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uniss UNIVERSITÀ DEGLI STUDI DI SASSARI veterinaria DIPARTIMENTO DI MEDICINA VETERINARIA

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLA SARDEGNA "G. Pegreffi"

FONDAZIONE INIZIATIVE ZOOPROFILATTICHE & ZOOTECNICHE

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19-22 Giugno 2019

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73 CONVEGNO SISVET

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I contributi presenti negli Atti del
73° Convegno SISVet 2019
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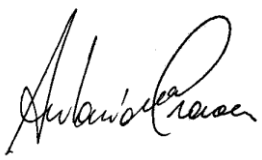
Relazione del Presidente

Carissimi,
come la maggior parte di voi sanno il 21 Novembre 2018, a Brescia, con atto a rogito del Notaio Guido Cirilli, è stata formalizzata la Federazione SISVET con la sottoscrizione del nuovo Statuto da parte dei 12 Presidenti di Società Scientifiche Veterinarie che hanno aderito. La scelta di Brescia per la costituzione della nuova Federazione non è stata casuale, Brescia, infatti ha sempre rappresentato unmomento di comunione tra l'accademia e tutte le altre componenti della Veterinaria, grazie alla Fondazione Iniziative Zooprofilattiche e Zootecniche che ha sempre sostenuto la SISVET sia con iniziative editoriali di pregio sia garantendo sede e segreteria amministrativa alla Società sin dal lontano 1946, dopo che i nostri Maestri fondarono a Bologna la Società Italiana delle Scienze Veterinarie. A Brescia, dai Presidenti delle Società, che per il nuovo statuto, rappresenteranno i Soci della Federazione, è stato eletto anche il nuovo Comitato scientifico ed il nuovo Comitato esecutivo composto da me in qualità di Presidente, dalla Prof.ssa Adriana Ianieri, in qualità di Vice Presidente, dal Prof. Ezio Ferroglio, Amministratore Economo, dal prof. Gaetano Oliva, Segretario Generale, oltreche dai componenti Proff.ri Paolo Ciaramella, Giuseppe Iovane, Giovanni Lacalandra, Serenella Papparella e Giuseppe Radaelli, che insieme all'Assemblea dei Presidenti delle Società governerà per ilprossimo futuro la Federazione. Negli anni passati ci eravamo proposti di attuare per la SISVET un profondo rinnovamento volto principalmente a creare le condizioni che permettessero alla Società di costituire una sorta di collante tra le varie componenti del mondo delle Scienze Veterinarie, cercando di rafforzarne il ruolo "politico" anche fuori dello stretto ambito Accademico. Alcuni degli obiettivi sono stati raggiunti altri necessitano ancora di ulteriore definizione. Compito del nuovo comitato direttivo sarà quello di confermare quanto raggiunto e mettere le premesse per la realizzazione di nuovi traguardi in collaborazione con la Conferenza dei Direttori dei Dipartimenti di Medicina Veterinaria, con i Dipartimenti Universitari, con il Ministero dell'Istruzione, Università e Ricerca Scientifica, con il Comitato

Universitario Nazionale, con il Ministero della Salute, con la Veterinaria Pubblica (Istituti Zooprofilattici, ASL), con la Federazione Nazionale dei Medici Veterinari e con Sindacati, entità le cui finalità spesso coincidono e necessitano sempre più di integrazione e sinergia. Ulteriore particolare attenzione sarà dedicata ai giovani, ai rapporti internazionali e ad una maggiore integrazione tra la Federazione e le altre Società Scientifiche non accademiche e della libera professione per creare veramente le condizioni di una Veterinaria Unica. Primo banco di prova della nuova Federazione sarà il prossimo Congresso che si terrà ad Olbia dal 19 al 22 di Giugno 2019 il cui programma scientifico, concordato con il nuovo Comitato Scientifico, coordinato dalla Prof.ssa Maria Laura Bacci, dovrà essere arricchito da contributi frutto dell'impegno dei nostri migliori ricercatori con l'obiettivo di innalzare ulteriormente il trend, in costante crescita negli ultimi anni, sia in termini numerici che di qualità. In questo momento di transizione corre l'obbligo di ringraziare il Comitato direttivo e il Comitato scientifico che ci hanno preceduti e che tanto hanno fatto per il raggiungimento dei nuovi traguardi. In particolare, desidero ringraziare il prof. Bartolo Biolatti che fortunatamente continuerà a sostenerci con la sua esperienza, competenza e dedizione, nel suo nuovo ruolo istituzionale di *Past President*.
Un in bocca al lupo a tutti!

Antonio Crovace
Presidente SISVet



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PROGRAMMA GENERALE

ABSTRACT

WORKSHOPS

and

Main Lectures

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

Workshop 1/ECM

Mercoledì, 19 Giugno 2019

IL SISTEMA IMMUNITARIO E LA DIETA

In collaborazione con **ARNA**

Con il Patrocinio ***SINU****: Società Italiana di Nutrizione Umana*

Responsabile Scientifico: Prof. G. Bertoni (Presidente ARNA)

14.00	**Saluto e patto d'aula**
14.30	Acidi grassi omega 6 e omega 3 e loro ruolo nelle risposte immunitarie **Valerio Chiurchiù** *Università degli Studi di Roma*
15.00	La dieta mediterranea: gli effetti funzionali dell'olio extravergine di oliva **Lino Natale Frega, Deborah Pacetti** *Università Politecnico delle Marche*
15.30	Alimenti, colesterolo e ossidi del colesterolo: immunità e infiammazione **Giovanni Lerker, Massimo Cocchi** *Università degli Studi di Bologna*
16.00	La dieta come determinante nei processi di infiammazione: effetti positivi e negativi **Francesca Danesi** *Università degli Studi di Bologna*
16.30	Pausa caffè
17.00	L'aumento delle patologie legate al glutine può essere attribuito al miglioramento genetico del grano? **Pasquale De Vita** *CREA*
17.30	Come valorizzare gli alimenti dal punto di vista nutrizionale: dai "*claim*" al digitale **Claudio Truzzi** *METRO Cash and Carry*
18.00	Test finale

Omega-6 and Omega-3 fatty acids and their role in immune responses

Valerio Chiurchiù e **Mauro Maccarrone**

Università Campus Bio-Medico di Roma

Inflammation is an immune response that works as a contained fire that is pre-emptively sparked as a defensive process during infections or upon any kind of tissue insult, and that is spontaneously extinguished after elimination or termination of the damage. The cells involved in this process are indeed immune cells, both of the innate (i.e. neutrophils, monocytes/macrophages) and adaptive branch (i.e. T and B lymphocytes) of immunity and that together interact and cooperate to effectively remove the insult and also to restore tissue homeostasis. However, a persistent and uncontrolled activation of the immune system act as a wildfire that promote chronic inflammation, unresolved tissue damage and, eventually, chronic diseases. Of note, all immune processes are governed by a wide network of soluble mediators, among which endogenous omega 6 and omega 3 fatty acids and their metabolites, metabolized by most immune cells, are arguably the most important biologically active mediators not only to be implicated in all phases of inflammation but also to be involved in the regulation and fine-tuning of its course and cessation. The main families of omega 6- and omega 3-derived fatty acids that control immune responses are classical eicosanoids, specialized pro-resolving mediators (SPMs), lysoglycerophospholipids/sphingolipids and endocannabinoids (eCBs). These are all generated from ω-6 or ω-3 essential polyunsaturated fatty acids precursors (arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid), that upon dietary assumption are esterified into membrane lipids and act by binding to and activating specific G protein-coupled receptors. In the event of tissue insults or infections, innate immune cells, such as granulocytes and monocytes/macrophages, are recruited to the damaged site and rapidly generate classical eicosanoids, the class of lipid mediators that is responsible for acute inflammation (or angiophlogosis) characterized by the so-called “cardinal signs” of inflammation: redness, heat, swelling, pain, and loss of function. Classical eicosanoids are thus highly pro-inflammatory and ignite the fire during inflammation, with the aim of removing injurious stimuli, a fire that, however, needs to be self-limiting and, eventually, promptly extinguished upon cessation or elimination of the noxious stimulus. During the last process, referred to as “resolution of inflammation” or catabasis, the very same innate immune cells recruited in the inflammatory milieu, where they produce classical eicosanoids, undergo a temporal lipid mediator class switch and start producing another class of bioactive lipids, the newly discovered SPMs. These lipids actively terminate inflammation and drive the restoration of full tissue homeostasis by activating

the signs of resolution: removal, relief, restoration, regeneration, and remission. When the fire of inflammation is not properly extinguished, due to impaired resolution, it turns into chronic inflammation (or histophlogosis), resulting in aberrant tissue remodeling and organ dysfunction. In this context, the outcome of inflammation depends also on the other two families of bioactive lipids, i.e., lysoglycerophospholipids/sphingolipids and eCBs, which regulate numerous cellular processes that are important for triggering those mechanisms that underlie cell and tissue adaption to inflammatory events. These processes demonstrate the importance of a balanced diet between omega 6 and omega 3 fatty acids in the regulation of several immune processes that underlie the outcomes of inflammation and the underlying pathological conditions derived from an impairment in their assumption, metabolism and function.

HOW TO VALORIZE FOOD FROM A NUTRITIONAL POINT OF VIEW: FROM CLAIMS TO DIGITAL

Claudio Truzzi

Head of METRO Italia Cash and Carry S.p.A. Via XXV Aprile, 25 20097 San Donato Milanese (MI)

In Europe, the proportion of overweight people remains high and about 7% of European health spending is used in the treatment diseases related to obesity - such as diabetes, high blood pressure, cardiovascular diseases, etc. overweight is attributable to a variety of factors that are related to lifestyle; similarly, another factor is represented by a diet that does not consider the nutritional quality and the complex of foods consumed during the day. Therefore, through nutritional and communication policies it is possible to change eating habits, directing consumers towards more correct and conscious choices about food. The only reformulation of food from a nutritional point of view, leaving the sensory characteristics and the shelf life unaltered, is not sufficient, it is necessary to give visibility and transparency to the characteristics of the reformulated product. EC Reg. 1924/2006 harmonizes the claims, however to avoid that the information and communication work is not in vain, we need a good innovative system and solutions that are more consistent with the market, such as fast, sustainable digital systems that allow us to measure feedback from end users in real time. E-commerce in Italy has been growing steadily for several years: online purchases are always higher and the turnover generated exponentially increases. The Italian market continues its growth also in 2018 with a value of online purchases that will reach 27 billion at the end of the year (+15% compared to 2017). The Food & Grocery sector in particular is considered one of the most strategic with an increase of +34%, (from € 0.83 billion in 2017 to € 1.1 billion in 2018). Metro Italia, in the "quality" section of its website, deals the issues related to: supply chains, certifications, HACCP, safety data sheets and product recall. Through these insights, Metro Italia sensitizes its customers to all the controls that are implemented throughout the entire chain in order to guarantee a high quality standard of the raw material and of the finished product both from a sensorial, microbiological and nutritional point of view. In particular, the nutritional aspect this year will begin a new project in collaboration with ARNA aimed at promoting products with particular nutritional claims that will be included in specific menus and proposed in restaurants. The purpose of this project is to educate our customers on a healthy diet, thanks to specific in-depth information on the site, and to promote a balanced diet without give up on taste.

Workshop 2

Mercoledì, 19 Giugno 2019

VETTORI E PARASSITI

In collaborazione con **SOIPA**

Moderatori:

Prof. Fabrizio Bruschi (Università di Pisa)
Prof. Ezio Ferroglio (Università degli Studi di Torino)

14.30	**Vector-borne pathogens of dogs and cats: transmission times and disease control** Domenico Otranto ***Università degli Studi di Bari "Aldo Moro"***
15.00	Peaks and troughs in vector control within the global fight against malaria **Marco Pombi** *Università degli Studi di Roma "La Sapienza"*
15.30	A national survey of ixodidae ticks and transmitted pathogens in dogs in Italy **Maria Paola Maurelli** *Università degli studi di Napoli "Federico II"* **Stefania Zanet** *Università degli Studi di Torino*
16.00	Microbiota of insect vectors: a tool for diseases control? **Guido Favia** *Università degli Studi Camerino* **Paolo Rossi** *Università degli Studi Camerino*

Vector-borne pathogens of dogs ad cats: transmission times and disease control

Domenico Otranto

Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Valenzano, Italy

Feeding is an important concept to be investigated, at cellular, individual and even population level, in any parasite-host interaction, while this interaction is usually optimised by the parasite in the attempt to survive, without causing major harm to the host. Vector-borne pathogens have evolved a close relationship with blood feeding arthropod ectoparasites by exploiting a huge variety of vector transmission routes. Indeed, several bacteria, viruses, protozoa and helminths have taken advantage of the biology of blood feeders to ensure their transmission and distribution to receptive hosts, both at individual and population level. Therefore, the arrays of vector-borne transmission pathways represent one of the most complex examples of interaction among pathogens, hosts and vectors, evolved under the pressure of a range of ecological and environmental drivers. Undoubtedly, knowledge of feeding habits of arthropod vectors, as well as the array of ecological and environmental factors influencing the interactions between them, the pathogens transmitted and the hosts, is of paramount importance in medical and veterinary medicine. This is not only a fascinating field of study in parasitology, but it is pivotal for exploring future strategies for controlling vector-borne diseases (VBDs) and for understanding the reasons of some failure to achieve control (e.g., vaccines against arthropods). Though a better understanding of the biology, mechanisms and timing of pathogen transmission could provide interesting clues for the control of VBDs, information about these modalities is still limited to laboratory reports. Depending on the compound used, alone or in combination, their action may i) prevent attachement (before it starts – repellence through contact); ii) disrupt contact between the arthropod parasite and the host (also referred to as “expellence”); iii) cause the death of the arthropod parasite after the blood begins (killing effect); iiv) interfere with egg fertility and development of off-host life-cycle stages (growth inhibition). The fast speed of kill exerted by systemic isoxozaline, as well as the repellent effect of pyrethroids have renewed the interest of the scientific community and pharmaceutical companies towards reducing the burden of vector borne diseases under field conditions. However, endosymbionts and vaccines targeting arthropod or pathogen antigens should be further investigated as control strategies towards the goal of achieving an effective integrated strategy for vector-borne diseases.

Otranto, D., 2018. Arthropod-borne pathogens of dogs and cats: From pathways and times of transmission to disease control. Vet. Parasitol. 251, 68-77.

A National survey of Ixodidae ticks and transmitted pathogens in dogs in Italy

Stefania Zanet (1), Elena Battisti (1), Paola Pepe (2), Maria Elena Morgoglione (2), Lavinia Ciuca (2), Liliana Colombo (3), Dimitris Countouris (1), Ezio Ferroglio (1), Laura Rinaldi (2), Giuseppe Cringoli (2), Maria Paola Maurelli (2)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali. (3) MSD Animal Health, Milano

The Ixodidae ticks can transmit viruses, bacteria, protozoa and helminths to animals, as well as humans, causing a wide variety of infections commonly referred to as tick-borne diseases (TBDs)[1]. Dogs, in particular may be useful as sentinel for monitoring tick population distribution and tick-transmitted pathogens (TPs)[2]. The aim of this study was to conduct a national survey of ticks and TPs distribution in owned dogs. In particular, detection of TPs was focused on protozoa belonging to the genera *Babesia* and *Theileria*, on bacteria belonging to the family Anaplasmataceae and to *Borrelia burgdorferi* sensu lato complex. The TPs were chosen due to their relevance for human and/or animal health.

Over a period of 20 months, 153 veterinary practices, from 64 Italian provinces, were asked to examine, for the presence of ticks, 5 different dogs per month *at random* and to complete a questionnaire for each enrolled dog. Differences in tick infestation associated with: sex, age and hair length; the dog's habitat (indoor or outdoor/kennel) and environment (urban or rural/sylvatic) were evaluated. The attachment site of ticks on the dog was also recorded. The questionnaire included also information regarding product used as ectoparasiticide, date of last treatment and date of sampling. Ticks were morphologically identified to species level and divided for DNA extraction into pools composed by specimens collected from the same dog and homogeneous for species, developmental stage, sex and engorgement status. For detection of TPs, a semi-nested PCR, targeting the V4 region of the 18S rDNA, was used for *Babesia* spp. and *Theileria* spp.[3], while PCRs targeting the 16S rDNA and the *Fla* gene were performed respectively for Anaplasmataceae[4] and for *B. burgdorferi* s.l.[5]. Positive samples were sequenced for species identification. The results were expressed as a minimum infection rate (MIR) [6].

A total of 3026 dogs were examined and 1383 (45.7%) were carrying at least one tick. Overall, 2439 tick samples were collected and a total of 14 species were identified, belonging to: *Rhipicephalus* (63.8%), *Ixodes* (36.7%), *Dermacentor* (0.7%) and *Haemaphysalis* (0.2%).

Rhipicephalus sanguineus group were the most predominant (63.6%), followed by *Ixodes ricinus* (30.6%) and *I. hexagonus* (5.6%). Twenty-four dogs had mixed tick infestations. Long-haired dogs had a higher tick infestation risk as dogs with outdoor and rural/sylvatic lifestyles. Finally, ectoparasiticide treatments were found significantly protective against tick infestation especially orally administered formulations. For detection of TPs, 1583 pools of ticks were analyzed. DNA of *Babesia* and *Theileria* was detected in 437 pools (MIR=27.61%) from 397 dogs. A significant higher prevalence was found in *I. ricinus*. Dogs living in urban environment had a significantly reduced risk of being parasitized by *Babesia/Theileria* infected ticks. Sequencing allowed to determine the presence of at least 9 species of the genus *Babesia* and 5 species belonging to the genus *Theileria*. The zoonotic *B. venatorum* was the most prevalent species (MIR=7.52%). The zoonotic *B. microti* group (MIR= 2.40%) and the enzootic *B. microti* "Munich-type" (MIR=0.25%) were also found. *B. canis* and *B. vogeli* that have the domestic dog as primary *reservoir* host were recorded with a lower prevalence (MIR= 0.38%; MIR=0.63%). DNA of *Anaplasma* and *Ehrlichia* was detected in 165 pools (MIR=10.42%) from 160 dogs. A significant higher prevalence was found in *I. ricinus*, while ticks of the genus *Rhipicephalus* were significantly less infected. The zoonotic *A. phagocytophilum* was identified in 80 pools (MIR=5.05%), while *A. platys* and *E. canis* were detected in 13 (MIR=0.82%) and 21 (MIR= 1.33%) pools, respectively. A higher infection prevalence, although not statistically significant, was found in ticks of dogs attending forest than in those from urban and rural environments. DNA of *B. burgdorferi* s.l. was detected in 10 pools (MIR=0.62%) from 10 dogs. Sequencing permitted to identify *B. burgdorferi* s.l. in 6 pools (MIR=0.38%) and *B. afzelii* in 4 pools (MIR=0.25%). No statistically significant differences were reported among tick genera or species due to the low number of positive samples. All dogs with *B. burgdorferi* s.l. positive ticks are housed inside with access to the garden. This study provides a comprehensive spatial coverage of the species of ticks and TPs in our country, useful to develop and plan effective control measures.

[1] Otranto et al. Ticks infesting humans in Italy and associated pathogens. Parasit. Vectors., 7:328, 2014. [2] Cardoso et al.. Molecular investigation of tick-borne pathogens in dogs from Luanda, Angola. Parasit. Vectors. 9: 1–6, 2016. [3] Zanet et al. Horses infected by Piroplasms different from *Babesia caballi* and *Theileria equi*: species identification and risk factors analysis in Italy. Vet. Parasitol. 236: 38-41, 2017. [4] Goodman et al. Direct cultivation of the causative agent of human granulocytic ehrlichiosis. N. Engl. J. Med. 334:209–15, 1996. [5] Skotarczak et al. Coexistence DNA of *Borrelia burgdorferi* sensu lato and *Babesia microti* in *Ixodes ricinus* ticks from northwestern Poland. Ann. Agric. Environ. Med. 9: 25-28, 2002. [6] Kramer et al. Detection of the agents of Human Ehrlichioses in Ixodid ticks from California. Am. J. Trop. Med. Hyg., 60: 62–65, 1999.

Workshop 3

SOIPA

Giovedì, 20 Giugno 2019

STANDARD OPERATING PROCEDURE (SOP) PER LO SVILUPPO E IDENTIFICAZIONE DELLE LARVE DEI NEMATODI GASTRO-INTESTINALI (NGI) DEGLI OVINI

Moderatori:

Prof. Giuseppe Cringoli (Università degli Studi di Napoli)
Prof. Antonio Scala (Università degli Studi di Sassari)

8.30	Identificazione dei generi dei NGI degli ovini e applicazioni pratiche **Antonio Varcasia** *Università degli Studi di Sassari*
8.50	SOP per l'allestimento delle coprocolture, isolamento e identificazione delle larve dei NGI degli ovini **Antonio Bosco** *Università degli Studi di Napoli*
9.05	*Performances* delle tecniche biomolecolari per l'identificazione delle larve dei NGI degli ovini **Philippe Jacquiet** *École Nationale Vétérinaire de Toulouse, Francia*
9.40	L'esperienza di un gruppo di lavoro CIRPAR **Manuela Diaferia** *Università di Perugia;* **Riccardo Lia** *Università di Bari "Aldo Moro";* **Claudia Tamponi** *Università degli Studi di Sassari*; **Gabriella Gaglio** *Università di Messina*; **Barbara Paoletti** *Università di Teramo*; **Alessandra Amadesi** *Università degli Studi di Napoli*

Gastro-Intestinal Nematodes in sheep: where are we going?

