as well as concerning the assessment of the animal welfare. Considering the lack of information, recommendations and a welfare specific legislation on the farming of this species, the aim of this study was to highlight possible critical aspects of this farm system and to assess animal welfare.

During each on-farm visit, a check list was fulfilled according to a designed specific score for: good housing and management (*bedding quality,* regular health checks, foot care, control of insects and rodents, weaning process, milking procedures), good nutritional status (BCS and water provisions), good health (integument cleanliness, injuries, fecal quality, lameness, swollen joints, signs of teeth abnormalities, udder and teat hygiene), behavior (stereotypies). Descriptive statistic of the observed parameters was calculated.

A total of 6 dairy donkey farms located in north-western Italy were visited during 2013 and 2014. The number of donkeys per facility ranged from 40 to 60; of a total of 206 females, 62 were lactating and 144 were dry jennies, aged between 36 and 48 months. A total of 6 stallions and 67 foals lived on the farms. The animals were kept on pasture with shelters during the day and stabled in pen during the night. They were grouped according to their production, with the exception of one farm where they remained all together on pasture. The visited farms were family run, only two farms had employees. The number of donkeys cared for by a single person varied from 5 to 103 animals. As far as good housing is concerned, straw bedding quality (depth and hygiene) was adequate in all farms. The bedding was changed with a frequency up to 4 times per month. The cleaning of the troughs and of the farm in general (walls and floors) was given a medium score. Regular health checks were provided. Antiparasitic treatments were administered in all farms and measures to control insects and rodents adopted.

For the milking procedures, foals were separated from their mothers four (n=1) six (n=3) or 10 hours (n=2) before milking. When separated, they could see or hear the mother. No farms used artificial milk; therefore, the foals received colostrum from and were nursed by their mother during 6-12 months. Additional feed was introduced between 0 and 12 months. The milking parlour was present in 4 farms, and all the farmers adopted a method of cleaning and disinfection of the teats. The average milk production was 1 litre for 1 daily milking. Forty per cent of the donkeys showed a good body condition score (BCS=3), a consequence of appropriate nutrition. In 4 farms the animals were provided with hay ad libitum, and complementary feedstuffs; only one farmer provided minerals and vitamins; all the animals showed no changes in colour or consistency of the manure. Concerning good health indicators, all donkeys were judged clean, more than 90% had no swollen joints, lameness, signs of teeth abnormalities. No animal showed any stereotypies.

In conclusion, the dairy donkey farms surveyed showed a good level of animal well-being. Such good welfare is possible thanks to the increased knowledge and growing awareness among farmers of the importance of welfare protection in order to achieve a better animal health.

MYSTERY CASES

THE UNEXPECTED MASS

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A 5-year-old female dog Yorkshire was presented at a private veterinary ambulatory clinic because of a severe abdomen enlargement.

Long term medical history referred the bitch has never been coupled, had regular heats, and lived in house as the only domestic animal.

The bitch was 4.1 kg weight with BCS 3/5. Clinical examination revealed: slight depression, normal mucosae and palpable lymph nodes, temperature 38.2°C, heart rate 90 bpm, respiratory rate 66 bpm. All organic functions were normal; haematological and biochemical values were within normal limits.

At abdominal palpation, a firm ovoid mass was detected; thus abdominal ultrasonography and thoracic radiology were performed.

Thoracic radiology was negative; ultrasound examination revealed an ovarian mass measuring 10x5.5 cm in size. The mass had distinct margins and showed a medullary parenchyma rich in anechogenic, ovoid, cystic areas, while the cortical one appeared dis-homogeneous because of a miscellaneousy hyperechogenic/hypoechogenic areas, leading to a suspicious diagnosis of ovarian neoplasia.

An ovary-hysterectomy was carried out and the excised mass was sent to the pathology lab for examination.

At gross examination, the mass was irregularly round-shaped, red brownish in colour, firm in consistence. On the cut surface, several haemorrhagic and necrotic areas were present in the cortical region, while many cystic structures varying in size from 2 to 15 mm in diameter and a serous content was were detected in the medullary region.

At histopathological evaluation, H&E stain revealed a solid proliferation characterised by nests of both round and polygonal cells, with cytoplasm borders difficult to be seen, but rich in lipid droplets, giving the neoplastic tissue a foaming feature, and ovoid nuclei.