Antonio Varcasia

Università degli Studi di Sassari

Gastrointestinal nematodes of sheep are widespread throughout the world and belong to different genera and species. They can be a limiting factor in sheep breeding and for this reason each year monitoring protocols and anthelmintic treatment are usually performed by practicioners and farmers. The interest on these parasites has definitely increased following the numerous reports of anthelmintic resistance, especially in some regions of France and the United Kingdom. What is the state of the art on this topic? Is it possible to make a specific diagnosis? Can it be useful for clinical purposes and to prevent drug resistance? Are the protocols available adequate to the Italian epidemiological situation? These are some of the questions we will try to answer in this brief report and in the following symposium.

Workshop 6

Giovedì, 20 Giugno 2019

AGGIORNAMENTI IN MEDICINA D'URGENZA DEI PICCOLI ANIMALI

Moderatore:

Prof. Francesco Staffieri (Università degli Studi di Bari)

15.30	**Sepsi e terapia antibatterica nei piccoli animali** ***Massimo Giunti*** *Università degli Studi di Bologna*
16.00	**Valutazione del paziente in emergenza: triage, score di gravità e indici prognostici** ***Angela Briganti*** *Università di Pisa*
16.30	**Utilità dell'ecografia polmonare e dell'ecocardiografia nelle emergenze cardiologiche del cane** ***Tommaso Vezzosi*** *Università di Pisa*
17.00	**Gestione delle emergenze nei cuccioli e gattini neonati** ***Maria Cristina Veronesi*** *Università degli Studi di Milano*
17.30	**Emostasi: una cascata di eventi da Giulio Bizzozero ad oggi** ***Antonio Borrelli*** *Università degli Studi di Torino*
18.00	**Shock settico: identificazione e trattamento** ***Roberta Troia*** *Università degli Studi di Bologna*
18.30	**Approccio clinico e terapeutico allo stato di male epilettico** ***Gualtiero Gandini*** *Università degli Studi di Bologna*

Emergencies management in newborn puppies and kittens

Maria Cristina Veronesi

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria

Because of the multisystemic immaturity and the metabolic instability of newborn dogs and cats, very often puppies and kittens require an emergency management. However, due to their small size, and anatomic and phisiologic characteristics, the management of emergency in newborns is a challenge for the clinician. Emergency could be required at birth, or during the neonatal period when diseases, or prolonged starvation, can affect the chance of survival. The most frequent conditions, needing emergencies, can be hypoxia, hypothermia, hypoglycemia, dehydration, bacterial infections [1].

At birth, hypoxia can predispose to bradicardia, affecting the cascade of neonatal adaptation. Therefore, when, at birth, a puppy is not breathing efficiently and does not display clear vocalizations within 1 minute after birth, respiratory assistance is required. Although a short period of hypoxia is physiologic in newborns, it must be contrasted stimulating respiration, only after having removed fluids from the airways. Oxygen can be administered by facial masks, while laringeal masks should be restricted to large size newborns. Repiratory analectics and xantines should be administered only to contrast apneas and not to improve oxygenation. The cardio-circulatory efficiency in newborns depends on respiration, with heart contractility and rate depending on tissues oxigenation. However, when necessary, epinephrine can be administered in the attempt to improve heart function. Although the initial hypothermia protectsthe brain from hypoxia, the gradual resolution of hypothermia must also be performed to contrast hypoxia [2].

During the neonatal period diseases, unfavorable environmental conditions, and starvation, can lead soon to at life-threating conditions, often concurrent, developing because of the multi-systemic immaturity, metabolic instability, scarce energy and fat stores and the high surface/mass ratio. Newborns contain a great quota of extracellular water, maintained by the regular milk intake. The key points for fluid therapy must be adapted to the newborns, in which the simple evaluation of the dehydration deficit can be difficult, because of the neonatal characteristics, even if, very often, the water deficit exceeds the 5-10%, and it must be corrected by infusion of crystalloids, as first-line fluids choice. Frequently, because of the concurrency of dehydration with hypoglycemia, the fluid administration must also provide energy, when the patient is hypothermic or not able to suck or swallow efficiently. In these cases, dextrose solutions can be administered.

In newborns, the elective route for fluid administration is the intraosseous one, due to the particular characters of long bones *pars spongiosus*, providing a quick absorption and distribution, similar to the intravenous one. Also, the rehydration monitoring is challenging in newborns, and often rely upon the frequent assessment of body weight and urines characters. When possible, the enteral administration of milk can be used to correct low degrees of dehydration, providing also sugar support. Although enteral feeding by nursing represents the best choice, sick or weak neonates frequently require oro-gastric tube-feeding, with diameter of tube adapted to the size of the neonate. Volume and frequency of feeding must be adapted to the specific condition, considering the age and the body weight of the neonate [1,2].
Hypothermia must be corrected slowly, with an increasing body temperature of about 1° C within 1 hour. When bacterial infections are detected or suspected, antibiotic treatments must be immediately initiated, possibly on the base of bacterial culture and antibiotic sensitivity test results. It must however take in consideration the list of antibiotics useful in newborns and that the exact dosages and frequencies of administration are not well understood because of the lacking knowledge about canine and feline neonatal pharmacodynamic and pharmacokinetics characteristics [1,2].

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Workshop 7/ECM

Giovedì, 20 Giugno 2019

LA FILIERA DEL LATTE OVINO E IL TERRITORIO: DALLA PRODUZIONE ALLA TRASFORMAZIONE

Responsabile Scientifico:

Prof. Sanna Passino Eraldo (Università degli Studi di Sassari)

Prof. Mario Colombo (Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia)

15.30	**Saluto delle Autorità**
15.45	**Tecnologia della trasformazione** ***Antonio Pirisi*** *Agenzia Regionale della Ricerca, Sardegna*
16.15	**Sanità della mammella e qualità del latte** ***Agnese Cannas*** *Istituto Zooprofilattico della Sardegna*
16.45	**Caratteristiche nutrizionali del latte ovino** ***Marcello Mele*** *Università di Pisa*
Pausa Caffè	
17.30	**Quale organizzazione di filiera per la valorizzazione del comparto ovino sardo?** ***Antonello Carta*** *Agenzia Regionale della Ricerca, Sardegna*
18.00	**Food Safety Management experience and perspectives in the sheepmilk chainin Sardinia (Italy)** ***Enrico Pietro Luigi De Santis*** *Università degli Studi di Sassari*

Food Safety Management experience and perspectives in the sheep milk chain in Sardinia (Italy)

De Santis E.P.L., Scarano C., Spanu C., Piras F.

Università di Sassari

The Food Safety management experience developed in Sardinia, the Italian region that is accounted for highest dairy sheep livestock, milk and cheese productions, could be of interest as a reference for this sector. The regional sheep milk chains framework and specializations, the available economical and technical resources, determine different approaches and performance, also on food safety management and, as consequence, on associated critical issues. The "industrial" chain involves SMEs, private or cooperative, that collect and process a large part of the sheep milk produced in Sardinia. The industrial companies play the leading role on products valorization on the international markets and on the large scale retail distribution. The cheese-making companies management and quality assurance sectors, are responsible to develop food safety programs, that are expected to cover the whole chain. Currently they are strongly engaged on improving their hygienic management, to meet regulatory and voluntary standard requirements. Inspection, audit and verifications by competent authority and certification bodies are mainly focused on hygienic design and procedures, raw materials income and products compliance at plant level. Significant improvement of some hygienic (TBC) and udder health indicators (SCC) in the tank bulk sheep milk occurred in the last 15 years, as a results of the regional programs on farmers training and best practice spreading at farm level. However, enforcement of preventive controls at primary production level is less considered, and the food safety and verifications programs currently provides only a partial involvement of farmers. This approach, together to other conditions, contributes to widen the gap between different links of the sheep milk chain, reducing their coherence, and the awareness of farmers as FBOs and as partners involved on certification process. Improvements on the food safety of the sheep milk chain require a revised and coherent approach of policy makers, companies and stakeholders, to develop more performance-based whole chain food safety management programs, involving farmers on the objectives and adopting new and effective communication strategies. New perspectives require subsidies supporting innovative services, to develop an integrated (also including welfare and animal health), preventive approach at farm level, collecting certifiable evidence. E.g., Italian Health Ministry provides voluntary program based on qualified veterinary consultants, supported by the ClassyFarm database to strengthened risk based approach for official controls at farm. Official controls should also consider a more holistic approach on the whole chain including FBOs responsibility at farms level, where it is advisable a more risk based and technical approach, less formal, as provided by flexibility principle.

An overview on food safety critical issues on the sheep milk chain and products, should consider that the risks for consumer is currently minimized by the extensive use of milk thermization and pasteurization, the medium or long term cheese ripening, together to considerable improvement of the process control. However some weakness points could be highlighted and give evidence of incorrect procedure or hazard control loss. At farm level some critical procedures are pointed out, as preventive control for some contaminants or residues of veterinary medicines, milk sampling, the management of late term lactation milk, etc. There is still evidence that further efforts are required to the cheese-making plants, e.g. to manage the bacterial growth in the milk storage before processing; to validate milk thermization or pasteurization; to control at plant level environmental condition and related biofilms, or niches harbouring pathogens; the availability of data supporting the shelf-life from a food safety point of view; some critical issues are related to ricotta cheeses, not fully covered by preventive measures. In a more comprehensive approach some issues could be regarded as shared between a large number of the stakeholders of the sheep milk chain. Some critical interventions on food safety could have a huge impact on the competiveness, on the internationalization process and the markets access (e.g. formal risk assessment reports), but requires large amount of resources and their effectiveness makes essential the involvement of joint cheese-making plants network and multidisciplinary contributes.

Workshop 8 / ECM

Sabato, 22 Giugno 2019

In collaborazione con

AIPVET – AIVPA - UNISVET

ALGORITMO DIAGNOSTICO E GESTIONE DELLE PATOLOGIE DEL TRATTO GASTROENTERICO DEL CANE E DEL GATTO

Moderatore:

Prof. Andrea Boari (Università degli Studi di Teramo)

10:30	**Microbiota intestinale e Disbiosis Index, quanto ne sappiamo e come utilizzarli al meglio nella valutazione del paziente enteropatico** Matteo Cerquetella (Università degli Studi di Camerino)
11:00	**La medicina di laboratorio nella diagnostica delle patologie gastrointestinali del cane e del gatto: valutazione "critica" delle potenzialità dei differenti markers** Paola Scarpa (Università degli Studi di Milano)
11:30	**L'esame endoscopico quale importante ausilio diagnostico in corso di gastroenteropatie del cane e del gatto: quando e come eseguirlo valutandone criticamente le potenzialità** Deborah Cattaneo (UNISVET)
12:00	**La valutazione istopatologica del campione bioptico: elementi essenziali del referto ed accortezze che aiutano il clinico nella gestione del paziente** Giacomo Rossi (Università degli Studi di Camerino)
12:30	**Approccio terapeutico alle patologie gastrointestinali del cane: stato dell'arte e nuovi algoritmi** Marco Pietra (Università degli Studi di Bologna)
13:00	Pausa Pranzo

Moderatore:

Prof. Antonio Crovace (Università degli Studi di Bari)

14:30	**Corretto approccio dietetico in corso di patologie gastrointestinali del cane e del gatto: quali ingredienti, che regime scegliere e perché** Eleonora Fusi (Università degli Studi di Milano)
15:00	**Nuove prospettive di impiego del trapianto fecale per il trattamento delle enteropatie del cane e del gatto: lo stato dell'arte ed esperienze personali** Fabrizio Rueca (Università degli Studi di Perugia)
15:30	**Le cellule staminali nella terapia delle enteropatie croniche dei piccoli animali: un approccio molto promettente** Eva Maria Perez Merinos (Universidad de Estremadura)
16:00	**Patologia gastroenterica e problemi comportamentali: può un disturbo comportamentale generare un'enteropatia? Evidenze cliniche e approccio terapeutico** Raffaella Bestonso (AIVPA, Libero Professionista)
16:30	Discussione - Chiusura del Congresso

Stem cells in the treatment of chronic enteropathies in small animals: a very promising approach

Eva Maria Perez Merinos

Universidad de Estremadura

While the established therapy for IBD has been focus on inflammation control and on inmunosupression, the optimal IBD therapy should also enhance proliferation and coordinate remodeling during the healing process. The application of mesenchymal stem cells (MSCs), as alternative treatment for IBD, to achieve this aims is a recent concept Successful preclinical studies using MSCs in animal models of colitis have paved the way for clinical trials. Since then, in human luminal Crohn's disease, intravenous MSCs have been tested in clinical trials demonstrating the prospective efficacy and safety of intravenous infusion of MSCs. In the veterinary field, a study of the use of intravenous MSC therapy in a spontaneous feline entheropaty has shown safety and a positive clinical response.

Accordingly these reports, our starting hypothesis was that allogeneic MSCs could be also beneficial in the treatment of canine IBD.

Therefore, our research was structured in two parts:

Stage 1: aimed and designed to establish the safety and efficacy of the intravenous infusion of allogeneic adipose-derived stem cells (ASCs) on the clinical and laboratorial manifestations of canine IBD.

For this part, criteria inclusion was: persistent (>3 weeks duration) or recurrent gastrointestinal signs, inadequate response to dietary and symptomatic therapies alone (Jergens et al., 2003) and histopathological confirmation of the presence of gastrointestinal inflammatory infiltrates. These dogs had received standard treatment (elimination diet, corticosteroids, antibiotics, antidiarrheal and antiparasitic drugs), which did not result in a full response to treatment or in recurrences. There was at least a 3-week washout period before entering the study.

No acute reactions to the allogenic adipose derived stem cells infusion or side effects were reported in any dog. These patients were monitored for 42 days after transplantation using CIBDAI and CCECAI clinical scoring systems and laboratory biomarkers like folate, cobalamin, albumin and C-reactive protein (CRP). Treatment significantly improved clinical scores, serum albumin, folate and cobalamine levels compared to baseline values. Endoscopic reassessment of these animals showed the improvement of the macroscopic gastrointestinal injury and a slight reduction of the histological gastrointestinal inflammation.

Long-term follow-up of these patients (1 and 3 years after the treatment) have proven the persistency of clinical improvement in all of them and the safety of the therapy in the long run.

Stage 2 (currently under way): aimed to increase the number of treated patients in order to give consistency to the clinical, laboratory and endoscopic results. We also try to deepen in the pathological mechanism of the disease and the effects of this therapy on it. For that reason, different samples are also being taken to determine their impact on oxidative stress, citoquines and microbiota.

For this part, different dosages and treatment regimes are being tested. Also, dogs in this part can be refractory patients that are concurrently receiving inmunosuppressive drugs.

MAIN LECTURE

Histopathological and proteomic characterization of ileal lesions in sheep with active infection by Mycobacterium avium subps. paratuberculosis in multibacillary and paucibacillary disease

Stefano Rocca (1), Maria Filippa Addis (2)

(1) Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari.

(2) Dipartimento di Medicina Veterinaria, Università degli Studi di Milano

Paratuberculosis (PTBC) or Johne's disease is a chronic, contagious enteritis of ruminants caused by Mycobacterium avium subspecies paratuberculosis (MAP) [1]. PTBC is especially relevant in farmed ruminants, including sheep, for the economic consequences caused by increase in mortality, decrease in milk production, and weight loss. In addition, viable MAP can be found in pasteurized milk and milk products with a potential risk of zoonotic transmission. MAP and its role in PTBC have been the subject of numerous studies on disease progression and evolution in cattle, but the pathogenesis of PTBC in sheep requires further elucidation and more effective tools for disease diagnosis and control are still needed [2]. MAP transmission can occur by the fecal–oral route, in utero, and by ingestion of contaminated colostrum or milk. Once the bacterium reaches the intestine, it is taken up by M cells and translocated across the intestinal mucosa, where it is internalized by naive macrophages and can lead to persistent infection. The best diagnostic approach remains the post-mortem evaluation based on the histopathological investigation, which represents the best indicator to confirm PTBC and define its stage. The outcome of MAP infection is a type of widespread granulomatous reaction. A scoring system classifies histological signs into mild lesions represented by small focal granulomata of epithelioid cells limited to the Peyer patches (type 1) or extending to the adjacent mucosa (type 2), and more severe lesions with a multifocal cellular infiltration in mucosal areas not associated with lymphoid tissues and extending into the submucosa, with thickening of the mucosa and atrophy of villi (type 3a). According to Pérez and coworkers [3], symptomatic animals with the end-point disease (clinical disease) present two different types of lesion, lepromatous (type 3b) or tuberculoid (type 3c). Based on the degree of colonization, lesions can also be classified into paucibacillary, with few or no acid-fast bacilli (AFB), and multibacillary, with abundant AFB. Type 1, 2 and 3c lesions are paucibacillary, while type 3b are multibacillary. Type 3a lesions are mainly paucibacillary, but multibacillary patterns can also be present, indicating that a crucial "transition stage" may occur in animals with this type of lesions. Actively infected sheep with the subclinical disease can either remain such for their whole life, acting as MAP

reservoirs and shedders, or develop clinical disease by showing either paucibacillary (3c) or multibacillary (3b) lesions. At present, factors, pathways, stages and dynamics of disease progression are not completely understood [4-5]. Based on current knowledge, PTBC progresses as follows:

• subclinical phase lasting from 6 months to 4 years, with dissemination of the bacterium (process active even in the absence of obvious symptoms), and decline in production;

• paucibacillary phase (evident in sheep and can remain so for the entire duration of life);

• symptomatic multibacillary phase (typical in cattle where it generally represents the evolution of the paucibacillary phase; in sheep it can appear as such immediately).

Subclinical infections are the real challenge, and obtaining a certain early diagnosis is fundamental. A careful histopathological analysis of multi and paucibacillary subjects associated with a clinical examination of the flock can offer useful hints for innovative diagnostic strategies on categories of asymptomatic subjects. Omics approaches can significantly help in understanding the differences among disease stages. Specifically, shotgun proteomics can decipher host and pathogen protein repertoires expressed in the context of a naturally occurring infection. When compared with gene expression approaches, information is more reliable, since some proteins are regulated at the translational or post-translational level. In addition, proteomics can reveal changes in tissue localization which can then be further investigated and validated by histological studies. A comparative analysis of healthy ileal tissues with tissues actively infected with MAP, both in the clinical and in the subclinical paucibacillary forms, can provide novel data on host-pathogen interactions and uncover specific protein patterns linked to disease progression to support histopathological diagnosis. By integrating shotgun proteomics with molecular and histopathological procedures, we carried out a detailed characterization of tissue protein alterations induced by MAP in sheep ileal tissues [6-7]. This generated a detailed picture of the protein abundance changes occurring along disease progression. New data on pathological processes and biochemical pathways involved in disease have been collected, and several host proteins that are absent in healthy tissues but abundantly present in MAP-infected tissues have been identified, with abundance levels or tissue and cell localizations relating to severity. These marker proteins can improve lesion characterization and staging with classical histopathological and immunohistochemical approaches, support the validation of studies carried out with other omic approaches or by means of *in vivo* and *in vitro* experiments, and open new perspectives for developing new diagnosis and prevention tools. Adding to this, we have identified several MAP proteins expressed during natural infection *in vivo*, possibly with a role in pathogenicity. Our presentation will summarize the current knowledge on sheep paratuberculosis, illustrate our combined research approach, present its results and discuss their implications and perspectives.

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2. Arsenault RJ, Maattanen P, Daigle J, Potter A, Griebel P, Napper S (2014) From mouth to macrophage: mechanisms of innate immune subversion by Mycobacterium avium subsp. paratuberculosis. Vet Res 45:54
3. Pérez V, García Marín JF, Badiola JJ (1996) Description and classification of different types of lesion associated with natural paratuberculosis infection in sheep. J Comp Pathol 114:107–122
4. Marquetoux N, Mitchell R, Ridler A, Heuer C, Wilson P (2018) A synthesis of the patho-physiology of Mycobacterium avium subspecies paratuberculosis infection in sheep to inform mathematical modelling of ovine paratuberculosis. Vet Res 49:27
5. Whittington RJ, Begg DJ, de Silva K, Purdie AC, Dhand NK, Plain KM (2017) Case definition terminology for paratuberculosis (Johne's disease). BMC Vet Res 13:328
6. Pisanu S, Cubeddu T, Uzzau S, Rocca S, Addis MF (2017). Proteomic changes in the ileum of sheep infected with Mycobacterium avium subspecies paratuberculosis. Vet J. 219:1-3
7. Pisanu S, Cubeddu T, Cacciotto C, Pilicchi Y, Pagnozzi D, Uzzau S, Rocca S, Addis MF (2018). Characterization of paucibacillary ileal lesions in sheep with subclinical active infection by Mycobacterium avium subsp. paratuberculosis. Vet Res. 49:117.

MAIN LECTURE

THE SIGNIFICANCE OF MICROBIOTA AND MICROBIOME IN VETERINARY REPRODUCTION

Slavcho Mrenoshki, Maria Elena Dell'Aquila, Daniela Mrenoshki, Giovanni Michele Lacalandra

As veterinarians our efforts are focused to protect animal health from pathogenic microorganisms that are usually non-residential of animal's body. But we disregard the fact that animal health is maintained and also can be disrupt by homeostasis or dysbiosis of resident commensal or mutualistic microorganisms *(the microbiota)*. The sum of microbiota, their genes and metabolites *(the microbiome)*, effects the residential body organs or systems leading to their normal functioning or provoke pathologic conditions; in addition, the locally produced metabolites by circulation effect distant organs in positive or negative way too. Such associations between microbiota and health is increasingly documented in humans as well in animals and veterinary medicine. Although the majority of research is focused on gut microbiota, the significance and interest for microbiomes of other organs and systems is growing too. This literature review is mostly focused on the influence of microbiome in reproductive health and assisted reproduction technologies, with aim to introduce and motivate veterinarians and researches to consider the reproductive microbiota/microbiome as significant aspect in their professional activities.