Immunohistochemistry was negative for cytokeratin and HBME-1, whereas strong immunoreaction with vimentin, calretinin and inhibin was observed.

Gomori histochemical stain highlighted an abundant reticulin network among neoplastic cells.

According to all these findings, a diagnosis of thecoma was issued.

THE "DISRUPTIVE" PATIENT

Bianca Conturba

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SIGNALMENT: BREED: Italian Saddle. SEX: gelding. AGE: 11 years. MANTLE: bay. WEIGHT: 482 Kg. ATTITUDE: show jumping.

ANAMNESTIC INFORMATION

The Veterinarian reports, on the day following vaccination against Influenza and tetanus (adjuvant vaccine with immunostimulatory complex ISCOM, suspension for injection, in single dose), the onset of a systemic symptomatology, characterized by fever, depression, anorexia, generalized myalgia, jaundice, associated with neurological symptoms suggestive of involvement of the central nervous system (apathy, alteration of mental state with depression of central nervous responses and disorientation, postural abnormalities, ataxia and incoordination of movements). This clinical picture is considered consequent to an adverse reaction to vaccination, and therefore reported to the Ministry of Health by completing and sending the appropriate Reporting Forms of Suspected Adverse Reaction. None of the other treated horses, in addition to the subject considered, showed a similar reaction. The reports indicates also that the symptomatology lasted for about 14 days, during which a symptomatic and supportive therapy was undertaken with partial improvement of the clinical conditions. However, depression, apathy, ataxia, and incoordination persisted, accompanied by a progressive weight loss. Therefore it was decided by the Veterinarian to confer the horse to the Department of Medicine of the Horse Veterinary Hospital of the University of Milan, Azienda "Polo Veterinario di Lodi" for further investigations.

PHYSICAL EXAMINATION

At the general examination, the subject showed fair nutrition conditions (Body Condition Score: 3/5, body weight: 482 kg). The skin and coat showed no alterations; the presence of a state of edema affecting the region of the distal shin and the hindquarters of both hind limbs was detected. The rectal temperature was 37.2° C and the mucous membranes appeared congest; the capillary filling time was less than 2 seconds. There was mild lymphadenomegaly of the right intermandibular lymph node. On palpation, arterial pulse was normal, with a frequency of 32 bpm. The cardiac stroke was perceivable and, at the auscultation, the second tone was split; the heart rhythm was with no pericardial noises. At the functional examination of the breath, the respiratory frequency was 12 per minute, and the breath was normal. At the auscultation of the thorax, through the use of a respiratory bag, no anomalous lung noises were found.

NEUROLOGICAL EXAM

The mental state was normal, but the sensory appeared depressed. An alteration of posture with the limbs articulated and swaying of the rear train with proprioceptive deficit, tetraparesis and widespread muscle tremors were detected. Upon examination of the region, a deficiency of the 5th, 7th and 8yh pair of cranial nerves was detected (slight rotation of the head and low deviation of the bottom downwards, with a reduction of the sensitivity of the nasal septum and nostrils). The static examination of the neck and front limbs showed a limit of sensitivity at the dorsal surface of the forearm, carpus and metacarpus. The static examination of the region of the trunk, the hind limbs, the anal region and the tail shows no significant changes. On the dynamic examination, a moderate bilateral weakness of the hind limbs (positive wave test) was found, with difficulty in repositioning and resistance to the traction of the tail, marked ataxia, which worsened with increasing gait (from step to

trot) , following a sudden stop and retreat, and bandage. In addition, the limb crossing test was positive for both the anterior and hind limbs. A lack of proprioception, primarily in the front limbs, was detectable for both the right and left hands.