MAIN LECTURE

Biomolecular techniques for the identification of NGI larvae of sheep

Milhes M., Bordes L., Grisez C., Prevot F., Jacquiet P.

UMT Santé des Petits Ruminants, Ecole Nationale Vétérinaire de Toulouse

The technique of larval culture is required to differentiate gastrointestinal nematode species in sheep. Despite several contributions, the species identification of third stage larvae (L3s) has intrinsic limitations such as the inability to unequivocally identify and differentiate particular genera and/or species especially *Teladorsagia* and *Trichostrongylus*. There have been significant advances in molecular methods for the genus and or species-specific identification of gastrointestinal nematodes in sheep. The ability to rapidly identify and rank nematodes according to their numerical contributions by a qPCR approach could represent a major advantage over routine methods in the monitoring of anthelmintic resistance in the field. In the present study, we propose a new real-time PCR approach to identify the third stage larvae of the three main gastrointestinal nematodes genera in sheep (*Haemonchus, Teladorsagia* and *Trichostrongylus*). Thanks to LNA technology, the specificity of this molecular tool was confirmed on monospecific starins and on adult worms collected in slaughterhouse. Moreover, a quantitative approach of qPCR is permitting to rank the different genera in order of importance. The different applications of this tool are considered in epidemiology, in exploration of anthelmintic resistance in sheep farms.

MAIN LECTURE

The nanotechnology approach to microbial detection and identification

J.A. Ikonomopoulos
School of Animal Science and Aquaculture, Agricultural Univ. of Athens Botanikos, Athens, Greece

The last two decades of the 20th century was a period during which the polymerase chain reaction (PCR), revolutionized the field of diagnostics. Expectedly the broad application of PCR revealed some of its draw-backs:
Decreasing the minimum limit of detection (MLD) to minute amounts of target-DNA imposed vigorous precautions to avoid the carry-over effect. Additional quality control measures were required to minimize and detect false-negative results generated by the fragmentation of the target-DNA or the presence PCR inhibitors. Effectively the reliable use of PCR proved costly.
In decreasing laboratory diagnostic testing, the use of nanotechnology offers considerable advantages, since the chemical bio-compatibility of nanoparticles combined greatly with the fact that they can be constructed in a variety of sizes. Being by definition smaller than 100nm, nanoparticles can be adjusted in size from that of the width of the double helix of DNA i.e. approximately 1-2 nm, to that of proteins, viruses or the smallest of bacteria, i.e. around 100 nm.
Depending on the properties of the material one is interested to make use of, there are a number of metal or polymer nanoparticles from which to choose. In the last years, nano-diagnostics have focused more on colloidal gold nanoparticles (AuNPs) and cadmium selenide (CdSe) quantum dots (QDs) that are being produced commercially in a robust manner. Hence, the specific nanoparticles exhibit characteristics of stability and performance that is consistent with their use even in routine diagnostic applications, targeting different pathogens and/or their immunogenic footprint.
Colloidal AuNPs range in size from 3 to 100 nm, and exhibit strong size-dependent optical resonance. Photo-activation results to a huge enhancement of their electromagnetic field that causes scattering. The negatively charged AuNPs can be chemically (using in most cases salt) or electrically (using micro-circuits) directed to selective deposition, detectable by change of colour (visual detection) or energy (construction of biosensors). Linkage of two or more properly modified for conjugation AuNPs that will hybridize to adjacent regions of an analyte, such as a nucleotide target, will also result to aggregation due to the decrease of intra-particle distance.

Notably the use of AuNPs for optical detection of analytes offers some unique advantages compared to fluorescent dyes: AuNPs are not prone to photodecomposition, they are not toxic, and most significantly they demonstrate a very precise correlation of their chemical/physical properties to their optical characteristics, which if accurately measured can confer improved MLD and sensitivity.

QDs are semiconductor crystals with physical dimensions not larger than a few nanometers. CdSe nanoparticles are already becoming increasingly popular, especially around the size range of 2-6 nm, which makes them dimensionally more compatible with nucleic acid and proteins. When a photon of visible light hits such a semiconductor some of their electrons are excited into higher energy states. A photon with a frequency that is characteristic of the semiconductor is emitted when the electrons return to their ground state. The ability of QDs to exhibit size-dependent fluoresce-emission wavelengths is the foundation of their use as biodetectors, which can be greatly improved by the incorporation in their outer surface of a “shell” such as ZnS or silica. Notably the latter can be easily linked to bioconjugators such as avidin, which allows incorporation of these nanaparticles into several already established diagnostic assays. In summary the QD shells can protect the core from oxidation, they minimize or even eliminate core-derived toxicity and they improve water solubility. CdSe may be size-tuned very accurately and thus it can be used for the simultaneous detection of multiple targets with a single excitation wavelength.

ORAL COMMUNICATIONS

AIPVET

DIFFERENCES IN MICRO RNA EXPRESSION BETWEEN MAST CELL TUMOUR AND HEALTHY ADJACENT TISSUE

Valentina Zamarian (1), Damiano Stefanello (1), Roberta Ferrari (1), Valeria Grieco (1), Giulietta Minozzi (1), Fabrizio Ceciliani (1), Raffaele Calogero (2), Maddalena Arigoni (2), Cristina Lecchi (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria. (2) Università degli Studi di Torino, Dipartimento di Biotecnologie Molecolari e Scienze per la Salute

Mast Cell Tumour (MCT) is the most common skin neoplasm in dogs, representing up to 20% of canine skin tumors. It arises from mast cells in cutaneous and subcutaneous tissue. The specific pathogenesis is not well understood and the etiology is still unknown [1,2]. The clinical behavior is very variable, making complex the prognosis. For this reason, an investigation on molecular profile of MCT can improve the knowledge about the behavior and support the diagnosis. MicroRNAs (miRNAs) are post-transcriptional modulators, involved in the processes of tumor progression and metastasis; thus, they are candidate biomarkers for early diagnosis [3, 4]. A retrospective study on Formalin Fixed and Paraffin Embedded (FFPE) canine MCT samples was carried out. Using a brightfield microscope, samples of tumors and, as intra-patient control, were collected with a biopsy punch. In order to obtain the complete miRNomic profile, a small-RNA sequencing using the Illumina platform was performed on 18 samples, including 7 healthy and 11 MCTs. Differentially expressed miRNAs (DE-miRNAs) were validated on all sequenced 18 samples and on other 13 MCTs and tumor-adjacent normal counterpart samples by quantitative PCR (qPCR). Gene ontology (GO) and KEGG Pathway analysis were exploited to investigate the biological functions of DE-miRNAs using miRNet. Sixty-three DE-miRNAs, 18 up- and 45 down-regulated, were detected in tumor compared to control margins. Nine DE-miRNAs were selected for further qPCR validation, showing that 2 were up- and 3 were down-regulated in tumor compared to control; 4 were not statistically different. In particular, miR-379 and miR-21 were up-regulated in MCT (P=0.0005, $\log2FC_{MCT/Healthy}$ = 2.61; P=0.004, $\log2FC_{MCT/Healthy}$ = 2.84, respectively) and miR-885, miR-338 and miR-92a were down-regulated (P=0.008, $\log2FC_{MCT/Healthy}$ = -2.53; P=0.025, $\log2FC_{MCT/Healthy}$ = -0.86; P=0.021, $\log2FC_{MCT/Healthy}$ = -0.78, respectively). GO analysis reveals that up-regulated miRNAs play a role in negative regulation of apoptosis, programmed cell dead and in myeloid cell differentiation. Down-regulated miRNAs are involved in RNA catabolism and metabolism, G1/S transition and negative regulation of transcription. KEGG analysis shows that up-regulated miRNAs are involved in MAPK signaling pathway and apoptosis and down-regulated miRNAs in cell cycle, p53 and TGF-beta signaling pathways. Both are involved in cancer pathways. In conclusion, results highlight that miRNomic profile drastically change in tumour microenvironment compared with healthy tissue, but in order to obtain a more specific profile an in depth molecular characterization is necessary.

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MACHINE LEARNING APPLICATIONS IN CANINE MAMMARY CANCER: A RETROSPECTIVE STUDY USING ELECTRONIC RECORDS (CANCER REGISTRY)

Andrea Gabrieli (1), Elisabetta Antuofermo (2), Pierfranco Demontis (2), Giovanni P. Burrai (1)

(1) Department of Veterinary Medicine, University of Sassari, Italy. (2) Department of Chemistry and Pharmacy, University of Sassari, Italy.

Cancer is the leading cause of death in companion animals, and mammary tumors (MTs), the most common neoplasm in female dog, represents a serious issue in worldwide veterinary practice and is a matter of concern for both oncologists and pathologists [1]. With the advent of new technologies, large amounts of cancer-related data, mainly derived from electronic medical records such as cancer registries, have been collected and are available to the medical research community. Therefore, one of the main problems in translating this massive amount of big data into relevant scientific information resides in the data analysis itself [2]. Machine learning (ML) is a branch of artificial intelligence research that combines statistical, probabilistic and optimization tools and can be employed to classify and categorize new data from past experiences [3]. Furthermore, ML techniques have been used in human healthcare systems to develop various cancer prediction models improving the accuracy of cancer detection, cancer outcome and the overall diagnostic and prognostic performances [4].
Therefore, in this work, three different machine learning techniques, namely Support Vector Machines (SVM), Random Forest (RF) and Stochastic Gradient Boosting (SGB), were compared in order to assess their ability to predict the biological behavior (benign vs malignant) of canine mammary tumors using the breed and the age as features. SVM, RF and SGB models were built on a dataset of 272 MTs and their performances were evaluated considering the area under the receiver operating characteristic curve (AUC), the F-measure and the balanced accuracy. The statistical analysis of the data-set revealed that benign neoplasms are more frequent in small pure breed dogs of younger age, whereas malignant occurred in larger breeds and older dogs ($P<0.05$). Overall, all the ML models investigated showed comparable performances with an AUC, F-measure and a balanced accuracy, in the ranges 0.60-0.75, 0.40-0.68, 0.52-0.71, respectively, meaning that veterinary clinicians can predict the biological behavior of the mammary tumors with good accuracy using only two macro parameters, such as the dog's breed and the age. This study demonstrated, for the first time in veterinary oncology, that the integrated machine learning methods can be a helpful tool in the clinical decision also for canine mammary tumors. Therefore, an extensive survey with an increasing number of patients and with comprehensive clinical-pathological variables, such as tumor size and microscopical features, could substantially improve the overall accuracy of the predictions of MTs.

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TUMOR INFILTRATING LYMPHOCYTES (TILS) IN CANINE MELANOCYTIC TUMORS: AN INVESTIGATION ON B AND T LYMPHOCYTIC POPULATIONS

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The attention on cancer immune environment has been rapidly increasing in recent years, as demonstrated by the growing number of studies on this topic, aimed both at understanding the relationship between neoplastic and immune cells as well as at the identification of immunotherapy targets.[1] Despite being considered one of the most immunogenic tumor types, melanoma can progress in the presence of abundant lymphocytic infiltration, suggesting that the immune response is not able to efficiently control tumor growth.[2] Lately, in human medicine, a distinct subset of B cells has been described and designated as B regulatory cells (Bregs). These cells seem to exert immune-modulatory functions through the secretion of immunosuppressive cytokine, such as TGF-β and IL-10. Breg cells may also promote neoplastic progression and metastasis by converting resting $CD4^+$ T cells into Treg cells.[3] To the authors' knowledge, no studies have been performed to characterize lymphocytic populations within canine melanocytic tumors. Therefore, our current purpose is to preliminarily characterize Tumor Infiltrating Lymphocytes (TILs) in these tumors, and their main B- and T-cell subpopulations. Immunohistochemistry using anti-CD3 and anti-CD20 antibodies was performed on 101 canine melanocytic tumors to evaluate the two main populations of TILs. The results of our study show that tumor-infiltrating lymphocytes are present in a large proportion of canine melanocytic tumors, being more commonly found in oral melanoma than in cutaneous melanoma and melanocytoma ($P=0.001$) and are mainly represented by $CD3^+$. Both TILs populations examined were significantly associated with some negative histologic prognostic factors, such as the mitotic count, the cellular pleomorphism and the percentage of pigmented cells. Remarkably, a high infiltration of $CD20^+$ TILs was associated with tumor-related death ($P<0.001$), presence of metastasis/recurrence ($P<0.001$), shorter overall survival ($P<0.001$) and disease-free time ($P<0.001$). Additionally, a high infiltration of $CD20^+$ TILs was also associated to a higher hazard of death ($P=0.001$) and of developing recurrence/metastasis ($P<0.001$). Our results clearly showed the promising utility of $CD20^+$ TILs as a prognostic marker in canine melanocytic tumors. Therefore, further studies should be performed to confirm these results and fully characterize immune populations within melanocytic tumors. Although further studies should be performed to characterize immune cells, our preliminary data suggest the presence of a presumptive Breg population in canine melanocytic tumors and a potential role of these cells in canine melanomagenesis and progression.

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CARNITINE PALMYTOIL TRANSFERASE 1 A AS NEW DRUGGABLE TARGET FOR TREATMENT OF CANINE MAMMARY TUMORS

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Canine mammary tumors (CMTs) are the most frequently occurring cancer in bitches. CMTs share a similar biologic behavior to that in humans and dogs, due to short lifespan and rapid progression of mammary cancer represent an excellent and valuable model correlate to human breast cancer [1]. Our previous study performed by using immunohistochemistry, western blot and qRT-PCR analysis showed that canine mammary cells and tissues express Carnitine Palmytoil Transferase 1 A (CPT1A), the rate-limiting enzyme of fatty acid oxidation (FAO). FAO has not been fully investigated as glycolysis or glutaminolysis, even though several malignancies (prostate and breast) strongly rely on FAO for their growth and survival [2,3]. Targeting FAO for cancer therapy may be achieved by inhibiting CPT1A. Several irreversible inhibitors of CPT1A (Etomoxir and Perhexiline) have been evaluated both *in vitro* and *in vivo*. Although the promising results on rodents, clinical use of these drugs is avoided due to their serious side effects observed in humans [4]. In the last years, a reversible selective CPT1A inhibitor named ST1326 (Teglicar) has been developed and it is under investigation both in a phase II clinical trial for the treatment of type 2 diabetes and *in vitro* preclinical studies for the treatment of leukemias [5]. For this reasons, in this study we assessed the cytotoxic effects of Etomoxir or ST1326 on canine mammary tumor cell lines (CMT-U27, CMT-U309 and P114). Cytotoxicity studies were performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay whereas western blot analysis was used to assess the effect of CPT1A inhibitors on cell viability. A significant reduced cell viability was observed only in P114 and CMT-U27 cells treated with etomoxir (10-147.59 μM), whereas no effect was observed on CMT-U309. On the contrary, the exposure of ST1326 (1-54.7 μM) markedly decreased cell viability of all three cell lines analyzed. Mechanistically, the effect of CPT1A inhibitors seems to involve MAPK pathway (at least in part by downregulating p-ERK) and an increased apoptotic cell death after drugs exposure (at least in part by downregulating p-AKT). Our results suggest that CPT1A inhibitors exert cytotoxic effects in canine mammary tumor cells and open the avenue for additional therapeutic modalities such as the use of cell metabolism-modifying therapies.

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TOWARD THE DEFINITIVE DIAGNOSIS OF CANINE PLASMA CELL TUMOR: COMBINING IMMUNOHISTOCHEMISTRY AND CHROMOGENIC IN SITU HYBRIDIZATION (CISH)?

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The variety of morphologic features of canine plasma cell tumor (PCT) makes their differentiation from other round cell tumors sometimes challenging. Immunohistochemistry (IHC) for lambda (L) and kappa (K) immunoglobulin (Ig) light chains is often equivocal due to high background staining [1]. Recent findings support that multiple myeloma oncogene 1 (MUM1) specificity for PCT is not as high as traditionally believed [2]. The chromogenic in situ hybridization (CISH) technique for the detection of light chain expression has shown a higher sensitivity compared to IHC in a short PCT series [1]. The aim of our study is to evaluate the diagnostic potential of CISH for Ig light chains in canine PCTs, in conjunction with routinely used IHC markers.
Fifty-four canine round cell tumors (40 cutaneous, 9 mucocutaneous, 5 oral) morphologically compatible with PCTs were submitted to automated CISH with L and K light chains probes and IHC (L light chain; MUM1; CD45; CD3; CD20; Iba-1) performed on the Ventana BenchMarck ULTRA stainer [1]. Thirty-three out of fifty-four (61%) cases showed demonstrable monotypic light chain production by CISH, 31/54 (57%) expressing L and 2/54 (4%) K chain. In the remaining 21/54 (39%) cases, a clear CISH signal was not detectable in tumor cells. IHC confirmed L light chain expression in 26/54 (48%) cases and resulted negative in 15/54 (28%); the remaining 13/54 (24%) cases were not assessable due to high background staining. Fifty-two out of 54 (96%) PCTs showed unequivocal nuclear positivity for MUM1 while in 2/54 (4%) cases positivity was weak and inconstant. The doubtful cases for MUM1 showed clear CISH staining for L light chain. All 54 cases were negative for CD3 and Iba-1; CD45 and CD20 were variably expressed, in 43/54 (80%) cases and 21/54 (39%) cases, respectively. Our study confirms that CISH has a higher sensitivity compared to IHC in detecting light chain expression in canine PCTs and allows an easier interpretation of results. The absence of a clear CISH staining for either light chains in a subset of tumors (which resulted negative or equivocal by IHC for L light chain) may be due to pre-analytical processing and/or a lower light chain production by tumor cells. Based on our data, IHC for MUM1 remains the most sensitive technique for canine PCTs. Nevertheless, CISH for L light chain allowed confirmation of plasma cell origin in two tumors with unclear MUM1 staining. MUM1 expression has also been demonstrated in histiocytic tumors [2]. Therefore, we recommend MUM1 to be always used in conjunction with other markers (i.e. Iba-1). In conclusion, the identification of less differentiated canine PCTs requires the assessment of a panel of IHC markers, with the potential support of CISH for Ig light chains.

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IMMUNOHISTOCHEMICAL AND MOLECULAR ANALYSIS OF C-KIT IN CANINE ORAL MELANOMA PATHOGENESIS

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Canine Oral Melanoma (COM) is one of the most life-threatening disease of older dogs and a potential powerful pre-clinical model for human mucosal melanoma (hMM). Although clinical trials in both dog and human beings showed low or null response to kinase-inhibitors [1], the Proto-Oncogene Receptor Tyrosine Kinase KIT is considered a promising target for anti-cancer therapy. In hMMs, c-KIT has been found constitutively activated as a consequence of point mutations and amplifications. In particular, single nucleotide polymorphisms (SNPs) have been reported in exons 13, 17 and 18 [2]. While amplification of c-KIT has been recently reported in COMs, the mutational landscape has been poorly deepened and its role is far from being fully understood. Twenty formalin-fixed, paraffin-embedded blocks of COMs were selected, their DNA extracted and subjected to array Comparative Genomic Hybridization (aCGH) analysis. Those samples with sufficient residual genomic DNA were submitted to a PCR-amplification with primers designed for exons 13, 17 and 18 and the amplicons obtained were Sanger-sequenced. Immunohistochemical staining (IHC) was performed on 4µm-thick sections with an anti-KIT polyclonal antibody (Dako®), using a mast cell tumor as positive control. The aCGH analysis revealed amplification of the gene c-KIT in 35% on the COMs examined. The PCR-amplification was successfully obtained in 100% of the samples, and the Sanger-sequencing did not reveal SNPs in the specimens, not even in those not affected by c-KIT amplification. The IHC analysis showed a general inconsistent result for c-KIT protein expression. The c-KIT amplification reported in one third of the COMs is confirmed by studies reported by other independent groups. The lack of SNPs in exons 13, 17 and 18 suggests that point mutations are not a common pathway of c-KIT activation in COMs, as reported by studies on exon 11 [3]. Nevertheless, it is noteworthy that no mutation was found in those samples characterized by c-KIT amplification, suggesting a potential mutual-exclusive relationship between the mutational status and the chromosomic aberration in this gene. In contrast with other works [3], IHC positivity did not allow consistent results, even in samples with c-KIT amplification. Although technical yet unrevealed pitfalls may be not ruled out, our data suggest a major involvement of amplification than mutations for c-KIT in COM's pathogenesis. Further studies are still needed to find out if gene amplification always causes protein overexpression, an assumption that is currently strongly debated. Future analysis aimed to identify other possible hidden mechanism of c-KIT activation are under development.