INSTRUMENTAL EXAMINATION

An electrocardiographic examination was performed, which showed no changes in heart rhythm or myocardial conduction activity, and haematological and hematochemical tests, showing a slight decrease in the count of erythrocytes and hematocrit (RBC: 6.78 x 10^6/µL; , 3%), leukocytosis (WBC: 11.08 x 10^3/µL) with relative and absolute neutrophils (neutrophils: 88%, 9.8 x 10^3/µL) and mild relative and absolute lymphopenia (lymphocytes: 10%; 1, 1x10^3/µL), mild hyperbilirubinemia (total bilirubin: 2.8 mg dl), mild hypercalcaemia (calcium: 12.5 mg/dl), hypophosphoremia (phosphorus: 1.7 mg/dl), hypomagnesemia (magnesium: 1.21 mg/dl), slight decrease in iron concentration (iron 30 mg/dL), slight increase in the enzymatic LDH activity (LDH 569 U/L) and hyperfibrinogenemia (fibrinogen: 395 mg/dL). Protein electrophoresis showed a total hypoproteinemia (total protein: 5.7 g/dl) with hypoalbuminemia (albumine 3 g/dl, 52.7%) and a decrease in the fraction of α1-globulins (0.1 g/dl, 1.7%). The finding of neutrophilic leucocytosis and hyperfibrinogenemia suggests the presence of an active systemic inflammatory state.

The parasitological examination of faeces was negative.

In addition, a nasal swab and blood collection (serum) were performed for PCR examination of type 1-4 Equine Herpesvirus, neurotropic Flaviviruses and Equine Viral Arteritis, which resulted all negative. Serial blood samples were also collected for culture test, which scored negative.

Subsequently, an X-ray and an ultrasound examination of the cervical spine were performed, which showed no alteration that would justify the observed symptoms.

The cerebrospinal fluid was collected performed, at the lumbosacral level following sedation and containment. A PCR test was performed for type 1 and 4 Equine Herpesvirus DNA, neurotropic Flaviviruses, Equine Viral Arteritis and *Borrelia burgdorferi*, with negative results; a microbiological test showed a negative result, while the cytological examination and the microprotein dosage showed neutrophilic pleocytosis (30%) and a significant increase in total proteins (300 mg/dL).

The electromyographic examination was normal.

DIAGNOSIS

On the basis of the medical history and the results of the investigations carried out, the diagnosis of immunocomplex associated meningitis and active systemic inflammation, referable to the condition of hyperreactivity of the immune system, was obtained, as a probable result of an adverse reaction to the administration of polyvalent vaccine.

RECOVERY AND THERAPY

Initially, the clinical symptomatology was controlled by administration of Flunixin Meglumine (1.1 mg kg I.V. once a day) and, subsequently to the negativity of the microbiological tests (blood and liquor) and of the PCR tests (nasal swab, blood and liquor), the anti-inflammatory treatment was modified with the use of dexamethasone according to a scalar protocol starting from 0.06 mg/kg administered once a day, associated with a systemic antibiotic treatment with ceftiofur at a dose of 2.2 mg/kg intramuscularly every 12 hours for 8 days, for prophylactic purposes. Subsequently, the anti-inflammatory treatment Flunixin Meglumine was modified at the dose of 1.1 mg/kg I.V., once a day, and after a few days substituted with Fenilbutazone (4.4 mg/kg once a day) orally. Considering the possible side effects due to protracted administration of non-steroidal anti-inflammatory drugs, during the hospitalization the renal function was periodically monitored and at the same time a therapy with gastroprotectors and enteroprotectors (oral sucralfate at the dosage of 6 g every 12 hours) was

adopted. Over the period of hospitalization, the evolution of the clinical picture was monitored through periodic neurological examination and laboratory tests, that showed a positive response to the therapeutic protocol, as showed by the progressive, though partial, regression of neurological deficits and re-entry within the normal range of the main haematological and hematochemical parameters (fibrinogenemia and leukocyte counts).

Subsequently, during the period of hospitalization, attempts were made to gradually reduce the anti-inflammatory drug therapy, which each time determined a relapse of the systemic symptoms (depression and hyperthermia) and of the orthopedic, ophthalmological and cardiological signs described below. These events forced the immediate resumption of anti-inflammatory treatment and the institution of the treatment that was more appropriate in relation to the arising complications.

COMPLICATIONS RELATED WITH THE STATE OF HYPER-REACTIVITY OF THE IMMUNE SYSTEM, AND RELATED TREATMENT

Orthopedic complications

Fifteen days after admission, acute onset of 5/5 grade on the left hind limb was observed. Bilaterally, at the hind limbs, edema was present in the distal region of the shin and the hobble. On the other hand, no changes appeard to the front limbs. The palpation of the hind limbs was negative; the ultrasound examination of the plantar region of the shin showed the presence of a slight increase of anecogenous synovial fluid inside the synovial sheath of the flexor tendons.