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EXPRESSION OF TRANSFERRIN RECEPTOR IN FELINE MAMMARY TUMOR

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The Transferrin receptors (TFRs) are transmembrane glycoproteins, whose role is to internalize iron ions within cells[1]. TFR1 and TFR2 are two subtypes of TFRs: TFR1 is ubiquitously expressed in generic cells, whereas TFR2 is especially expressed in liver cells [2]. It is known that iron plays crucial roles in various physiological and pathological processes. The disorders of iron metabolism relate with many kinds of diseases including various cancers where TFR1 has been verified to be abnormally expressed in comparison with healthy tissue[3]. In addition, some experimental and clinical drugs and antibodies targeting TFR1 have showed strong anti-tumoral effects, herein TFR1 probably become a potential molecular target for diagnosis and treatment for cancer therapy[3]. In veterinary oncology, the TFR expression has been investigated only in canine lymphoma[4], whereas no information pertaining TFR's expression level in cats, both in tumors and in cancer cell cultures, is reported. The aim of this work is to investigate the level of TFR's expression in feline malignant mammary tumor in formalin fixed paraffin embedded (FFPE) samples as well as *in vitro* on primary and metastatic feline mammary cancer cell lines. For identifying TFR's expression in tissue, immunohistochemistry (IHC) has been performed on 28 feline tissue samples, histologically classified as: mammary gland healthy tissue (7 cases), primary malignant mammary carcinoma (7 non metastatic and 7 metastatic) and lymph node metastases of mammary tumor (7 cases). In addition, on feline primary mammary tumor cell line (FMCp) and on feline metastatic mammary cancer cell line (FMCm) both immunofluorescence (IF) and flow cytometry (FC) were carried out. The antibody's cross reactivity for cat species was validated using Western-Blotting analysis. IHC results, analysed with a H score, revelated a higher and more intense expression of TFR in tumor specimens and in metastatic lymph nodes compared to healthy mammary gland ($p=0.0018$ and $p=0.0108$, respectively). No statistical difference was observed between TFR expression in mammary carcinomas and lymph nodes metastases. On FMCp IF confirmed both cytoplasmatic and membrane positivity. FC results showed a higher expression of TFR in FMCm compared to FMCp. In conclusion, the study revealed the presence of TFR in feline healthy mammary gland, primary malignant mammary tumors and in metastases. It showed a higher level of TFR1 expression in cancer cells compared to healthy mammary cells and in cells which greater tendency to metastasize. Further studies will evaluate and quantify these levels of expression.

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AUTOCRINE EFFECT OF EXTRACELLULAR VESICLES ON MAMMARY TUMOR OF DOG AND CAT

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Mammary tumors are among the most frequent tumors in intact bitches and queens. These tumors are often malignant and have a high tendency to metastasize, especially in the queen [1]. Extracellular vesicles (EVs) are enclosed membrane vesicles released by cells. They work as mediators in intercellular communication and they take part to many physiological and pathological process. In the past few years, many studies focused on EVs and on their effect on tumorigenesis in human medicine. It has been shown that EVs take part to many processes, including cellular proliferation, migration, angiogenesis and apoptosis[2].

The aim of this study was to evaluate the autocrine effect of EVs produced by canine and feline mammary cancer cell lines studying their *in vitro* migration, proliferation and invasive potential.

EVs were isolated by ultracentrifugation from a canine (CIPp) and a feline (FMCp) mammary cancer cell lines. Wound healing assays, transwell migration assays, transwell invasion assays and proliferation assays were performed on CIPp and FMCp treated with CIPp-derived EVs and FMCp-derived EVs, respectively. Images of the wound-healing assay were analyzed with ImageJ, migrated cells in both transwell migration assay and transwell invasion assay were stained with crystal violet and counted underneath a microscope. In the proliferation assay cells were counted using an automated cell counter (Countess II ThermoFisher) and luminescence and absorbance were measured using Cell-Titer-Glo and the MTS assay respectively. FMCp cells treated with FMCp-derived EVs showed increased migration in both the wound healing assay after 5 hours (p value<0.001) and 10 hours (p value<0.001) and the transwell migration assay after 6 hours (p value<0.01). Additionally, they showed increased migration/invasion in the transwell invasion assay after 24 hours (p value<0.05). CIPp cells treated with CIPp-derived EVs showed a higher migration only in the wound healing assay after 14 hours (p value<0.001). Cell proliferation was apparently not increased after EVs addition in none of the two cell lines.

These results suggest an interesting autocrine effect of mammary cancer cells-derived EVs on cell migration and cell invasiveness which is more evident in the feline mammary cancer cell line. More studies are needed to better understand the EV role within tumors in veterinary medicine and to improve EVs isolation and purification.

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A WEB-BASED APPLICATION TO EVALUATE FORENSIC ACTIVITY IN THE PATHOLOGY SERVICE AT THE ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLE VENEZIE (IZSVE)

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Forensic veterinary medicine is becoming increasingly important in this modern, litigious world. The medical/legal interface, both civil or criminal, is becoming increasingly challenging and it is essential that there are practitioners available to the courts who have both the requisite knowledge and experience to perform the examinations in a satisfactory manner and who can also provide evidence to enable the court to reach its verdict [1]. At National level a working group in the Italian Association of Veterinary Pathology started in 2015 to define guidelines for forensic autopsies. In this framework we developed a system to evaluate the possible impact of forensic cases in the competent territory, the northeast of Italy. The aim was to associate the reason for submitting and possible legal reflections to the final results of the necropsy. The expected output was to provide evidences for implementing new diagnostic tools and specific training activities for the pathologists to improve the appropriateness of the diagnostic service as well as to protect towards professional responsibility. The approach takes advantage of the tools already available at IZSVe for process management strategies that include the QlikView®, a business intelligence product, and the IZSVe Laboratory Information Management System (IZILAB). The tool is applied for the dogs and cats that are submitted to the IZSVe provincial laboratories for necropsy. At acceptance the veterinarian in charge collects anamnestic data and basic information (e.g. diagnostic suspect of the referral veterinarian and possible legal implications) are inserted in the IZILAB in addition to traditional data. At necropsy the case is categorized in syndromes. Each syndrome refers to the appropriateness protocols developed at IZSVe for the diagnostic process and includes a panel of analyses that help the diagnostician to reach the final diagnosis. These categories are divided by species and syndrome (e.g. puppies, cardio-circulatory, respiratory, digestive). A web-based dynamic reporting system is available to each veterinarian with the information at IZSVe, laboratory and individual case level. In this work, we present the design of the system and the results of its application that started in 2018. During the period January 2018 to March 2019, 1036 dogs and cats were submitted to the IZSVe provincial laboratories and, after a testing period of the tool, 578 have become available for reporting. Cases are categorized by the reason of sampling, anamnesis and syndrome. Design of the approach and preliminary results will be presented. This approach demonstrates how data combination allows to monitor the extend of the phenomenon and to support decision-making in order to implement a forensic pathology service and define evidence based approaches for diagnosis and sample management.

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THE SUPPORT OF FLOTAC IN THE DIAGNOSIS OF DROWNING IN FORENSIC VETERINARY PATHOLOGY

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Drowning mechanisms and the associated lesions have been described extensively in human forensic medicine [1], but very rarely in veterinary medicine [2,3], and no specific diagnostic lesion have been well-defined. The diatom test [2] is currently considered the "gold standard" for the diagnosis of drowning, but it still has a high possibility for false positive results. FLOTAC and Mini-FLOTAC represent highly sensitive techniques in parasitological diagnosis [4]. Our hypothesis was that FLOTAC and Mini-FLOTAC can be used also for the detection and count of diatoms in the organs of drown animals. Based on these considerations, this work has the following aims: 1) to evaluate the role of anatomo-pathological assessment in the diagnosis of drowning; 2) to investigate the differences in number and location of diatoms between drown and non-drown animals; 3) to assess the feasibility of FLOTAC and Mini-FLOTAC devices for diatom detection and count. Twenty-four dead adult dogs were used for the study and divided in 5 groups. Group A included drown dogs (n=4); Group B included non-drown dogs (n=5); groups C, D and E included dogs dead for causes other than drowning and subsequently immersed in water for 24, 48 and 72 hours, respectively (n=5 each). On each animal, a complete macroscopic and histological examination, diatom test and FLOTAC/Mini-FLOTAC techniques (using zinc sulphate specific gravity 1350 as flotation solution) were performed. Macro and microscopic findings for each group were superimposable and consisted mostly in pulmonary congestion, oedema and hemorrhages of the lung. Diatom test and FLOTAC/Mini-FLOTAC techniques allowed us to detect and count diatoms in the lungs, liver and kidneys of dead animals of group A, C, D and E but not in group B. We observed differences between drown animals and all the experimentally drowned groups and control animals regarding diatom numbers recovered from tissue samples ($p<0.05$). These results suggest that the histological and anatomopathological findings in drowning cases are not specific because they can be observed in a wide range of causes other than drowning, the diatom test is a valid tool to support the diagnosis of drowning and the FLOTAC and Mini-FLOTAC techniques should be considered as practical and safe method for the isolation and the count of diatoms, thus opening a new potential use for these tools. Nevertheless, further studies should be conducted to better understand the applicability and performance of the FLOTAC and Mini-FLOTAC devices to support the diagnosis of drowning in veterinary forensic pathology.

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POST-MORTEM BIOCHEMISTRY: AQUEOUS HUMOR AS AN ALTERNATIVE MATRIX FOR BIOCHEMICAL ANALYSIS IN DOGS

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Biochemical analyses are useful to determine the cause of death or to better understand the meaning of many anatomic lesions found during necropsy [1]. However, ante-mortem biochemical results are not always available to the pathologists and post-mortem serum samples are considered unsuitable for biochemical analyses [2]. Aqueous humour (AH), a blood filtrate, is located in a protected anatomical site and its deterioration occurs more slowly than other body fluids [3]. The aim of this study was to evaluate AH as an alternative matrix to perform biochemical analysis in dead animals and to evaluate its stability after storage at room temperature. AH samples were collected from the eye's anterior chamber of 15 dogs within 1 hour from the death of the animals. To evaluate the stability of the analytes over time, each sample was divided in 4 aliquots, kept at room temperature for 0 (t0h), 24 (t24h), 48 (t48h) and 72 (t72h) hours and then frozen. The following analytes were measured: alkaline phosphatase (ALP), alanine aminotransferase (ALT), creatinine, urea, creatin kinase (CK), total protein (TP), albumin, microalbumin, paraoxonase1 (PON-1), C-reactive protein (CRP), glucose, sodium (Na), potassium (K), magnesium (Mg), phosphorus (P) and chloride (Cl). Ante-mortem serum biochemical results were available for 9 dogs. At t0h, urea, creatinine, ALT and glucose levels in AH were correlated to the serum levels. Moreover, microalbumin at t0h in AH was correlated to both serum albumin and TP. The other analytes in AH and in serum were not correlated. At t0h, albumin, TP, ALP, ALT and glucose levels in AH were significantly lower than serum, whereas Cl was higher. No differences were found for the other analytes. ALT activity at t72h and creatinine levels at t48h in AH were higher compared to t0h, on the contrary CK activity at t24h and at t72h and Na levels at t24h were lower compared to the samples immediately frozen. AH could be useful to evaluate kidney and liver functions and to predict serum glucose and proteins concentration of dogs at the time of death. Moreover, the majority of analytes in AH were stable after 120 hours at room temperature. However, we collected and stored AH separately from the eyes, thus we cannot exclude that post-mortem alteration of whole body and/ or the eyes could affect the AH composition.

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INSIGHTS INTO DOLPHINS' IMMUNOLOGY: IMMUNO-PHENOTYPIC STUDY ON MEDITERRANEAN AND ATLANTIC STRANDED CETACEANS

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Immunology of marine mammals is a relatively understudied field and its monitoring plays an important role in the individual and group management of these animals, along with an increasing value as an environmental health indicator [1,2,3]. This study was aimed at implementing the knowledge on the immune response in cetaceans stranded along the Italian coastline to provide a baseline useful for assessing the immune status of bottlenose (*Tursiops truncatus*) and striped (*Stenella coeruleoalba*) dolphins. In particular, since the Mediterranean Sea is considered a heavily polluted basin [4], a comparison with animals living in open waters such as the Atlantic Ocean was made. Formalin-fixed, paraffin-embedded spleen, thymus and lymph node tissues from 16 animals stranded along Italian and 11 cetaceans from the Canary Island shores were sampled within 48 hours from death. Information regarding stranding sites, gender, and age as well as virologic, microbiological, and parasitological investigations, and the cause and/or the death mechanism were also collected in order to carry out statistical analyses. Selected tissues were routinely stained with hematoxylin-eosin and with immunohistochemical techniques (IHC). For IHC analysis, anti-human CD5 monoclonal mouse antibody to identify T lymphocytes, CD20 monoclonal mouse antibody for the identification of mature B lymphocytes and HLA-DR antigen (alpha-chain) monoclonal mouse antibody for the identification of the major histocompatibility complex type II were previously validated for both species by Western-blotting technique. T TEST method applied to quantitative evaluation of IHC positive cells showed a significant relationship between the number of (expression) of CD20 stained lymphocytes and normal and hypoplastic lymph nodes, respectively. No other significant correlations were noticed. Analyses for organochlorines (OC) compounds were performed in animals (n=5) having frozen blubber tissue available. Even if the limited number of the investigated cetacean specimens do not allow definitive conclusions, a simple linear regression was calculated to predict if the amount of OCs could influence the number of inflammatory cell subpopulations and a moderate negative correlation was found between the presence of high quantity of contaminants and the number of T lymphocytes. Future analysis should be aimed to understand the effect of the major immunomodulatory pathogens on sub-populations of B and T cells.

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TESTUDINES INTRANUCLEAR COCCIDIUM: THE PARASITE THAT BREAKS A BIOLOGICAL PARADIGM. IMMUNOHISTOCHEMICAL CHARACTERIZATION OF LESIONS AND HOST IMMUNE RESPONSE

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Testudines Intra-Nuclear Coccidiosis (TINC) is an infectious emerging disease in chelonians, caused by a coccidium ascribed to the *Eimeriidae* family [1]. Vertebrates coccidia commonly have endozoic cytoplasmic development, although at least 11 species of *Eimeria*, *Isospora* and *Cyclospora* are caryotropic, having intranuclear life stages [2]. This represents a unique behavior where an eukaryotic cell (parasite) enters into the nucleus of another eukaryotic cell (host), breaking a biological paradigm and opening new questions about parasite-host interaction. The aim of the present work was to characterize tissue and cellular lesions, uncovering the host defense and immune response mechanisms. In order to explore these aspects we performed immunohistochemistry (CD3, CD21, F4/80, S100, IL-1 and TUNEL assay using formalin-fixed paraffin-embedded tissues (kidney, intestine, liver, lung, spleen) from five dead patients ascribed to the species *Astrochelys radiata* (n=2), *Cuora aurocapitata* (n=1), *Stigmochelys pardalis* (n=2) with TINC generalized infection positive histology. TINC PCR was also performed in three patients from formalin-fixed paraffin-embedded tissues to verify the presence of the parasite. Anamnesis referred lethargy, anorexia and hematological data showed mild to severe anemia and leukocytosis. Histology revealed intranuclear protozoans (different endozoic forms) mainly localized in liver, kidney and lungs. Parasitism was accompanied by small foci of hepatic and renal tubular epithelial cells death with interstitial lymphomonocytic infiltrate and numerous activated melanomacrophage centers (MMCs). In particular, immunohistochemistry results revealed $CD3^+$ T cells and $F4/80^+$ macrophages. $S100^+$, IL-1 $^+$ intranuclear structures, morphologically compatible with parasite endozoic forms, were highlighted in hepatic, bile ducts and renal tubular epithelial cells. Parasite infected cells generally showed $TUNEL^+$ signal. PCR results confirmed the presence of the parasite in one patient. Lesions immunophenotyping unveils the cell-mediated nature of the host immune response characterized by a strong antigen presenting cells multi-organ surveillance, as suggested by the numerous activated MMCs. The parasite high potential systemic invasion is probably the result of an evolved strategy that takes advantage from the phagocyte cells network as a cellular “Trojan horse”. The $S100^+$, IL-1 $^+$ intranuclear structures reveals from one side the existence of parasite motility adaptation in the eukaryotic nuclear context and from the other the host response, activating the caspase-1 dependent trigger to pyroptosis, with DNA fragmentation as demonstrated by the TUNEL assay.

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EXPRESSION OF AVIAN BETA-DEFENSIN AND HISTOPATHOLOGICAL EVALUATION OF CHICKENS' GUT FOLLOWING SALMONELLA ENTERITIDIS ΔZNUABC ADMINISTRATION

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Contaminated poultry and eggs are major sources of Salmonella infections in humans. The decrease of Salmonella foodborne infections is dependent on the reduction of *Salmonella* burden at primary production stages. Defensins are major antimicrobial peptides, effectors of innate immunity playing a key role in the protection of mucosal surfaces of animals and humans through multiple modes of action [1]. Aim of the work was to investigate if the infection of one-day-old chickens with *S. Enteritidis* deleted of ZnuABC transporter (*S. Enteritidis* ΔznuABC) used as a vaccine candidate modifies the course of avian β-defensin genes expression during the first 4 weeks of chicken life. Forty white Leghorn hens were divided into two groups of 20 animals (treated and control group). Experimental groups were allocated in separate rooms under natural day–night rhythm with ad libitum access to commercial feed and tap water. Experiment was authorized by national authority in according to Italian and European regulations (D.L. 116/92, 86/609/EEC, Decreto 225/2009-B) and was carried out under the supervision of certified veterinarians. One mL of sodium bicarbonate buffer containing 105 CFU of *S. Enteritidis* ΔznuABC was administered by oral gavage to each animal of the treated group; animals of the control group received 1 mL of sterile sodium bicarbonate buffer. Five animals in each group were sacrificed on day 3, 7, 17, 32 and 86 post-administration. Liver, caecal tonsils and colonwere aseptically removed and processed for bacteriological, biomolecular and histological analyses. Data were analysed by GraphPadPrism Software. Effects of time and treatment group were evaluated by means of Two-Way ANOVA, followed by Sidak's post tests. $P<0.05$ was considered statistically significant.The results of the microbiological analyses showed a decrease in the concentration of Salmonella in the intestine in the first 32 days and a complete disappearance in about 3 months. This result highlights the attenuation of the vaccine strain in the chicken and its possible use in animals with long production cycles, in accordance with the data reported in the literature [2]. The biomolecular analysis showed a pattern already found in several studies for the AvBD1, AvBD2, AvBD4 and AvBD7 genes, not significantly modified by vaccination. In contrast, AvBD9, AvB10 and AvBD14 showed a new pattern of expression in the control group, not observed in treated animals, but without significant differences between the two groups. Histological semiquantitative analysis showed an increase in haemorrhages in the colon and caecal tonsils in treated animals and an activation of lymphoid follicles of the colon at day 7. These results highlight some possible issue of the vaccine (haemorrhages). Epithelial and haemorrhagic damage was also observed in control group, thus requiring further investigations.

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HISTOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF WHITE STRIPING MYOPATHY IN BROILER CHICKENS

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White Striping (WS) is an emerging myopathy of broiler chickens characterized by white striation appearance of muscle [1]. Despite the recent evidences, the pathomechanism underlying the WS still remains elusive, and the consequences on the quality of the meat are not yet clear [2]. The aim of this study was to characterize the morphological findings of WS. 30 breast muscles with macroscopic evidences of WS were collected at a CE authorized slaughterhouse from two batch of 55-day old ROSS 308 broiler chickens with a mean weight of 3,2 Kg. According with the severity of the lesions [1], chicken breasts were divided in moderately (group 1) and severely (group 2) affected. Fresh-frozen sections were studied using hematoxylin and eosin, Engel trichrome, reduced nicotinamide adenine dinucleotide tetrazolium reductase, succinate dehydrogenase and cytochrome oxidase stains. Furthermore, the phenotypes of inflammatory cell infiltrate and the expression of MHC classes I and II in WS-affected breast muscles were evaluated using chicken-specific antibody anti-Bu1 for B cells, CD3 for T cells, CD4 for T helper, CD8 for T cytotoxic, TCRγδ for γδ T cells, Monocyte/Macrophage-antigen for macrophages, anti-MHC I and MHC II. The severity of atrophy, necrosis, inflammation and mitochondrial alterations were semi-quantitatively scored from 0 to 3 (0=absent, 1=mild, 2=moderate, 3=severe). The differences between groups were evaluated with t-test. Myopathic features included mild to severe inflammation, necrosis with sarcoclastosis, fibrosis, excess fat cells, regeneration and atrophy associated with coarse intermyofibrillar network, target fibers, and aggregation of mitochondria. Necrosis, inflammation and mitochondrial alterations were more severe in group 2 compared with group 1 ($p<0.05$). Immunohistochemistry showed a leukocyte population mainly composed by macrophages, cytotoxic T cells and gamma delta T cell and scattered T helper and B cells. Ninety percent of the muscle fibers expressed intense MHC I positivity on the sarcolemma but they were negative for MHC II. These findings were consistent with an immune-mediated inflammatory myopathy. So, we hypothesize that myopathic features could recognize an ischemic injury of muscle due to inadequate vascular system of rapid growing muscle [2] and that the proinflammatory microenvironment following infarcts could favor autoimmune responses to tissue antigens breaking tolerance mechanisms [3]. The association between hypoxia-related lesions and autoimmune response have been already reported in the literature both in relation to myocardial infarctions [4], stroke and traumatic brain injury [3]. Further studies are needed to understand the role of immune response associated with WS.

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NEBRODI BLACKPIGS: EVIDENCES OF THEIR RESERVOIR ROLE IN THE MYCOBACTERIUM BOVIS INFECTION.