A cold shower several times a day of the involved limb and a compression bandage were applied. Over the next three days, a clear clinical improvement of the lameness was detected, until complete recovery. Three days after the remission of the symptoms of the hind left limb, however, the onset of an acute lameness of grade 5/5 of the opposite limb (hind right) was detected. The affected limb showed hot, painful edema of the distal region of the shin and the hobble. The palpation of the hind limbs was negative; the ultrasonographic examination of the plantar region of the shin showed the presence of a central anechoic area at the distal third of the deep flexor tendon of the phalanges, compatible with an acute tendinitis, accompanied by a modest increase of anecogenous synovial fluid inside the synovial tendon sheath. In addition, the ultrasound examination of the plantar region of the moth showed a thickening of the digital plantar annular ligament, suggesting the presence of a chronic annular ligament lesion. We then started with a symptomatic treatment similar to that used for the left hind limb (repeated hydrotherapy and compression bandage) associated with the topical application of a gel based on escin and diethylamine (Reparil Gel). During the first days, the animal was kept at rest in the box. Subsequently, the monitoring through a physical and ultrasonographic examination showed a gradual improvement, characterized by progressive resorption of edema, recovery of limb support and disappearance of the lameness (15 days after onset), with ultrasound results suggestive of a progressive partial regeneration of the injured tendon fibers (35 days after onset). At the same time, in order to obtain a faster recovery, an orthopedic shoeing was performed, and a daily walk session of a few (5-10) minutes repeated three times a day was introduced.

Ophthalmologic complications

Sixteen days after admission, an acute onset of a severe right eyelid swelling accompanied by ocular purulent discharge, marked conjunctival hyperemia, complete corneal opacification, hyphaema and total visual deficit was detected. The fluorescein test for corneal ulceration resulted negative, both for the right and left eyes. Because of the complete corneal opacification, ophthalmoscopic examination resulted impossible to visualize the fundus of the right eye; hyperemia of the papilla of the left optic nerve was found. The ultrasound examination showed the presence of intensely hyperechoic material inside the hind chamber and the anterior chamber of the right eye, a marked edema and retinal detachment and severe chorioid hemorrhage; no clinically significant ultrasound changes were

detected in the left eye. The alterations highlighted by the investigations performed were compatible with a diagnosis of severe irido-cyclo-corioid-retinitis of the right eye. The treatment included an oculoconjunctival lavage with sterile physiological solution and the topical application of an ophthalmic antibiotic ointment based on tetracycline, chloramphenicol and colistimethate four times a day, of eye drops with tropicainamide-based midriatic action, and of an flurbiprofen-based anti-inflammatory collyrium, twice a day. This therapeutic regimen was maintained for the entire duration of the admission; this treatment was subsequently integrated with the topical application of a dexamethasone-based ophthalmic ointment. The evolution of the ophthalmic condition in the right eye was monitored by a periodic ophthalmological, ophthalmoscopic and ultrasonographic examination. There was a progressive reduction in acute inflammatory symptomatology with disappearance of ocular drainage (after 5 days), edema, hyperemia and corneal opacification, accompanied by a gradual moderate atrophy of the eyeball and enophthalmos (progressively during the following 25 days onset). The visual impairment persisted. At the ophthalmoscopic examination, the visualization of the fundus of the eye was still impossible; the ultrasound examination showed progressive reduction of the accumulation of inflammatory material which, in the organization phase, appeared inside the hind chamber of the eye, with persistent retinal detachment.

Cardiological complications

As the last complication (49 days after admission), the appearance of a severe tachyarrhythmia (fc maximum 120 bpm) occurred. The electrocardiographic pattern showed an atrioventricular dissociation, characterized by the presence of premature ventricular complexes in pairs, triplets and in flaps, which evolved periodically in phases of persistent ventricular tachycardia. During the whole period of continuous monitoring by electrocardiographic tracing and Holter recording (lasting 48 hours), the persistent arrhythmia pattern alternated with short periods of spontaneous return to sinus rhythm. The return to a stable sinus rhythm occurred within 24 hours after the onset of arrhythmia, after restoration of anti-inflammatory therapy, without further recurrence. The echocardiographic examination, performed during the arrhythmia period and after the recovery of the normal sinus rhythm, showed thickening and hyperhogenicity of the valvular flaps of the aortic valve, associated with aortic regurgitation in the diastolic phase. The results of the diagnostic investigations carried out suggested the presence of a picture of endocarditis/acute myocarditis.