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Bovine tuberculosis (bTB) continues to represent an emerging disease among farm animals and wildlife in many parts of the world. Nebrodi Black pigs, an autochthonous free- or semi-free-ranging pig breed may be responsible for the maintenance of bTB infection in this area where TB prevalence in cattle is high [1, 2]. The authors report two cases of generalized *Mycobacterium bovis* infection involving the nasal cavity and mammary glands in Nebrodi Black pigs. An abattoir survey was carried out in the province of Messina (Italy) in Nebrodi Black pigs to further address their role as a potential reservoir host for bTB. After post mortem examination, bTB lesion-like samples were submitted to bacteriological and histological exams. Genotyping of the isolates was performed. Granulomatous lesions were evident in multiple lymphoid structures (tonsils, mandibular, retropharyngeal, parotid, bronchial, intestinal and mammary lymphnodes), abdominal and thoracic organs, mammary glands, vertebrae, spinal cord and cerebral meninges. On histopathology these lesions were classified as type 4 granulomas, characterized by necrotic calcified center and peripheral fibroplasia. Interestingly, in two cases nasal cavity, turbinates and frontal sinuses were affected by smaller disseminated granulomas, microscopically characterized by necrotic center or neutrophilic aggregates surrounded by epithelioid cells and a variable number of multinucleated giant cells, macrophages and lymphocytes; moreover, calcifications were recorded. Bacterial isolates were identified as M. bovis. Generalized chronic infections in pigs are rarely reported and the involvement of the nasal cavities is even rarer. Our results show the presence of granulomatous lesions in the nose, with mucosal erosions and submucosal chronic inflammation which might suggests the potential transmission of bacteria to other susceptible species via nasal secretions. Moreover, the presence of granulomatous lesions in the mammary glands, the isolation of the mycobacteria in faeces and the identification of new *M. bovis* Spoligotype indicates new routes of transmission of the bacteria from an unusual reservoir, the Nebrodi Black pig which might play a role in the maintenance of bovine tuberculosis infection in Sicily.

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MORPHOMETRIC CHARACTERIZATION OF NECROSIS INDUCED BY PERFUSED AND NON-PERFUSED RADIOFREQUENCY THERMAL ABLATION NEEDLES IN LIVER ON A SWINE *IN VIVO* MODEL

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RFA (Radiofrequency thermal ablation) is a technique presently applied in human oncology, based on tissue coagulative necrosis. Thermal energy is usually applied through electrodes; recently, perfused needles have been developed to improve ablations [1]. The aim of this work was to characterize necrosis induced by perfused and non-perfused RFA needles on the liver in a swine *in vivo* model. The *in vivo* trial (aut. n. 885/2016-PR, 22/09/2016) followed two *ex vivo* pilot studies and involved eight 4-months-old Landrance x Large White sows, weighing about 45 kg. Under general anaesthesia, all animals underwent median celiotomy, from xiphoid cartilage to a point 5 cm caudal to the umbilicus; a monolateral or bilateral paracostal incision was added to improve visibility when needed. Subsequently, the needle was placed in each single liver lobe under ultrasound guidance, and the RFA procedure was carried out under different conditions. A series of 18G internally cooled needles (RF Medical Co. Ltd., Seoul, Korea), either perfused (P) or not (NP), with fixed time of delivery of thermal energy (60 seconds) was used. The P needle was tested using four solutions: saline 0.9% (P 0.9%), hypertonic saline 3% (P 3%), 7% (P 7%) and 10% (P 10%), and six replicas for each condition were obtained. At the end of the procedure and still under general anaesthesia, the animals were euthanized by an overdose of thiopental sodium i.v. The lesions created by the needle were transversally and longitudinally cut, and pictures were obtained for macroscopic morphometry. Morphometric analyses were carried out by ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA). Samples were formalin fixed, and H.E. stained histological sections were obtained. Statistical analysis was applied to evaluate differences in the characteristics of the lesions induced by the different treatments. Following RFA, ablated areas showed macroscopically a central white area surrounded by a red hyperaemic halo. The necrotic volume varied depending on the type of needle used, being smaller with the NP one and increased along with the saline concentration of P needles; P 7% and P 10% produced the significantly largest lesions ($p<0.5$ and $p<0.01$, vs NP, respectively). The central liver parenchyma in the lesions was histologically characterized by the presence of lacunae, with detachment of hepatic plates, shrunken hepatocytes and pyknotic nuclei. The lesion was surrounded by an area of congestion and disseminated haemorrhages. Moreover, the surrounding tissues showed a mild vacuolization. The findings showed that perfused needles induced larger necrotic lesions, increasing progressively with higher saline concentrations. NaCl concentrations higher than 10% were not investigated, but P 7% and P 10% gave similar results, thus suggesting to be a useful choice to be further investigated.

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ORAL COMMUNICATIONS

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OCCURRENCE OF *LISTERIA* SPP. AND *L. MONOCYTOGENES* IN INDUSTRIAL AND FARMSTEAD SHEEP'S CHEESE-MAKING PLANTS

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The cheese-making processing environment is frequently contaminated with *L. monocytogenes*. The aim of the present study was to investigate the occurrence of *Listeria monocytogenes* and *Listeria* spp. contamination in the environment of Sardinian sheep's cheese-making plants. Samples were collected from four industrial plants and three farmstead cheese makers. Each facility was visited once for samples collection, which was performed during the production runs. Environmental testing was conducted using commercial sponge sampling kits. Food contact (raw milk filter, moulds, drainage tables, shelves, conveyor belts and inner part of equipment) and non-food contact surfaces (floor, floor drains and outer part of equipment) were collected from different areas such as curd production, whey heating, moulding, salting, storage/ripening and packaging. Sponges were also scrubbed on the surface of pecorino cheeses and ricotta salata wheels. Detection of *Listeria* was conducted using ISO 11290:2017. Up to five colonies were isolated from each positive samples for species identification conducted with phenotypic and biochemical methods. *L. monocytogenes* identification and major serovars was confirmed by multiplex PCR. Overall were collected 75, 84 and 36 sponges respectively from food contact, non-food contact and food samples. *Listeria* spp. was recovered from six out seven facilities from 48 samples (30.1%). Industrial plant showed *Listeria* spp. contamination in 45 samples (34.3%), while it was detected in 3 (10.7%) in farmstead cheese making facilities. *L. monocytogenes*, other *Listeria* spp. and their simultaneous presence accounted respectively for 27 (16.9%), 11 (6.9%) and 10 (6.3%) of contaminated environmental samples. Of the 152 confirmed *L. monocytogenes* strains 111 (73.0%) belonged to serovar 1/2a while 41 (27.0%) to serovar 1/2b. Out of 92 *Listeria* spp. isolates 37 (40.2%) were *L. welshimeri*, 29 (31.5%) *L. innocua*, 19 (20.7%) *L. ivanovii*, 5 (5.4%) *L. grayi* and 2 (2.2%) *L. seeligeri*. The greater *Listeria* contamination was in the following areas: product washing (85.7%), product salting (50.0%) while the sampling sites most frequently contaminated were the washing machine (84.6%) and the floor drains (32.4%). Pecorino cheese was positive in 1 swab for *L. monocytogenes* (2.7%) and in 3 swabs (8.3%) for *Listeria* spp. Industrial plants showed a likelihood of contamination (odds ratio) 3.4 greater than farmstead production. The presence of *L. monocytogenes* was associated with other *Listeria* spp. in 87.5% of positive environmental samples, confirming that they share a similar ecology. Therefore, it is essential for an effective monitoring program testing also for *Listeria* spp. as an indicator of the potential presence of *L. monocytogenes*. Floor drains represent a sentinel site for the detection of *Listeria* contamination in the processing environment. The results highlight the potential risk of transfer *Listeria* surface contamination to the paste during the successive portioning and packaging steps.

ISO 11290-1: 2017. Horizontal method for the detection and enumeration of *Listeria monocytogenes* Part 1: Detection method.

TRACEABILITY AND VIRULENCE DETERMINANTS OF *L. MONOCYTOGENES* ISOLATED FROM OVINE CHEESE MAKING PLANTS

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The aim of the present work was to study the potential virulence of *L. monocytogenes* (*LM*) strains isolated from environmental and food samples in different cheese making plants. To investigate the possible source of the contamination and the microorganism spreading to different working areas, a traceability study was also carried out. Samples were collected from 6 Sardinian sheep's milk cheese making plants. Sampling was conducted on food contact surfaces, non-food contact surfaces and food samples. Detection of *LM* was performed according to ISO 11290-1:2017. Up to 5 colonies from positive sample were picked and submitted to PCR for species identification. Multiplex PCR was used to identify *LM* major serovars. To study the genetic diversity and traceability of *LM* isolates within each cheese making plant, a cluster analysis was performed by PFGE [1]. Strains with 100% of similarity were included in the same cluster. The presence of the *actA*, *hly*, *inlA*, *iap*, *plcA* and *plcB* virulence genes was investigated on a selection of *LM* strains. In order to avoid overrepresentation of potential clones, strains selection included one isolate for each PFGE cluster. Isolates grouped in the same cluster with different serovar were also included. A total of 218 environmental and food samples were collected. Presumptive *LM* was detected in 57 (26.1%) samples. Two hundred two strains were isolated and confirmed as *LM* by PCR. The serovar 1/2a was the most represented with 144 strains (68.6%), followed by 4b with 41 strains (19.5%) and 1/2b with 10 strains (4.8%). Only 7 *LM* strains (3.3%) belonged to the 1/2c serovar. PFGE results showed a greater genetic diversity in 4 out of 6 premises, with strains grouped from 6 up to 8 different clusters within each plant. A lower variability was detected in the remaining 2 premises with strains grouped in 2 and 4 clusters, respectively. The presence of identical PFGE profiles in different environmental sites was observed in all facilities while only 2 plants shared the same PFGE pattern between environment and food. To investigate the virulence profile, 41 *LM* strains were retained. All the isolates carried *actA*, *hly*, *iap* and *plcB* virulence genes, while the *plcA* gene was detected in 78% (32/41) of the strains. The *actA* gene showed a genetic polymorphism (268 and 385 bp). Twenty six strains carried the 385 bp variant, while the remaining 15 the 268 bp variant. Results showed that *LM* is frequently isolated from the cheese making plants environment. Traceability study demonstrated a cross contamination along processing environment and to the final product. This pose a risk for human health, since isolates of the present study showed a moderate or high potential virulence and could be responsible of Listeriosis after consumption of contaminated cheeses.

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PRESENCE OF *LISTERIA* SPP. AND *L. MONOCYTOGENES* IN CHEESE- MAKING PLANTS PRODUCING PDO TALEGGIO CHEESE

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Listeria monocytogenes is an important human pathogen in the dairy chain that may find favourable conditions for its survival and growth in cheese-making plants environment[1]. Aim of the present study was the evaluation of the presence of *Listeria monocytogenes* and *Listeria* spp. in four facilities (A: producer and seasoner of PDO Taleggio cheese, B and C producers of PDO Taleggio cheese that is then seasoned in the plant D). Environmental testing was conducted during the production using a commercially available pre-sterilized sponge collection kit in addition, Taleggio cheese samples were taken. A total of 409 environmental and 10 food samples were obtained in three sessions carried out during May, July and October 2017. Enumeration and detection of *L. monocytogenes* was conducted using ISO 11290:2017. Species confirmation of *Listeria* spp. isolates was obtained by phenotypic methods and biochemical test (API Listeria, BioMérieux, France). The *Listeria* spp. prevalence, differed according to the plant (plants A and D resulted in significantly higher prevalences if compared to B and C, $P<0.01$) and the sampling session (October>July>May, $P<0.01$), it was 8.9%, 20.5%, and 67.9% in plant A in May, July and October, respectively; 4.3%, 5.4%, and 15.6% in plant B; 13.5%, no detectable and 4% in plant C; 17.1%, 25.7%, and 55.2% in plant D in May, July and October, respectively. *Listeria* spp. was isolated from different environmental sites including floor drains, drainage channel and equipment. *L. monocytogenes* was detected exclusively from plant D in two different sampling sites (1.6%) from the piercing and cutting areas of blue cheese, which was also produced in the same facility. Out of 127 *Listeria* spp. isolates speciated 100 *(*78.7%) were *L. innocua*, 12 (9.4%) *L. ivanovii*, 7 (5.5%) *L. grayi*, 3 (2.6%) *L. seeligeri* and 3 (2.6%) *L. welshimeri*. *Listeria* spp was never detected from Taleggio cheese samples. The wide environmental contamination with *Listeria* observed in Taleggio PDO cheese-making plants should be regarded as a potential risk of the presence *L. monocytogenes* due to common favourable conditions. An additional concern is the low tolerance policy adopted by some importing countries with respect to *Listeria* spp. environmental contamination. The results confirm that the highest *Listeria* spp. prevalence was observed in ripening rooms environment (plants A and D), and some concerns arise as no further decontamination of the final product before packaging is provided. The simultaneous presence of several typologies of cheeses, other than PDO Taleggio, in the same plant (D) may be a potential source of cross-contamination of this cheese. The results suggest that Taleggio PDO cheese-making plants should adopt effective *Listeria* environmental monitoring program.

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LISTERIA MONOCYTOGENES MITIGATION STRATEGIES FOR SAFETY AND SHELF LIFE EXTENSION OF RAW READY-TO-EAT FISH: CHALLENGE AND MODELLING APPROACHES

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An increasing number of the worldwide population consumes raw ready-to-eat (RTE) fish products, which represent a high risk for causing foodborne illnesses. Potential *Listeria monocytogenes* contamination of raw RTE fish is primarily from raw material, although the processing plant might also play an important role (1). In order to comply with the EU regulation, which accepts the limit of 100 CFU/g for *L. monocytogenes* at the end of shelf-life for those RTE unable to support the pathogen growth, it is necessary to obtain raw materials with low levels of contamination as well as to adopt strategies for preventing the pathogen growth at refrigerated temperatures.
In this study the growth potential of three different *L. monocytogenes* strains in artificially inoculated raw fish tartare containing a buffered vinegar product, was investigated. In addition, to accurately establish the pathogen growth probability using a modelling approach, the minimal inhibitory concentrations (MICs) of undissociated acetic acid (UA) was determined for these strains. Samples of ground tuna, amberjack fish and salmon, prepared using mixtures of NaCl (2.5%) and Verdad (0.75%), were packed in a modified atmosphere and then inoculated (100 CFU/g) with three pooled *L. monocytogenes* strains, previously adapted to low temperatures. The pathogen growth potential was estimated according to the EURL document (2). MICs of UA for the three strains were determined in a pH range of 5.5-6.0 by a Mejlholm & Dalgaard (3) equation, whereas estimation of the concentrations of UA in fish was based on a Wemmenhove et al. (4) equation. *Praedicere Possumus* (PP), a validated application for predictive microbiology (5) was used for the growth probability prediction. The challenge testing results show that the three fish products were unable to support the *Listeria* growth, at least when the storage temperature was 4°C and the storage time was 11 days. Differences in MICs of UA were found between the *Listeria* strains, with values being in the range of 5.7-6.46 mM, consistent with the MICs determined by Coroller et al. (6), but different from the MICs showed by Wemmenhove et al. (7). Applying PP to estimating growth probability, the fish products were predicted to be unable to support the growth of *L. monocytogenes* at 4°C. Therefore, these products should be categorized as foods that do not support the growth of *Listeria* and a tolerance level limit of 100 CFU/g should be applied. In addition, with the adoption of accurate MICs limits, the PP application proved to be a valuable tool for producers in identifying and validating mitigation strategies for preventing the pathogen growth, as well as for determining the compliance of RTE products with the EC safety criteria.

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SANITARY RISKS DUE TO THE PRESENCE OF PARALYTIC SHELLFISH POISON (PSP) TOXINS IN MUSSELS BREED IN SARDINIA (ITALY)

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Paralytic shellfish poison (PSP) is one of the major toxins causing seafood toxicity and represents a serious health hazard to humans, with a worldwide distribution. PSP belongs to a neurotoxins group known as saxitoxins (STXs), that constitute a set of related tetrahydropurines. To date 57 analogues have been reported (1); they are specific agents that can block sodium channels in excitable cells, suppressing ion permeation. This action can subsequently cause a range of concentration-dependent responses within 24 hrs of consumption of the toxic shellfish, including nausea, ataxia, shortness of breath, paralysis and death through asphyxiation (2). PSP is mainly produced by dinoflagellates belonging to the genera *Gymnodinium*, *Pyrodinium* and in particularly *Alexandrium.* In order to protect the consumer health, the European Commission settled that the maximum allowable concentration in live bivalve mollusks must not exceed 800 µg saxitoxin equivalent per kg in any edible part (800 µg STX eq./kg e.p.) (Regulation N. 853/2004). Based on the Regional plan of control of live bivalve mollusks production and marketing (3), the aim of our paper was to document the sanitary risk due to the presence of PSP over the legal limits recorded in Sardinia in 2018, in relation to the presence of *Alexandrium pacificum* Litaker. Mussel samples were analyzed for PSP toxin presence by using the mouse bioassay (MBA) (AOAC 959.08) (4). A total of 910 mussel samples were analyzed for PSP toxins from January to December 2018. Four samples resulted over the legal limits (2780 µg STX eq./Kg e.p as maximum concentration). All the positive samples were observed on May concomitantly with the presence of *A. pacificum*. For the first time, in 2018 there has been the presence of PSP over the legal limits also in the south Sardinia (Santa Gilla area). Previously, in Sardinia there has been reported several cases of PSP positivity. First cases were observed in 2002 in the Gulf of Olbia, even then on May (5). Since then there has been several other cases, rather constantly until 2011 Olbia area and between 2006 to 2012 in the Gulf of Oristano (5). Most of positivities were reported in winter (between November to February). Even if in Sardinia the PSP were absent from 2012 to 2018, a strictly application of monitoring plans, both in the mussels and breeding water, reduced sanitary risks in the seafood consumers. Therefore, outcomes from this study highlight the importance of Sardinian regional plan in preventing human pathologies.

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BIVALVE MOLLUSCS AND MARINE ENVIRONMENT AS TOOL FOR MONITORING ANTIMICROBIAL RESISTANCE IN *SALMONELLA ENTERICA* SUBSP. *ENTERICA* AND *ESCHERICHIA COLI* PHYLOGROUPS: PRELIMINARY DATA FROM THE MOLLUSCS PRODUCTION AREA OF FERRARA

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Bivalve molluscs represent an important tool for monitoring antimicrobial resistant (AMR) bacteria (1). The study presents the AMR findings in *Salmonella enterica* subsp. *enterica* (n=102) and *Escherichia coli* (n=79) isolated in the production area of Ferrara, Italy, with the aim to confirm the role of molluscs as AMR indicators.
A total of 102 *Salmonella* spp. (72 *S.* Typhimurium and 30 *S.* Typhimurium 1,4,[5],12:i:) and 79 *E. coli* were collected in different species of bivalve molluscs and sea and brackish water, respectively from 2001 to 2017 and from 2016 to 2018, in the same 5 sub-areas of production, classified as Long-line, Lupini, B-Out, B-in and Sacca. The isolates were screened for their resistance against a panel of antimicrobial agents (AA) by the agar diffusion method (2). Epidemiological cut-off values proposed by EUCAST were used as first choice, followed by EUCAST and CLSI clinical breakpoints for *Enterobacteriaceae*. *E. coli* isolates were tested for their phylogentic group affiliation by the ClermonTyping (3). Overall, 81% of *Salmonella* spp. and 75% of *E. coli* isolates were resistant to at least one AA and respectively 54% and 51% were multidrug resistant (MDR). Monophasic variant and *S.* Typhimurium accounted respectively 97 and 72% of resistant strains to at least one AA and 75 and 44% of MDR strains. Both serovars showed resistance to aminoglycosides (97 and 43%), penicillin (80 and 40%), tetracyclines (67 and 36%). Resistance to sulfonamides (60%) was observed for monophasic variant whereas for *S.* Typhimurium resistances were to carbapenems (24%) and phenicols (21%). Over the years, the proportion of *Salmonella* isolates resistant to at least one AA decreased from 94 to 72% but the MDR increased from 25 to 61%. The most common resistances of *E. coli* isolates were to penicillin (56%), aminoglycosides (52%), sulfonamides (30%) and third-generation cephalosporins (24%). The majority of *E. coli* isolates belongs to B1 (51%), A (14%), C (14%) and to the other B2, D and E (21%) phylogroups. Most resistant and MDR strains belonged to phylogroups C (91 and 80%) and A (82 and 56%): phylogroup C showed the highest resistances to almost all the considered AA. In relation to spatial trends analysis, the B-in area presents the higher resistant and MDR levels both for *Salmonella* (87 and 58%) and *E. coli* (85 and 65%) isolates, whereas in the Sacca area the lower AMR level was reported for *Salmonella* (70 and 29%) and *E. coli* (67 and 41%) isolates. No difference was observed between sources (molluscs versus water). Very high to extremely high resistance and MDR levels were observed in *Salmonella* spp. and in *E. coli* isolated from molluscs, sea and brackish water in the production area of Ferrara. Varying occurrence AMR levels were between *S.* Typhimurium and its monophasic variant, *Salmonella* and *E. coli, E. coli* phylogroups, and the sampling area: these findings confirm bivalve molluscs as an important tool for monitoring spatial and temporal trends in AMR and MDR.