DIMISSION AND FOLLOW UP

At the time of dismissal, the subject appeared in good nutrition status, with a body weight of 516 kg; the sensory condition was alert and the large organic functions were normal.

As regards the neurological conditions, the initial deficit regressed to 80%. At the neurological examination, the sensory condition and the mental state appeared normal. Occasionally, a postural alteration was found, with the left anterior limb maintained in abduction position. At the head region, no alterations related to the nervous system were detected. On static examination there were no changes in the region of the neck, forelimbs, trunk, hind limbs, anus and tail. On dynamic examination, a moderate proprioceptive deficit were left, that could be detected primarily in the hindlimbs and could be evoked in a narrow vault with greater severity in the right hand.

As regards the orthopedic conditions, the clinical and ultrasound examinations showed a progressive improvement of the tendon lesion of the right hind limb. It was suggested to continue the walk for 10-20 minutes a day, preferably twice a day, and to perform concomitant hydrotherapy of the hind legs with cold water. It was also recommended to perform a night bandage with rest bands on both hind limbs. Finally, it was recommended to ascertain the evolutionary state of tendinopathy by performing periodic ultrasound examinations, possibly modifying and/or integrating the therapeutic protocol in agreement with the Veterinarian.

As regards the ophthalmological conditions, the clinical examination of the right eye showed regression of the acute inflammatory symptomatology, to which was associated however a persistence of the atrophy of the eyeball and enophthalmos, without recovery of full vision. In this regard, the prognosis *quoad functionem* remains poor, due to the retinal detachment. On ophthalmoscopic examination, the visualization of the fundus of the eye was prevented by the accumulation of inflammatory material during the organization phase inside the hind chamber of the eye, which coul also be detected by ultrasound. It was recommended to continue for at least two weeks with a local therapy consisting of topical oculo-conjunctival application twice a day of Visumidriatic eye drops 1%, followed 10 minutes after the application of Colbiocin Unguento Oftalmico and Luxazone Unguento Oftalmico, and (after further ten minutes) and of Ocufen Collirio. It was recommended, at the end of the two weeks, to perform a clinical re-evaluation, and to suspend or modify the therapy after agreement with the Veterinarian.

As regards the cardiac conditions, monitoring of myocardial conduction and aortic insufficiency was suggested by periodic monitoring.

The recurrence of systemic symptoms and the appearance of serious autoimmune complications, in response to any attempt to reduce the anti-inflammatory treatment, highlight the instability of the current clinical situation, the need to prolong the treatment with anti-inflammatory drugs for at least 1 month starting from dismissal, and to consider the prognosis as questionable.

In the follow-up, which took place by telephone after 2 months and 6 months from the date of dismissal, we were informed of the stabilization of the clinical picture after the suspension of anti-inflammatory treatment, with gradual recovery and a slight sporting activity.

DISCUSSION

The close temporal relationship between the evident and severe clinical symptomatology (24 hours) and the administration of the vaccine, suggests the probable occurrence of an adverse reaction to the vaccine.

The current legislation defines "adverse drug reaction" as "any harmful and unwanted effects resulting from the use of a medicinal product". The current definition, introduced in the European community in 2010 by the European Directive 2010/84 / EU, was implemented in Italy on July 2nd, 2012. This definition implies the manifestation of the phenomenon in relation to use, in compliance with the indications contained in the authorization to placing on the market, treatment errors and/or not compliant use according to the indications contained in the Marketing Authorization, such as overdose, improper use ("off label") and abuse.

In particular, the occurrence of neurological symptoms associated with fever, the day following the vaccine administration, can be considered a "serious side effect".

Such a serious adverse reaction is to be considered a rare occurrence, reported by some authors in humans and sporadically in some species in the veterinary field, but not mentioned in the literature in the equine species. Given these premises, it appears reasonable, based on some possible similarities with what is described in humans and other animal species, to consider what is reported in the literature, in order to explore the possible mechanisms underlying this reaction.

In humans, dogs, sheep and cattle (Mayhew, 1989; Furr and Reed, 2008; Zani et al., 2009), meningitis are reported secondary to vaccine administration performed in the paraspinal musculature: the inflammatory reaction, of granulomatous type, that develops in the muscular planes near the vertebral apophysis can, by contiguity, deeply involve the meninges, causing the formation of abscesses or epidural collections. The affected animals show a rise in temperature, lethargy, paraplegia or paraparesis and ataxia, and show haematological tests characterized by neutrophilic leukocytosis. In addition, cytological examination of the liquor is characterized by moderate

neutrophilic pleocytosis and a normal protein content, with a negative bacteriological examination. In all the described cases, the diagnosis was only possible at the autopsy or through RMI, since in no subject the radiographic examination showed any alteration.

Other possible causes reported in literature as rare vaccine complications are reactions of immune origin (Tizard 2013). In particular, in humans, aseptic meningitis induced by the administration of some categories of drugs are reported; in a single case this symptomatology followed a vaccination against Hepatitis B. The immunological mechanisms involved seem to be linked to Type III hypersensitivity, with the formation of immune complexes in the meningeal vascular endothelium (Moris et al., 1999). These reactions could be caused by the vaccine or by the adjuvant (Tizard 2013).

Also Guillain-Barré syndrome, an autoimmune neurological disorder in humans, can be triggered by the administration of the flu vaccine. It is also reported a single case in a dog that, following the administration of a polyvalent vaccine, showed a symptomatology attributable to the Guillain-Barrè syndrome, with the development of a polyneuritis secondary to the production of autoantibodies against the phospholipids of the peripheral nerves (Tizard 2013).

Uveitis and retinocorioiditis developed in the horse about 1 month after vaccination, in association with bilateral hind arthrosinovitis, which could be caused by a Type III hypersensitivity reaction; in this case, the systemic diffusion of an excess of antigen-antibody complexes, beyond the removal capacity of the mononuclear-phagocytic system, may have determined the deposition of immunocomplexes at vascular level in different districts of the organism, and the consequent development of vasculitis (Tizard, 2013). Such reactions could be caused by the vaccine or by the adjuvant (Tizard, 2013). The administered vaccine contained an ISCOM adjuvant classified as "immunostimulant", that acts by promoting the production of cytokines by the antigen-presenting cells, to enhance the humoral and cell-mediated immunity. Some authors (Andersen et al., 2012) report that this type of vaccine induces an acute phase response of greater magnitude than vaccines with "depot" adjuvants (based on aluminum hydroxide). The greater immunogenicity of this adjuvant, associated with a hypersensitivity of the subject, could justify the immunocomplexes reactions.

As regards the tendon injury at the right hind limb, it is possible that the excessive mechanical stress, resulting from the lack or reduced support of the left hind limb associated with neurological deficits, may have led to its onset.

Even the severe tachyarrhythmia appears to be due to the hyperreactivity of the immune system. In particular, episodes of autoimmune endocarditis/myocarditis have been reported in humans and in various experimental animal models, depending on the administration of particular vaccine and adjuvant antigens. Gamma-globulin and complement deposition in the myocardium causes a variety of lesions, including inflammatory cell infiltration with areas of myocardial degeneration and valvulitis (Anard et al., 1983, Pericone et al., 2011).

As regards the differential diagnosis, it was possible to exclude neurotropic virus infections by PCR on liquor. As regards other infectious agents, the multisystem and nonspecific symptomatology associated with fever may be suggestive of a *Borrelia burgorferi* infection; however, the period of the year in which the symptoms occurred (January), as well as the geographical location of the subject (Northern Italy), make extremely difficult for a specific carrier to transmit the spirochete.

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A CASE OF MYSTERIOUS ILLNESS IN DONKEYS

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An outbreak of ulcerative stomatitis started in a donkey (*Equus asinus*) dairy herd. Fever and small nodular lesions, evolving into painful ulcers, were observed on the oral mucosa, tongue, and the skin around the lips in young animals (2 weeks to 4 months of age). Similar lesions were also observed on the mares teats and, sporadically, in the genital areas. The lesions typically recovered in 3-4 weeks. Hepersvirus DNA was detected using consensus herpesvirus primers. Upon sequence analysis, the virus was characterized as a member of *alpha-Herpesivirinae* sub-family within the genus *Varicellovirus*. These findings are relevant for the differential diagnosis of vesicular diseases of equids.

IT'S A MYSTERY CASE

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