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COMPARATIVE MITOGENOMIC ANALYSIS OF SPARIDS AND EVALUATION OF A NEW POTENTIAL DNA BARCODING MARKER FOR *DENTEX DENTEX*

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Dentex dentex is one of the most commercially caught *Sparidae* species in the Mediterranean Sea and Atlantic Ocean. It is very appreciated in European markets and consequently more subjected to species substitution frauds [1]. The currently mitochondrial (*mt*) DNA sequences used for fish species identification in prepared and processed products are cytochrome b-*CYTB*, cytochrome c oxidase I-*COI, 16S* and *12S* genes. Recent researches showed that the study of the whole *mt*DNA allows to identify new, effective and specie-specific barcode markers [2]. In particular, *NAD5* gene has high discrimination capacity for *Sparidae* species. However, the use of all these genes needs amplification and a sequencing process [2,3]. Therefore, a valuable species identification requires many laboratory steps and is time consuming. The aim of this research was to analyze and compare the whole mtDNA sequence of *Sparidae* species to find a barcoding marker useful to identify the sparid species *D. dentex,* avoiding the sequencing step. Thirteen *Sparidae* complete mitogenomes were compared in this study. They were aligned by UGENE software. Hamming Distance algorithm was used to evaluate in percent the genetic dissimilarity among species and genes. Overall mean *p*-genetic distance analyses were conducted using the Maximum Composite Likelihood model. The nucleotide sequence variability was determined by aligning gene-by-gene sequences of *Sparidae* species using MEGA 6.0. Specific primers for *D. dentex* were designed by eye after multiple alignment of the *Sparidae* complete *mt*DNA sequences using BioEdit Sequence Alignment Editor. Primers efficiency and specificity for *D. dentex* identification were tested using PCR reaction. Results of Hamming Distance, nucleotide sequence variability and *p*-genetic distance analysis showed the potentiality of *NAD2* gene as barcode marker for sparids. The PCR reaction confirmed the discrimination capacity of *NAD2* gene. In particular, the amplification of the selected *NAD2* fragment was possible only for the species *D. dentex*. In conclusion, *NAD2* gene showed high interspecific nucleotide dissimilarity to provide unambiguous results for *D. dentex* species authentication without sequencing, reducing time, costs and efficiency. In fact, species identification results can be obtained in a few hours of lab work. Therefore, competent national authorities responsible for monitoring and enforcing could improve and make full use of *DNA*-testing methods in order to deter operators from false labelling of seafood. In agreement with Regulation (EU) 1379/2013, this study contributes to the molecular traceability of fishery products.

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PREVENTING FRAUDULENT ADULTERATIONS: PRELIMINARY STUDY FOR THE SELECTION OF HISTOLOGICAL PARAMETERS FOR THE DISCRIMINATION OF FRESH AND FROZEN-THAWED OCTOPUS MANTLE

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The application of appropriate analytical techniques represents an important tool to prevent deliberate substitutions of fresh with thawed seafood. The histological method has already been validated to discriminate fresh from thawed fish [1,2], while it has been barely investigated on cephalopods [4]. The present study aimed at selecting histological parameters of freezing-induced structural alteration on Octopus (*O. vulgaris*), to be further used in the setting up of an operational grid to be used as analytical tool for sample discrimination. Ten whole fresh octopus (F), maintained at 0-2°C, were sampled at different time (24h, 72h, 144h and 192h) within octopus shelf life [3] obtaining 10 longitudinal and transversal mantle samples at each sampling. Samples were fixed in 10% formalin, embedded in paraffin and Hematoxylin-Eosin stained sections were observed to define fresh tissue morphological pattern and to highlight possible histological spoilage-related alterations. Then, 85 mantle tissue sections belonging to 20 fresh (F), 20 conventionally frozen in a laboratory freezer at -20°C for 15 days (CF), 25 industrially frozen in bulk blocks at -80°C (IFB) and 20 curled and Individually Quick Frozen (IQF) exemplars, were analogously processed and screened to select histological parameters descriptive of freezing-thawing process. Overall tissue structural organization (4x magnification), gaping among muscle fiber bundles and white round-oval-saccular spaces or clefts between and within the muscle bundles (10x magnification) were assessed on the central area of the longitudinal section, including radial, circular muscle bundles and a central connective tissue layer. Moreover, white spaces between and within muscles bundles were measured by morphometry and results expressed as percentage. Focal myofiber degeneration, shrinkage and swelling were histological modifications related to shelf-life. The increase of the overall tissue structure alteration, myofiber gaps and the presence of white spaces between and within muscle bundles were all statistically confirmed to be related to freezing as plausibly induced by the effects of water crystallization phenomena [4]. The mean white space percentages recorded in F (22.97%), CF (43.09%), IFB (46.57%), IQF (59.69%) highlighted significant differences between fresh and frozen tissue types. Thus, the study confirmed the suitability of all the aforesaid histological parameters as analytical indicators for the discrimination of fresh and frozen cephalopods.

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EXTREMOZYMES, POSSIBLE SOLUTION TO ADDITIVES USE IN FOOD PROCESSING

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Color is the most important factor that affect commercial value of tuna steaks and industries demand new solutions to improve it. In recent years has been highlighted the illegal use of nitrates via vegetable extracts for unprocessed fish □1□ because they could deceive the consumer about freshness and healthiness □2□. Besides, lately an opinion of the European Commission denounced the excessive use of antioxidant additives and set the limit at 300 mg/kg for ascorbic acid-ascorbates (E 300-302) because the improper use could mask or replace the use of preservatives. In this scenario, the aim of this study was to evaluate the color changes in thawed tuna fillets treated with antioxidant extremozymes. Extremozymes are produced by extremophiles, microorganisms capable to thriving in extreme environments, that have the ability to survive where mesophilic counterparts get inactivated. In this work was studied the class of extremozymes namely antioxidant/detoxifying enzymes superoxide dismutase (SOD) □3□ from *Aeropyrum pernix* (AP). Protein extracts were obtained from tomato cell lysates, where the coding genes were introduced. For the trial we used n=4 thawed tuna fillets (*Thunnus albacares*), each of 2.5 kg belonging to the same lot. Three brines with different compounds were tested: (i) BI (ascorbic acid 1% and acetic acid 0.5%), (ii) BII (APSODs 0.4%) and (iii) BIII (tomato cell extracts 0.4%). The brines were injected through a multi-needle automatic machine in n=3 different fillets. N=1 fillet was not injected and used as a control. The samples were vacuum packaged and stored at 4°C. At T0 (beginning) and T1 (7th day of storage), colorimetric evaluations with colorimeter Konica Minolta CR300 (Minolta, Osaka, Japan) and histamine determinations were performed on an aliquot of each fillet. After 7 days, colorimetric results showed that both additives and extremozymes act positively on color, without significant differences. Otherwise, during storage treaty sample with tomato cells and control fillet turned brown. Histamine values were lower than those set by the Reg. CE 2073/05. In conclusion, extremozymes have the same effectiveness of antioxidant. Even if natural compounds, their use could be considered only after the planned authorization process.

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ANISAKIS SPP. LARVAE IN DIFFERENT SEMI-PRESERVED FISH PRODUCTS SOLD IN EU RETAILS: PRELIMINARY DATA

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The presence of *Anisakis* larvae in fish represents a public health issue: effective risk management procedures as visual inspection of raw products and gutting should be applied to avoid heavily infected products before reaching the market. Moreover, despite freezing and treatments as salting assures no viable larvae in the fish products [1] the risk of potentially present allergens should be highlighted. Aim of the work was the evaluation of the presence of *Anisakis spp*. larvae in salted fishery products sold in EU territory. Twenty-one semi-preserved fish products (n=14 fillets of salted anchovies and n=7 fillets of salted sardine samples) belonging to 21 different brands were collected in different EU retails; for all samples visual inspection [2] and chloro-peptic digestion [3], in order to detect the presence of visible larvae, were performed. The whole content of the products was digested. Larvae detected after digestion were collected and submitted to morphological and to molecular identification. No larvae after visual inspection were detected; n=1 not viable larva was highlighted after digestion in n=1 salted anchovies sample (4.76% of the total samples, 7.14% of the anchovie samples) collected from FAO 27.8C. – Atlantic NE. The larva was identified as *A. simplex*. A mean number of 0.04 larvae per product (±0.21 SD) were found, in contrast with previous study [4]. According to EU legislation, unsafe food and obviously contaminated fish should not be placed on the market; in this case food business operator (FBO) has complied with EU law: beheading/partial gutting pre-salting and operator training during desalting and filleting ensure that anchovies result not obviously contaminated. If edible parts result obviously contaminated, the FBO has to apply normal sorting or processing procedures, including trimming, to ensure that the marketed product is not obviously contaminated [5]. Dead larvae in salted products should not be considered hazards but only defects because of the possible consumer rejection [6], taking into account the possible hypersensitization reactions in the sensitized people. Development of common modus operandi in sampling procedures, corrective measures and further studies on prevalence of parasites in semi-preserved fish products are needed.

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IC-HRMS ANALYSIS OF GLYPHOSATE, GLUFOSINATE AND AMINOMETHYLPHOSPHONIC ACID IN FOOD OF ANIMAL ORIGIN

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Glyphosate and glufosinate are broad spectrum herbicides [1], whereas aminomethylphosphonic acid (AMPA) is a degradation product of glyphosate. A controversy about toxicity of glyphosate exists, as it is not considered a probable carcinogenic by the European Chemicals Agency [2], in contradiction to the International Agency for Research on Cancer [3]. Nevertheless, AMPA was not considered. Scarce information is available about their presence in food. Their high polar nature makes extraction difficult, particularly in food of animal origin, rich of interferences, at residue levels. This challenge often resulted in the use of lengthy cleanup procedures involving derivatisation or chromatographic separation with different types of columns [4]. Based on these considerations, the aim of this study was to develop and validate a simple and versatile method for the analysis of glyphosate, its major metabolite AMPA and glufosinate, based on Ion-Chromatography coupled to High Resolution Mass Spectrometry (IC-HRMS) in three matrices: honey, bovine muscle and seabass. The extraction procedure was identical for the three matrices, by using only methanol and acidified deionised water (1% formic acid). The satisfactory LOQs in the range of 4.30-9.26 ng g^{-1} demonstrated high method sensitivity, compared to the few works present in literature; the mean recoveries ranged between 75 and 112%, indicating the efficiency of the extraction protocol; the matrix effect was modest with a percentage variation lower than the 20%, as well as for repeatability. The method was validated according to SANTE/11813/2017 guidelines [5]. Finally, the method was checked on 10 real commercial Italian samples of each edible matrix, randomly collected from different supermarkets. Any traces of the selected pesticides were not found in the analysed samples.

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APPLICATION OF A NON-THERMOCHROMIC MAGNETIC DECAY INK BASED LABEL AS A METHOD FOR COLD CHAIN TRACEABILITY

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The cold chain is a temperature-controlled transport and storage system, to ensure the safety and quality of refrigerated goods [1]. It is estimated that about one third of all food produced for human consumption in the world is lost or wasted due to inefficient cold chain management [3]. In Europe, about 10% of fresh imported products are wasted during transport [4], which appears to be the stage in the supply chain where multiple inefficiencies may occur relating to maintaining the correct temperatures [4]. For this reason, temperature monitoring plays a key role of the food industry, which has always been looking for new systems that operate continuously and ensure the possibility of intervening in real time on cold chain interruptions. There are various systems for controlling the temperature along the supply chain, among these smart labels, manufactured with special inks that, based on the chemical formulation, can interact with the surrounding environment detecting temperature changes [5]. The aim of this study was the test of a non-thermochromic magnetic decay ink based label as a method for traceability of the cold chain. Six tests were performed to assess the effectiveness of the labels in the food sector, with two different inks (high and low sensitivity). The first four were carried out as an effectiveness test in the laboratory, using 125 gr. pack size mozzarellas in three controlled refrigerators, simulating cold chain interruptions; the last two were performed as field tests, during regular distribution of meat products contained in a secondary packaging and frozen products of various types. In the first four tests and in the sixth, labels were printed with high sensitivity ink; in the fifth with low sensitivity ink. The carried out tests offered positive evidence both in the laboratory test phase and in the field test about reliability and versatility of the labels. Results showed the ability to offer data that are not influenced by episodic variations and responding in relation to conditions of concrete criticality of the process or the set of processes. Rapidity, simplicity and inexpensiveness of this smart label system make it propose for routinary temperature monitoring and as a tool for products' protection.

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SALMONELLA PREVALENCE AND CARCASS HYGIENE OF WILD BOAR FROM ASINARA NATIONAL PARK

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Salmonella is a zoonotic pathogen that affects humans, livestock, companion animals and wildlife. The importance of wildlife as *Salmonella* carriers has been highlighted on a variety of species including wild birds, reptiles and mammals [1]. Wild boar (*Sus scrofa*) are widely distributed in Sardinia and in the islands off its coast. Aims of the present work were to investigate the occurrence of *Salmonella* in wild boar from Asinara National Park and to assess the microbial hygiene of carcasses. The animals were trapped by mean of cage traps located inside the park, transported to a slaughterhouse and slaughtered the same day of the arrival. At slaughterhouse, sampling was conducted on five different days. During each sampling day, samples of colon content and mesenteric lymph nodes were collected immediately after evisceration from 15 wild boar, and submitted to *Salmonella* detection (ISO 6579:2018) in order to determine the carrier status of the animals. Moreover, to evaluate the hygiene of the slaughtering process, nondestructive sampling was performed by sponge (after dressing and before chilling) on each carcass at cheek, belly, back and ham sites. On carcass sponge samples, mean level (log_{10}/cm^2) of aerobic colony count (ACC, ISO 4833), *Enterobacteriaceae* (ISO 21528-2) and *Salmonella* detection (presence/absence) were determined. Overall, samples from 75 wild boar were collected. Presumptive *Salmonella* isolates were confirmed by a *Salmonella*-specific PCR. A subset of 25 *Salmonella* confirmed isolates was serotyped. *Salmonella* prevalence in wild boar differed between sampling days and was comprised between 33.3% and 80%. *Salmonella* was detected in lymph nodes and/or colon of 54.6 % wild boar (41/75) highlighting the potential role of this specie as reservoir and spreaders of the zoonotic agent. *Salmonella* was also detected in 2.6% (2/75) carcass samples during the same sampling day. The following *Salmonella* serotypes were identified: *S.* Abony (36%; 9/25), *S.* Hermannswerder (24%; 6/25), *S.* Derby (20%; 5/25), *S.* Agona (12%; 3/25), *S.* London (8%; 2/25) and *S.* Elomrane (4%; 1/25). Among the identified serotypes, *S.* Derby is often detected in wild and domestic pigs and is frequently associated to human salmonellosis [2]. The other serotypes are not associated to wild boar and the source of transmission should be clarified. The ACC and *Enterobacteriaceae* values indicate a good hygiene status of the carcasses with mean levels always ≤4 and 1.5 log_{10} ufc/cm^2 respectively for ACC and *Enterobacteriaceae*. The high prevalence of *Salmonella* in wild boar can pose a risk for human health. However, our data shows good slaughtering hygiene procedures, with acceptable contamination levels and low prevalence of *Salmonella* on carcass surfaces.

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DIVERSITY AND ANTIMICROBIAL RESISTANCE OF PATHOGENIC AND NON PATHOGENIC *ESCHERICHIA COLI* ISOLATED IN THE BROILER PRODUCTION CHAIN

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Avian pathogenic *Escherichia coli* (APEC) causes extraintestinal diseases in broilers and share common virulence factors with extraintestinal pathogenic *E. coli* (ExPEC) isolated in humans suggesting the zoonotic potential of APEC [1]. APEC are characterised by a high intra-population diversity in terms of serogroup, MLST and virulence genes [2]. The aim of the present study was to investigate the diversity and antimicrobial resistance of *E. coli* isolated from lung, spleen, pericardium and brain of broilers affected by colisepticemia and treated with enrofloxacin. In three different farms with ongoing infections, at day 0 (before starting the treatment), day 5 (last day of treatment), day 10 and day 20 (after treatment), 10 birds were humanly euthanized and their organs investigated for *E. coli* isolation by standard microbiological procedures. A total of 179 *E. coli* were isolated and submitted to pulsed field gel electrophoresis (PFGE) and minimum inhibitory concentration (MIC) to gain insights on their genetic diversity and antimicrobial susceptibility. Based on metadata as well as MIC and PFGE data, 31 isolates were whole genome sequenced on MiSeq platform (Illumina, 250 bp reads, paired ends). Genomic diversity by SNP calling and in silico MLST as well as antimicrobial resistance and virulence-associated genes were analysed with publicly available bioinformatics pipelines. According to PFGE results, in all three farms the genetic diversity was overall low before the treatment and increases along and after. At day 0, *E. coli* isolates can be gathered in 3 - 6 clusters at 95% similarity. Whereas at day 5, 10 and 20 the number of clusters rises up to 10-16 with the majority of single-isolate clusters. Similar results were confirmed by MLST and SNP calling analyses on the sequenced genomes. MIC data showed a significant increase of resistance to enrofloxacin and flumequine in *E. coli* isolates collected at day 5, 10 and 20 in comparison to day 0. Along with this drug, a significant increase of resistance to trimethoprim/sulfametoxazole (SXT) was observed in one of the three farms. These results suggest that the antibiotic was effective in eradicating the initial pathogen strains responsible for the colibacillosis infections. In all farms, fluoroquinolone resistant *E. coli* were collected after enrofloxacin treatment. In one farm, a high number of SXT resistant strains arose, although their SXT resistance genes were genetically unrelated to enrofloxacin resistance genes/mutations. Finally, the gross pathological lesions encountered before the treatment on diseased birds disappeared after the treatment in all organs except for lungs, suggesting the lower virulence of *E. coli* isolates collected after the treatment from spleen, pericardium and brain.

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EDUCATION IN VETERINARY PUBLIC HEALTH: A BRIEF ANALYSIS ON EIGHT DIFFERENT EUROPEAN PROGRAMS

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The changing role of the veterinarian and the occurrence of new emerging diseases of animal origin and human implications are strong reasons to encourage a set of adjustments in veterinary medicine educational programs [1]. Moreover, in the field of Veterinary Public Health (VPH) there is an urgent need of interdisciplinarity and collaboration (One Health Initiative) [2]. The OIE advised that potential veterinarians should be equipped with the necessary competencies to perform efficiently and to successfully support Veterinary Services [3, 4]. In order to develop a new curriculum, the programs of eight universities accredited by the European Association of Establishments for Veterinary Education (EAEVE) were examined and attention in this paper was given specifically to VPH. Educational methods, courses organization and differences due to the specific National Veterinary Systems were evaluated. Visions, focus and teaching objectives were analyzed according to the informative webpages, Self- Evaluation (SERs) and EAEVE final reports of eight Veterinary Schools in Western Europe, including Bern- Zurich, Bologna, Hannover, Helsinki, London, Madrid, Maisons-Alfort and Utrecht. The organization of National Health Systems and the national legislations determined relevant differences in veterinary curricula. Major disparities concerned the implementation of bachelor and master programs and the requirement of post-degree diploma to apply for positions in the Veterinary Public Services. Some schools have specific tracks that students can choose (e.g. Bern-Zurich, Utrecht) and use an integrated teaching approach. Basic concepts of epidemiology, preventive medicine, meat inspection and food hygiene are included in the different types of curricula, thus complying with the EAEVE standards, but not all programs have well-defined dedicated courses in VPH for all students (e.g. small animal tracks). In the area of Food Safety and Quality were found important differences as well, particularly in the number of lectures, seminars, self-directed and/or supervised lab- and desk-based training. The presence or absence of a dedicated tracking choice, different Masters and distinct requirements of post- degree diplomas have significant impact in the education systems. Differences in the teaching methods not only concern the hours of lectures vs self-directed and supervised training, but also the development of knowledge trough an interdisciplinary approach to the improvement of the professional skills.

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ORAL COMMUNICATIONS

AMV

AGE-DEPENDENT REGULATION OF OREXIN AND NPY IN THE BRAIN OF THE SHORT-LIVED FISH, *NOTHOBRANCHIUS FURZERI*

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The role of orexinergic and NPY-ergic systems during vertebrates aging has been widely studied in mammals. Here, we propose to employ a non-mammalian model species, the teleost *Nothobranchius furzeri,* to explore the conserved orexigenic role and the age dependent regulation of Orexin A (OXA) and its precursor hypocretin *HCRT*, and neuropeptide Y (NPY). OXA and NPY are two neuropeptides primarily involved in the regulation of feeding behavior and food intake in all vertebrates [1,2]. Furthermore, they orchestrate several physiological processes such as arousal, whole-body energy metabolism, reward seeking, autonomic function, sexual behavior, and ventilatory control [3]. Accumulating evidences document that they undergo age-related modifications, with consequences on metabolism, sleep/wake disorders and progression of neurodegenerations. We conducted experiments of short-term fasting in young and old animals, and measured levels of *HCRT* and *NPY* in the whole brain and dissected diencephalon of specimens. To better evaluate the metabolic stimulus of short-term fasting, we measured the levels of pS6, a marker of activated neurons. Remarkably, *HCRT* levels were unchanged either in young and old animals, whereas *NPY* was significantly increased only in young animals. Then, we evaluated the neuroanatomical organization of orexinergic and NPY-ergic neurons and the age-dependent regulation of *HCRT* and *NPY* in the whole brain of *N. furzeri.* Immunohistochemical and *in situ* hybridization experiments demonstrated that (a) HCRT and OXA and NPY mRNA and protein are localized in neurons of diencephalon and optic tectum, as well as in numerous fibers projecting through the entire neuroaxis, and are colocalized in specific nuclei; (b) in course of aging, *HCRT* and *NPY* mRNAs expressing neurons are localized also in telencephalon and rhombencephalon. Furthermore, quantitative analyses on the whole brain of young, adult and old animals documented that *HCRT* was not significantly regulated over aging, while NPY was remarkably overexpressed, in good agreement with the morphological observations.
These findings shed light on new aspects of orexinergic and NPY-ergic systems regulation in the brain of vertebrates, demonstrating an uncommon and unprecedented described regulation of these two orexigenic neuropeptides during aging.

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CHARACTERIZATION OF NESFATIN-1 IN THE BRAIN AND STOMACH OF THE TELEOSTEAN MODEL FOR AGING RESEARCH *NOTHOBRANCHIUS FURZERI*

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The anorexigenic peptide Nesfatin-1 (Nesf-1) and its precursor NEFA/nucleobindin 2 (NUCB2) showed a highly conserved structure between mammalian and non-mammalian species [1]. In Mammals, NUCB2/Nesf-1 were detected both in appetite-control hypothalamic nuclei [1] and in peripheral tissues involved in energy metabolism. In particular, NUCB2/Nesf-1 were described in gastric X/A-like endocrine cells and the highest mRNA expression allow to considered stomach as the main sources of circulating Nesf-1 [2]. In Teleosts, similarly to Mammals, NUCB2/Nesf-1 were mostly detected in feeding regulatory hypothalamic nucleus and in gut, with higher expression in the anterior gastrointestinal tract respect to the brain [3-4]. Although tissue distribution of NUCB2/Nesf-1 is well known in Vertebrates, there are few reports on their expression during ontogenesis. This study aimed to examine for the first time the age- related central and peripheral expression of NUCB2/Nesf-1 in the teleost fish *Nothobranchius furzeri*, a well consolidated model organism for aging research. Experiments were performed on brain and stomach of *N. furzeri* belonging to MZM 04/10 strain, at the following time points: 5 weeks post hatching (wph) (young-adult, age of sexual maturity) and 27wph (old). Phylogenetic analysis displayed that gene structure was well conserved in *N. furzeri*, showing 78% of similarity with medaka, the closest related species. Real-Time PCR revealed increased expression levels in old fishes compared to young both in brain and stomach. Then we carried out *in situ* hybridization against NUCB2 and immunohistochemistry against Nesf-1. Western blot on homogenates of brain confirmed the specificity of Nesf-1 antibody, revealing a band of about 40 kDa. Either in young and aged brains both NUCB2 mRNA transcript and Nesf-1 immunoreactivity cells were detected in the hypothalamic area. Interestingly mRNA expression and protein distribution were observed in non diencephalic regions, specifically telencephalon, optic tectum and semicircular tori at all age stages analyzed. Telencephalon, diencephalon and optic tectum are brain regions involved in appetite control of Teleosts [5]. Either in young and aged stomachs NUCB2 mRNA transcript was detected mainly in the lining epithelium while Nesf-1 immunoreactive cells were distributed in the submucosae. This study represents a step for understanding the regulation of NUCB2/Nesf-1 during vertebrate aging processes.

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EXPRESSION OF TRKA RECEPTOR IN ADULT ZEBRAFISH BRAIN

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In our previous study, the presence and distribution of nerve growth factor (NGF) were described in adult zebrafish brain [1]. In mammals, the signaling of mature NGF (mNGF) is transduced by the tropomyosin receptor kinase A (TrkA) and acts as mediator in many essential functions in the central nervous system. Since TrkA expression was described in developing zebrafish [2], the aim of the present study is to describe the pattern of distribution of TrkA mRNA in the brain of adult zebrafish. The investigation was conducted on brain fixed in paraformaldehyde. Sections were firstly incubated overnight with digoxigenin-labeled riboprobes for TrkA [2] and then with anti-digoxigenin Fab fragments conjugated with alkaline phosphatase. The chromogenic reaction was carried out by using Fast Red substrate. After counterstaining with DAPI, sections were visualized in fluorescence microscopy.

In the telencephalon, TrkA mRNA was found in the olfactory bulbs, specifically in the glomerular layer and in the external cellular layer. In addition, some positive round small neurons were seen in the regions of the dorsal area and rare positive neurons in the ventral area.

In the diencephalon, TrkA mRNA was seen in some neurons of the hypothalamus, in few neurons of mammillary body and synencephalon.

In the mesencephalon, TrkA mRNA was detected in neurons of the optic tectum, specifically in the periventricular grey zone and superficial grey and white zone, and in neurons of the semicircular tori.

Concerning rhombencephalon, TrkA mRNA was highly present in neurons of all regions of the cerebellum, and in neurons of cerebellar crest.

Our study highlighted the wide expression of TrkA receptor throughout all brain regions of adult zebrafish. In mammals, this receptor mediates the action of mNGF [3]. However, in zebrafish brain extracts, only the immature form of NGF (pro NGF) was found. These findings suggest a different NGF signaling in zebrafish or the presence of low and fleeting undetectable quantities of mNGF.

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MOLECULAR FEATURES OF EQUINE AMNIOTIC PROGENITOR MESENCHYMAL CELLS AND THEIR SECRETED MICROVESICLES

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Mesenchymal stem cells (MSCs) are non-hematopoietic adult multipotent cells capable of secreting a variety of bioactive molecules as part of their local trophic and immunomodulatory activities. Recent evidences have demonstrated that MSCs secrete extracellular vesicles (EVs) which can transfer protein, messenger RNA (mRNA) and micro RNA (miRNA) into cells. This makes EVs effective in tissue repair [1]. Recent studies have demonstrated that internalization of EVs from the equine amniotic mesenchymal cells (eAMCs-EVs) have a potential therapeutic application in equine tendon lesions [2]. The MSC surface is coated with a glycocalyx that regulates several aspects of stem cell biology and can be transferred to secreted EV surface. Since glycans are located on the cell and EV surface, their roles in EV function, biogenesis, release, and transfer are critical. In this study, the surface glycosylation pattern of both eAMCs and eAMC-EVs, as well as the morphology and surface protein markers of eAMC-EVs were analyzed. Equine AMCs were isolated from mare amniotic membranes and cultured to obtain conditioned medium from which EVs were isolated [2]. The glycosylation pattern of eAMCs and eAMC- EVs was investigated using a novel microarray-lectin procedure [3]. Extracellular vesicles protein markers were characterized by western blot analysis. Lastly, EVs morphometric analysis was performed using a Nanosight instrument (NTA) and a transmission electron microscope (TEM). Compared with eAMCs, glycomic investigation revealed that eAMC-EVs displayed i) enrichment in Galβ1,3GalNAc terminating O-glycans, α2,3-linked sialoglycans, and high-mannose N-glycans; ii) decreasing in N- acetyllactosamine, GalNAc, Gal, GlcNAc, and fucose terminating glycans; iii) no change in α2,6 linked sialoglycans content. Western blot analysis showed CD56, CD81, EphA2 and HSP70 receptors as well as an unusual HSP60 antigen in eAMC-EVs surface. At TEM analysis, EVs displayed a moderately electron- dense coat and appeared spheroid in shape with a diameter from 21.3 to 120.6 nm. These values matched with NTA data. In conclusion, the comparison of eAMCs and eAMC-EVs glycan patterns demonstrated that eAMC-EVs come from a specific portion of the parental cell membrane. In addition, the eAMC-EVs size together with the higher presence of CD81 than HSP70, suggest that eAMCs produce exosomes [4] that are characterized by a specific HSP60 receptor.

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LOCALIZATION OF THE APELIN SYSTEM IN THE OVARY AND GENITAL TRACT OF EWES SUBJECTED TO DIFFERENT NUTRITIONAL LEVELS

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The present work was aimed to evaluate the presence and localization of the apelin system in the reproductive system of the ewe. Apelin is a recently discovered adipokine mainly secreted by adipose tissue and also produced by several peripheral tissues. A biological activity of apelin on female reproduction was supposed since this molecule regulates gonadotropin release and steroidogenesis [1].

Moreover, it was evidenced in ovary and uterus with differences among species [2]. In this work, apelin and its receptor (APLNR) were evaluated by immunohistochemistry and PCR at the level of ovary, oviduct and uterus. The trial was carried out on 15 ewes grazing on a seminatural pasture and slaughtered at the maximum pasture flowering (MxF, 5 ewes) and at the maximum pasture dryness (MxD, 10 ewes). Five ewes of the second group were fed with 600 gr/die/head of barley and corn (1:1) in addition to the fresh forage (Exp). Since apelin is involved in energy metabolism, sheep were fed differently to assess whether the feeding can modulate the secretion of the apelin system. Accordingly, during the grazing period, concentrations of plasma apelin were evaluated every 15 days. Experimental procedures were approved by the Ministry of Health (No. of approval 95/2018-PR).

The transcript and protein for both apelin and APLNR were evidenced in all tissues evaluated. In the ovary the corpus luteum only showed a strong positivity for APLNR and a weaker staining for apelin while the other structures appeared negative. In the oviduct and uterus, both molecules were observed in the lining epithelium and in the uterine glands as uterus concerns. The highest levels of apelin and APLNR mRNA were detected in the MxD and EXP groups in the luteal phase of the estrous cycle in comparison to MxF group slaughtered during the anestrous phase. Pasture dryness had no effects on the plasma apelin levels.

The present data confirm that the apelin is involved in the reproduction function also in the ewe, being differentially distributed and expressed in the ovary and genital tract. The overlapping localization of both apelin and its receptor suggest a paracrine and autocrine action of apelin likely aimed at regulating the development and function of corpus luteum and the activity of genital tract such as epithelium proliferation and secretion. In addition, it can be supposed that apelin may be secreted in the lumen of oviduct and uterus playing a nutritional role for both oocyte and embryo respectively. Differences of expression evidenced among ewe groups are likely due to the cyclic activity of tissues rather than diet influence.

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HIGHPLEX IMMUNOFLUORESCENCE ON WHOLE SECTION AND TISSUE MICROARRAY FOR THE CLASSIFICATION OF MOUSE LUNG PARENCHYMA CELLS

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Single-cell omics are high-throughput molecular technologies currently used to perform screening or functional analysis in human diagnostics research [1]. Most of them require cellular dissociation, impeding the morphological allocation of individual cell types. Highplex immunofluorescence (IF), enable to analyze numerous epitopes in thousands of cells in a single experiment, providing a systematic view and maintaining the spatial and morphological features of normal/disease processes at the single- cell level. Our purpose is the translation of this high-throughput method from human diagnostics discovery process to animal model research. In this study we intend to classify, the murine lung structural cells trough highplex IF comparing the readouts from whole histological sections and Tissue MicroArray (TMA) cores. Sequential multiplex IF for structural and inflammatory antibodies (CD31, VWF, Cytokeratin panel, TTF-1, Collagen I, alpha-SMA, Desmin, SM22, Vimentin, MMR/CD206, CD3, Myeloperoxidase) were performed on FFPE archive mouse lung samples. Each multiplex IF round, was carried out on both whole section and TMA; Whole slide images (WSI) were digitally acquired (Nanozoomer S-60, Hamamatzu), the auto-fluorescence was subtracted [2] and each channel, in association with different marker, was co-registered based on DAPI staining (FIJI,[3]). A segmentation mask to extract single cell data from WSI was applied (Cell Profiler,[4]). Finally, the markers quantification and the spatial features of single cell, were extracted from each acquisition, combined and visualized using tSNE multidimensional reduction tools (HistoCAT,[5]).The highplex IF based cell classification was successfully obtained from both whole section and core histological substrates. The cells phenotype classification through tSNE allows a data dimensionality reduction transforming high dimension cell data into two dimension grouping similar cells. The obtained clustering of parenchymal cells from whole section and TMA showed high rates of concordance indicating, for the latter, an accurate representation of the donor tissue. Moreover, the maintenance of morphological and histological structures consents to investigate the presence and significance of interactions between neighboring cells. Combined highplex IF and TMA are preferable as they allow simultaneous analyses of multiple organs/animals or experiments improving standardization, avoiding experimental variability and technical artifacts.

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OREGANO DIET SUPPLEMENTATION ENHANCES SWINE GUT DEFENCE ABILITY

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Different studies have focused on use of natural compounds as alternative to synthetic additives to avoid the growing problem of antibiotic resistance. Among officinal plants *Origanum vulgare* L. has been shown to possess interesting antioxidant and antibacterial properties [1]. In addition, oregano is able to induce a higher glycoconjugate production in gut creating a physical barrier against microorganisms [2]. This study aimed to evaluate the effects of oregano aqueous extract (OAE) dietary supplementation on pig intestine complex carbohydrates, detected by conventional histochemistry, and oxidative stress, using as target molecule Bcl-2 Associate X protein (BAX). Thirty two pigs were divided in two groups and fed according to the following dietary treatments: (1) degermed corn-barley-soybean-based diet (CTR group); (2) CTR group diet supplemented (2 g/kg) with OAE (O group) (OPBA Approval E81AC.10/A). Glycohistochemical and immunohistochemical analyses were carried out on different gut tract: duodenum, ileum, caecum and colon. Glycohistochemistry was performed by staining with Periodic Acid-Schiff (PAS), Alcian Blue (AB) pH 2.5, AB-PAS, AB pH 1, AB pH 0.5, low iron diamine, high iron diamine. Adjacent serial sections were pre-treated with Sialidase V before staining with AB pH 2.5 (Sial-AB) preceded or not by saponification with 1% KOH in 70% ethanol to remove the acetyl groups (KOH-Sial-AB). BAX protein detection was performed by immunohistochemistry. Positive histochemical responses were observed at goblet cell level in all examined gut tracts; duodenal glands were also reactive. The statistical analysis of histochemical reactivity intensity evidenced significant differences between the dietary treatments. The most noticeable difference regarded the response to Sial-AB and KOH-Sial-AB histochemical analyses which induced differentiated behavior of goblet cells in the samples derived from O diets in which sialoglycoderivatives perform specific defense action. O diet increased the production of highly acid glycoconjugates in the gut which improve mucosal lubrication and the formation of a viscoelastic barrier, with an unspecific defense mean. Immunohistochemical analysis revealed a lower presence of BAX in the examined intestinal tracts of O group compared with CTR group. Findings show that OAE supplementation improves the production of the glycoconjugates able to enhance the protection of the pig intestinal mucosa. In addition, the reduced BAX immunostaining observed in O group swine respect to CTR group suggests an enhanced antioxidant action in oregano supplemented diet group. Outcome should be taken into account for studies aimed at enhancing defence ability in order to reduce antibiotic use and prevent antimicrobial resistance.

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HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL EVALUATION OF THE EFFECTS OF A DIETARY SUPPLEMENTATION WITH YEAST FRACTIONS ON THE JEJUNUM OF BROILER CHICKENS

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Among the different available prebiotics, yeast cell walls have been widely used in poultry and animal feeding; they comprise mannanoproteins, β (1,3)-glucans, β (1,6)-glucans, chitin and glycophospholipid surface proteins associated with the plasma membrane. Their dietary supplementation have been found to promote immunoglobulin production, to prevent diseases by pro-inflammatory responses, as well as to alter gut microbiota composition through competitive exclusion, production of antimicrobial agents and change of the fermentation pattern of gut microflora [1]. The present study aimed at evaluating the effect of the dietary supplementation with yeast fractions (mainly mannano-oligosaccharides and β- glucans from *Saccharomyces cerevisiae* as SafMannan®) on the general morphology as well as on the secretion of glycoconjugates in the jejunum of broiler chickens aged 42 days. Moreover, specific markers of jejunal inflammation were detected by an immunohistochemical approach. A total of 24 chickens fed a control diet (C) or the same diet supplemented with yeast fractions (Y), were slaughtered by cervical dislocation to sample jejunum for histological and immunohistochemical analyses. The study was approved by the Ethical Committee for Animal Experimentation of the University of Padova. All animals were handled in respect to the principles stated by the EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes. One sample of the jejunum wall was taken at the midpoint between the end of the duodenal loop and the location of the Meckel's diverticulum, fixed in paraformaldehyde in phosphate buffer saline (0.1 M, pH 7.4), dehydrated, and embedded in paraffin. Serial sections of 4 µm were obtained using a microtome and stained with: i) haematoxylin/eosin for morphometric evaluation, ii) Alcian-PAS staining for a quantitative and qualitative analysis of goblet cells, and iii) antibodies against CD3 intraepithelial T-cells and CD45 intraepithelial leukocytes. The villi length, the depth of the crypts and number of goblet cells were measured with an image-analysis software (Cell B, Olympus Soft Imaging Solutions, Co., Hamburg, Germany). At 42 d, nor villi height, crypt depth or villi to crypt ratio were affected by the dietary treatment. As regards glycoconjugate secretion, a mixture of neutral and acidic glycoconjugates were secreted by goblet cells whereas the number of goblet cells were significantly higher in animals fed the diet supplemented with yeast fractions. The density of CD45+ cells was significantly higher in control animals, whereas a trend was recorded in the case of CD3+, i.e. birds fed diet supplemented with yeast fractions had a lower density of CD3+ cells. Statistical anlysis was performed by a Generalized Linear Mixed Model (GLMM). In conclusion, the diet supplemented with yeast fractions improved the animal response in terms of increasing glycoconjugate secretion and lower jejunal inflammation (lower intraepithelial leucocytes density).

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EFFECTS OF BIOACTIVE MOLECULES ON ALVEOLAR MACROPHAGES AND GASTROINTESTINAL TRACT OF *SUS SCROFA*

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In the last years, great importance has been given to the beneficial effects of polyphenols [1]. Agricultural by-products are a rich source of polyphenols and have a potential suitable for development into dietary supplements and various food additives [2].
The objective of the present research was to investigate gut morphology, inflammation and immune response of adult pigs (Casertana strain) fed with polyphenols extracted from olive mill wastewater added to the standard diet, by *in vivo* and *in vitro* analysis. The 'Casertana' pig is an ancient autochthon genetic type and represents an experimental model suitable for semi-wild controlled breeding technique.
During the finishing period, pigs were randomly assigned in two groups: control group fed with a standard diet and treated group fed with standard diet supplemented with polyphenols extracted from olive mill wastewater (OMWW) using capsules (0.03 g/kg of feed per pig per day) [3]. The trial lasted for 120days. After slaughter, alveolar macrophages were extracted from pig lungs [4] and the superoxide anion assay was performed to test the anti-oxidant effects of polyphenols extracted from OMWW. Gastrointestinal tracts (stomach, duodenum, jejunum, ileum, caecum and colon) were collected from all animals, embedded in paraffin wax, serially cut in transversal sections and stained with hematoxylin– eosin for histomorphometric analysis.
No significant differences in the length of the jejunum-ileum villi and the depth of the colon crypts were detected between control and polyphenols fed pigs. COX-2 immunoreactivity in the gastrointestinal tract was more intense in the control group. The low level of expression of COX-2 in immunoreactive cells in the intestine of treated pigs could suggests a protective role of polyphenols, modulating and reducing the inflammatory response [5]. Superoxide anion production in alveolar macrophages was lower in pigs fed polyphenols ($p<0.05$).
In vitro studies suggested that OMWW polyphenols are potent antioxidants, while the interpretation of the *in vivo* experiments is more problematic and further studies are necessary on the interactions between bioactive feed compounds and intestinal status.

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THE EFFECT OF *CITRUS SINENSIS* POLYPHENOLS ON ADULT ZEBRAFISH GUT INFLAMMATION INDUCED BY ORAL INFECTION WITH *VIBRIO ANGUILLARUM*

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The polyphenols present in plants and their fruits have several biological and pharmacological activities, including anti-inflammatory properties [1]. The Tarocco orange (*Citrus sinensis*) is a typical fruit of the southern part of Italy, especially present in Sicily, but also in California, (USA), and Spain. *Citrus* fruits and juices can be considered among the most common dietary sources rich in poliphenols [2]. We have analyzed whether or not a natural extract from Tarocco orange (*C. sinensis*) is able to counteract the enteritis induced in adult zebrafish by *Vibrio anguillarum*, through live feed (*Artemia nauplii*). It is hypothesized that polyphenols from these fruits are able to play a role in attenuating inflammation due to a gram-negative infection. Adult male and female zebrafish (n=80) were divided into four groups. The first group was used as control; the second group as positive control for enteritis induced by *V. anguillarum* loaded *Artemia*; the third group, treated with *Citrus* Juices extract (CJe) (30 days), in order to detect the effects of extract on the intestine; the fourth group, treated with CJe (30 days), before the induction of enteritis using *V. anguillarum*-loaded *Artemia*. At the end of the experiment all fish were sacrificed and the gut was rapidly removed and dissected for the histological assay, immunohistochemistry and qRT-PCR. The third and fourth groups were sampled at 1, 3, 5 days post infection. Histological evaluation and qRT-PCR for IL-1β, IL-6, as well as TNFα, were performed to analyze the severity of inflammation in fish exposed to different experimental conditions. Fish infected through *V. anguillarum*-loaded *Artemia* showed histo-pathological evidence of enteritis in anterior and medium segments of the intestine and an over-expression of the main inflammatory cytokines. A previous treatment with CJe resulted in a remarkable reduction of tissutal inflammatory events, as well as a molecular down regulation of the inflammatory signs. The present findings strongly suggest that the treatment with natural extract of *C. sinensis* is able to counteract the structural evidence of enteritis and to modulate the expression of the main pro-inflammatory cytokines in adult zebrafish. Therefore, these natural polyphenols could be useful for the prevention of enteric pathologies.

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EVALUATION OF CANINE PLACENTA VASCULARIZATION AND ITS RELATION WITH NEWBORN BIRTH WEIGHT

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Neonatal mortality in dogs may be related to low birth weight and fetal growth restriction, as shown in humans where it is linked with placental factors (i.e. vascular perfusion) [1,2]. Placentas collected from twenty bitches, 9 toy-sized (< 5 Kg) and 11 small-sized (5 to 10 Kg), were included in this study. During natural delivery or c-section, newborns and the corresponding placentas were identified and weighed, the latter after removal of extraplacental adnexa; thereafter a picture was captured to macroscopically measure the Transfer Zone Area (TZA). A suitable sample including TZA was thus collected and, after paraffin embedding, it was sectioned for histology. On 5µm thick sections, immunohistochemistry was performed using a commercial anti CD31 antibody to identify blood vessels (PECAM-1 (H-3) mouse antibody, Santa Cruz Biotechnology, TX). Briefly, after antigen retrieval and endogenous peroxidase blocking, slides were incubated overnight at 4°C with the anti CD31 antibody diluted at 1:400 in PBS. The biotinylated horse anti-mouse antibody followed by Avidin-Biotin Complex and diaminobenzidine (Vector Laboratories) were used for color development. On five randomly selected fields, photographed at 40x, the area occupied by blood vessels was measured, and a vascularization index (VI, CD31 positive tissue on the image total area) was determined for each placenta. The Total Vascular Area (TVA) was thus estimated (VI*TZA). Newborn birth weight was positively correlated with placental weight ($P<0.001$, $r=0.689$), TZA ($P<0.001$, $r=0.772$) and TVA ($P<0.001$, $r=0,482$). Moreover, a positive correlation was found between placental weight and both TZA ($p<0.001$, $r=0.583$) and TVA ($p<0.001$, $r=0.333$). The VI was higher in toy-sized compared to small-sized bitches' placentas (15.08±2.50% vs 13.35±2.62%, respectively, $P<0.01$). Placental weight, TZA and TVA were significant determinants of birth weight in normal canine pregnancies as reported for humans [3]. PECAM-1, previously used for human placentas [4], was able to identify canine placental endothelial cells. Our data might provide reference values for placental weight, TZA, TVA and VI in toy and small-sized dog breeds.

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GAMETOGENESIS IN WILD AND CAPTIVE-REARED GREATER AMBERJACK *SERIOLA DUMERILI* (RISSO, 1810)

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Knowledge of gametogenesis is an essential pre-requisite to develop reliable technologies for the reproduction control of new candidate fish species for aquaculture. The greater amberjack *Seriola dumerili* (Risso, 1810) is a pelagic fish with rapid growth and excellent flesh quality, whose domestication represents an ambitious challenge for the European aquaculture. This research summarizes the results of some comparative studies on the process of gametogenesis in greater amberjack sampled from the wild and in individuals reared in captivity and exposed to handling [1-3; unpublished data]. Twenty-four individuals (12 males and 12 females) reared in captivity in a sea cage in Salamina (Greece) and 33 individuals (14 males and 19 females) caught from the wild around Pelagie Islands, were sampled during three phases of the reproductive cycle: early gametogenesis (late April- early May), advanced gametogenesis (late May-early June) and spawning (late June-July). Gonad samples were collected for histological and immunohistochemical analysis, and gonad and liver samples were collected for molecular analyses. Male proliferating and apoptotic germ cells were identified through the immunohistochemical localization of Proliferating Cell Nuclear Antigen (PCNA) and the TUNEL method, respectively. Vitellogenin (*vtga*, *vtgb* and *vtgc*) and vitellogenin receptor (*vtgr* and *lrp13*) gene expression was evaluated by qRT-PCR in liver and ovary samples, respectively. During the early gametogenesis phase, no apparent difference was observed between ovaries of wild and captive-reared fish; testes of captive-reared males exhibited seminiferous tubules of a smaller diameter, a decrease of spermatogonial mitosis, and a high level of apoptosis. During the advanced gametogenesis phase, captive-reared breeders showed extensive atresia of late vitellogenic oocytes and spermatogenesis arrest. Gene expression of *vtga*, *vtgb* and *vtgc* did not differ significantly between captive-reared and wild female greater amberjack; however, captive females exhibited low *vtgr* and *lrp13* gene expression.

The present study described the occurrence of a severe reproductive impairment in greater amberjack reared in captivity and handled during the reproductive season. The observed gametogenesis impairment arose during the early phase of gametogenesis, when males showed high germ cell apoptosis and transcription of vitellogenin receptor genes was reduced in females.

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RESEMBLING A NAÏVE ANATOMICAL ENTITY: THE HIGH POTENTIAL HELD BY A PRE-VASCULARIZED ENGINEERED DERMA

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The rapid development of a physiological vascular network thorough organ and tissue regeneration is a key event in the restoration of both morphology and function of an injured anatomical entity. Here we assessed the performance of a pre-vascularized bioengineered derma to be used to replace a damaged original one. First of all, we produced dermal tissue equivalent by seeding freshly isolated fibroblasts onto gelatin microbeads and by culturing these biohybrids into spinner flask bioreactors [1]. Afterwards, we seeded primary endothelial cells on biohybrids surface in order to obtain pre- vascularized dermis (PVD) [2]. Over the entire culture time we used confocal and multiphoton imaging techniques to screen respectively capillary-like tubule formation and collagen deposition. Once PVD samples displayed the proper evolution degree we implanted them subcutaneously on the back of nu/nu mice. One week later, we retrieved the biohybrids integrated with the host tissue and, after fixation in formalin or paraformaldehyde; we analyzed them for immunofluorescence and histological analyses. Animal studies were performed following the guidelines of EU (2010/63/EU). We characterized the biohybrids based on the detection of primary dermal fibroblasts embedded into their own extracellular matrix (ECM). Confocal imaging showed that endothelial cells included into the PVD were able to form capillary like structures (CLSs) displaying an inner lumen 7 days after seeding. The molecular profile of the analyzed PVD samples revealed that the development of CLSs within the biohybrid tissue was induced by VEGF-A secreted by the endothelial cells included in it. In addition, CLSs were totally embedded into the ECM produced by the fibroblasts also showing several bifurcations like those normally reported in physiological tissues. Concerning mechanical properties and translatability our biohybrid tissue is easy-to-handle, suitable to be sutured and able to be produced in a size which is adequate for clinical use. On the other hand, the analyses of the retrieved explants showed that the capillaries formed within the bioengineered constructs were perfused by mouse blood, thus proving the establishment of functional anastomosis between biohybrid CLSs and host vessels. These results highlight the crucial role of prevascularization in promoting the development of a functional vascular network into engineered anatomical entities and their rapid integration with the host tissue. Based on these findings, we now aim to feature the in vivo response of a full thickness skin model, which is currently under development, for wound healing applications.

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FEATURING MORPHOLOGY AND FUNCTION OF A NEWLY ONSET VASCULAR NETWORK

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Regenerative medicine (RM) has the ambitious goal to heal damaged tissues. A successful RM approach relies on acellular scaffolds to promote tissue growth. The ideal scaffold should recruit host cells without evoking immune response. To guide regeneration it is essential to sustain angiogenesis inside the scaffold since vascularization is needed to provide an adequate metabolic supply and waste disposal. Here we propose a porous trilayer scaffold made of Polycaprolactone (PCL) microparticles [1] loaded with VEGF165 (human recombinant vascular endothelial growth factor) in order to stimulate angiogenesis. To store the bioactive factor polylactic-co-glycolic acid (PLGA) depots are inserted into the scaffold. We proved that our scaffold is able to support the growth of Human Umbilical Vein Endothelial Cells (HUVECs) with a peak at 72 hours. To identify the minimal concentration of VEGF able to trigger angiogenesis we performed a Matrigel [2] tube formation assay using different VEGF concentrations (10, 20, 30, 40 ng/ml). The only concentration able to give a significant response vs control was 40 ng/ml resulting in 32.6±6.1% higher total tube length in respect to control (pvalue<0,003). The VEGF loading capacity was tested comparing known concentrations of VEGF to the factor extracted from PLGA nanoparticles. Results indicate that the loading efficiency is around 30% since the tube formation abilities of the microparticles loaded with 100 ng/ml of VEGF are 4,2±1,6% higher than the 20 ng/ml condition, but 8.2±1.6% lower than the 40 ng/ml one. This *in vitro* test candidates our scaffold as a promising biomaterial for *in vivo* applications. To score the *in vivo* function of our construct we selected MicroCT analysis of the retrieved explants based on the use of Microfil [3], a silicon rubber able to fill and contrast microvascular spaces. To evaluate angiogenesis within the scaffold we developed a new *layer-by-layer analysis.* This method dissects the Micro-CT file in three identical parts, corresponding each to a scaffold's layer, and analyze the vessel signal in each of them. This methodology compared to the one previously set up by our group [1] results in a significant improvement being much more versatile and time saving. Therefore here we propose the design of a biocompatible porous trilayered PCL scaffold loaded with VEGF able to trigger angiogenesis *in vitro and* set-up a new method to track vessel formation by Micro-CT analysis posing the basis for the fabrication of novel RM product with a great proangiogenic potential. The proposed methodology could add detailed information to a wide range of morphological and functional analysis.

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ORAL COMMUNICATIONS

ANIV

EVALUATION OF PASSIVE IMMUNITY IN CALVES FROM IMMUNIZED PREGNANT CATTLE WITH gE-DELETED MARKER VACCINE AGAINST BOVINE *ALPHAHERPESVIRUS* 1 (BoHV-1)

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To date, several European countries are aiming at controlling or eradicating BoHV-1 infection by using *marker* vaccines [1]. These products are known to induce a high humoral and cell-mediated immune response [2], while a few information is available as to passive immunity. The aim of the present study was to evaluate passive immunity acquired by calves suckled by their own dam, previously immunized with gE-deleted *marker* vaccines for BoHV-1. Eighteen Friesian cattle at fifth month of pregnancy and seronegative to BoHV-1 were divided into 3 groups of 6 heads each. Two groups were immunized with two different commercial inactivated gE-deleted *marker* vaccines (A, B) and the third one was kept as control. After calving, each group was increased by the respective six newborn calves and serum samples were collected from all animals at different times until post-calving day (PCD) 180. Antibodies against glycoprotein B (gB) and E (gE) to BoHV-1 were evaluated using commercial ELISA tests. In addition, neutralizing antibodies (NA) were evaluated by virus neutralization test, using the OIE protocol (2018). In the same period, colostrum or milk samples were collected from lactating cattle and the samples were tested using commercial ELISA tests (indirect, gB, gE). The experimental protocols were performed under the approval of the Italian Ministry of Health (no. 653/2018-PR). After immunization, no adverse reactions were observed in all pregnant cattle. On the day of delivery, the cattle evidenced a mean NA titre from 1:1568 (vaccine A) to 1:1195 (Vaccine B). The mean NA titre of cattle immunized with vaccine A decreased to 1:730 on PCD 2. At the same time, it started to steady decline on PCD 180 to antibody titers of 1:240. Otherwise, the mean NA titre of cattle immunized with vaccine B decreased gradually to 1:128 on PCD 180. Immediately after delivery, the newborn calves derived from cattle immunized with vaccine A, evidenced a mean NA titre of 1:781, decreasing to 1:560 on PCD 2 and declining to a mean titre of 1:14 on PCD 180. The calves derived from cattle immunized with vaccine B evidenced at birth a mean NA titre of 1:779 and, successively, a gradual decrease to a mean titre of 1:8 on PCD 180. All the animals remained seronegative to gE and seropositive to gB throughout the entire experimental period. No seroconversion was detected in the control group. The colostrum or milk samples were seropositive to gB and indirect ELISA, while were seronegative to gE. This study demonstrated that the two vaccines employed can be safely administered to pregnant cattle and induced a valuable humoral immunity detectable for at least nine months after vaccination. Moreover, the passive immunity transferred to calves can be detected up to PCD 180. Further studies will be necessary to assess the degree of protective immunity against the challenge.

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DIFFERENTIAL EXPRESSION OF ENDOGENOUS RETROVIRUSES IN EQUINE PLACENTA: PRELIMINARY RESULTS

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Endogenous retroviruses (ERVs) are proviral phases of exogenous retroviruses that have co-evolved with vertebrate genomes for millions of years [1]. Conservation of ERV genes through evolution suggests beneficial effects to their hosts' survival. An example of such positive selection is demonstrated by the *syncytin* genes that have a key role in placentation [2]. Until now, all the characterized syncytins have been associated with the highly invasive placentation type: endotheliochorial, synepitheliochorial and hemochorial [3]. Only recently, a study has found a retroviral *env* gene (EqERV) with syncytin-like properties in horses, having epitheliochorial placenta, and appraise its expression in different equine tissues (spleen, liver, lung, kidney and placenta), demonstrating that it is expressed at higher level in the placenta than in the other tissues [4]. Considering the type of placentation in equine species and the possible role of this gene, the exact localization of the EqERVs *env* gene expression could be relevant in the understanding of equine reproductive physiology and pathology. The aim of the current study was to show if there is a further different EqERVs *env* expression pattern in various areas of the same placentas (amnion, cervical star, corion). In order to better investigate the expression of EqERVs in target tissues, an improvement in the detection of the EqERVs *env* gene was performed, through an *in silico* analysis. Primers were designed on the *env* region of the candidate full-length ERV. Placentas were collected immediately after an eutocic delivery and a sampling of specific areas (amnion, cervical star, corion) of each placenta was carried out. Total RNA was extracted, treated with DNase I and reverse transcribed into cDNA, before qPCR reaction. The expression ratio of the gene of interest was normalized relatively to two reference genes. Among the selected full-length EqERVs, we found only one candidate showing an ORF longer than 300 aa suitable for downstream investigation. qPCR assay showed that this gene is regularly highly expressed in the placenta as expected for a candidate syncytin- like gene. Additionally, a different EqERV *env* pattern of expression also was found between amnion, cervical star and corion. These findings suggest a possible role of EqERV in placental tissue, which deserves further investigation. Moreover, because in horses there is no syncytiotrophoblast layer at their maternal-fetal interface, a putative immunological role of this EqERV in relation with maternal- fetal tolerance could be hypothesized. A possible interference with diagnostic activity for exogenous Equine Infectious Anemia virus should also be evaluated.

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ROLE OF CAPRINE *HERPESVIRUS*–1 (CPHV-1) IN THE INNATE IMMUNO-EVASION INTERFERON MEDIATE

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Caprine Herpesvirus-1 (CpHV-1) is a member of *Varicellovirus* genus within *Herpesvirus* family. CpHV-1 is the responsible of a disease that causes respiratory symptoms, balanopostitis, vulvovaginitis and abortion in adult goats. It also affects young goats with a systemic disease [1]. Type I (IFN-a/ß) and type II (IFN g) interferons, are the first weapons of the host to fight against viral infections. Type I IFNs induce the expression of more than 100 Interferon Stimulated Genes (ISGs) to establish an antiviral state that limits viral replication and dissemination. [2] Type II IFN is produced by activated immune cells and leads to the production of a different subset of ISGs via a distinct signaling pathway. Many viruses are able to subvert both type I and type II IFN-mediated antiviral responses. *Herpesviruses* are able to evade the IFN response by targeting different transcriptions factors of the interferon (IFN) signaling pathway [3]. There are no reports in literature about the role of CpHV-1 in IFN antagonism. Aim of the work: Herein, we describe a work to address the possible role of CpHV-1 as modulator of the innate immune response interferon mediate. Materials and methods. To investigate whether CpHV-1 interferes with type I interferon production, we performed an IFN-ß reporter assay, using a reporter plasmid that carries the IFN-ß promoter driving the expression of a firefly luciferase gene. A renilla-luciferase reporter plasmid was used as control. For the activation of the pathway we used the constitutively active N-terminal (2CARD) domain of RIG-I. To evaluate the potential CpHV-1-mediated inhibition of IFN a/ß signaling, we performed an ISRE54 reporter assay using a construct having an ISRE54 promoter driving the expression of firefly luciferase. A renilla-luciferase reporter plasmid was used as internal control. Results. Our results show that CpHV-1 infection strongly suppressed the activation of IFN-β promoter induced by RIGI 2 CARD domain. Moreover cells mock infected and treated with type I IFN showed a significant increase in luciferase expression, as expected compared with the cells that were not treated with type I IFN and were not infected. The cells infected with CpHV-1 and treated with type I IFN showed significantly reduced luciferase expression driven by the ISRE54 promoter.

These results showed that CpHV-1 is a strong inhibitor of type I Interferon production and signaling pathways.

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PROLINE RICH REITERATIONS IN MEQ PROTEIN AMINO ACID SEQUENCE OF ITALIAN GALLID ALPHAHERPESVIRUS 2 STRAINS ARE PREDICTIVE FOR MOLECULAR PATHOTYPING

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Marek's disease (MD) is a lymphoproliferative disease important for the poultry industry worldwide caused by Gallid alphaherpesvirus 2 (GaHV-2). GaHV-2 isolates virulence shifted over the years from mild to virulent, very virulent and very virulent + [1]. Nowadays the disease is controlled by vaccination, but field strains of increased virulence are still emerging worldwide. Economical losses due to MD are mostly associated with the acute form of the disease, characterized by visceral lymphomas. The GaHV-2 virulence determination by detection of molecular markers is a valuable method comparing to in vivo pathotyping assays which requires complex trials involving the use of a large number of experimental birds. The present study aimed to molecularly classify a group of 13 GaHV-2 strains detected in vaccinated Italian commercial chicken flocks during acute MD outbreaks, and to scrutinize the ability of predicting GaHV-2 virulence, according to the meq gene sequence. The full-length of the meq genes were amplified and the obtained amino acid (aa) sequences were analyzed, in comparison with five prototype strains, focusing mainly on the number of stretches of four proline molecules (PPPP) within the proline rich reiterations of the transactivation domain. A phylogenetic tree, based on the obtained meq gene aa sequences, on previously published sequences of Italian strains detected in backyard flocks and on selected GaHV-2 strains retrieved from GenBank, was built using the Maximum Likelihood method under the Jones-Taylor-Thornton model. All the analysed strains showed 100% sequence identity in the meq gene and encoded for a 339-aa long Meq protein including 4 PPPP motifs in the transactivation domain and a Proline-to-Serine substitution at position 218, interrupting a PPPP at third position. These features are typically encountered in highly virulent isolates [2]. Phylogenetic analysis revealed that the analysed strains belonged to a cluster in which are included high-virulence GaHV-2 strains detected in Italian backyard flocks and a hypervirulent Polish strain [3]. Our results support the hypothesis that the virulence of field isolates can be reliably predicted from the Meq protein aa sequence analysis. As in vivo pathotyping is not easily achievable, the meq gene molecular characterization turns out to be the most rapid and reliable way for pathotyping field strains. However, the molecular findings should be supported by clinical observations, necropsy findings and vaccination status.

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POTENTIAL OUTBREAK OF AUJESZKY'S DISEASE IN CATTLE IN NEBRODI AREA (SICILY): PRELIMINARY DATA

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Aujeszky's disease (AD) is a disease affecting mainly pigs, caused by Suid Herpes Virus-1 (SHV-1). Suids are the natural reservoir of SHV-1 whereas the disease is self-limiting in other species [1]. The disease is rapidly fatal in cattle, which is considered an "aberrant" host. The promiscuous husbandry system, typical of the Nebrodi area, is considered a critical issue in the application of adequate biosecurity measures for the control of AD. The purpose of this study is to describe the clinical and histopathological aspects of a suspected outbreak of AD in cattle in the Nebrodi area. The outbreak was recorded in a farm in which swine and cattle lived in a strict cohabitation sharing resting and feeding areas. In this farm immunoprophylaxis for AD in pigs was historically performed using an attenuated live vaccine and no signs of AD were recorded. After 20 days from the last vaccination, five bovines showed progressively anorexia and depression followed by intense pruritus in the hindquarters, mammary area and perineum with self-mutilation and restlessness. Moreover, aspecific neurological symptoms were present, such as stiff gait, hind limb hypometria, proprioceptive deficit, muscular clonic spasms, colic syndrome. The animals died 48-72 hours after the first manifestation of pruritus. Blood samples, oral and nasal swabs were collected. Serological investigations were performed. PCRs for SHV-1 detection targeting gB and gE genes was made on EDTA blood and swabs. ELISA test was used to detect antibodies anti gE and gB in serum. Serological and molecular techniques were carried out at the National Reference Centre for Aujeszky's disease (C.R.M.A.) as previously described [2]. Histopathology was performed on different organs. All the animals were seronegative for anti-gE antibodies and positive for anti-gB antibodies, confirming the suspected contact with the AD virus. gE and gB-PCRs were negative in all the blood samples but unfortunately, brain samples were submitted later and are now in progress. Moderate multifocal haemorrhages, non-suppurative myelitis, diffuse reactive lymphoid hyperplasia with depletion and severe systemic necrotizing vasculitis were histologically detected. Given the strict coexistence between pigs and cattle, an interspecific transmission of AD virus cannot be excluded. Further microbiological/biomolecular investigations are in progress to clarify the aetiopathogenesis of the disease.

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ANALYSIS OF THE 16S-23S rRNA ISR AND OF THE 16S rRNA GENE SHOWS HIGH GENETIC VARIABILITY OF *STREPTOCOCCUS EQUI* SUBSP. *ZOOEPIDEMICUS* INFECTING ITALIAN HORSES

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Streptococcus equi subsp. *zooepidemicus* (SEZ) is an opportunistic and zoonotic pathogen of horses [1]. SEZ diseases have been reported not only in equids but also in dogs, cats, poultry, and recently in alpacas with septicaemia [2]. Different genotyping techniques showed that SEZ are a genetically heterogeneous group in UK [3]. In order to evaluate the genetic intraspecies variations, SEZ strains isolated from different specimens of Italian horses were investigated by sequencing of the 16S-23S rRNA intergenic spacer region (ISR) and of the 16S rRNA gene. DNA archival samples of 95 SEZ isolated mainly from the respiratory and genital tracts of horses [4] were tested by PCR. Four PCRs (named A, B, C and D) were carried out with sets of primers targeting different regions of the 16S-23S rRNA ISR similarly as previously reported [3]. In addition, 16S rRNA gene was amplified with primers covering the variable regions from 1 to 5 (V1-V5). 16S-23S ISR rRNA type A1 was predominant, although a high rate of multiple products (30.5%) was obtained. Sequencing of 14 samples with an unexpected product in PCR named C showed some criticisms of the PCR protocol and classified them as type A1. Phylogenetic analysis of the 16S rRNA gene detected strains belonging to three genogroups (I, II and III). Although 16S rRNA variable regions V1 and V2 are the most variable regions in SEZ, at least V1-V5 regions should be investigated to avoid subgenotyping mistakes. These results show a high genetic variability in SEZ collected from different specimens of horses from various regions of Italy and serve as a basis for further genetic typing investigations by using methods with a higher discriminatory power.

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BORN TO BE WILD? DETECTION AND CHARACTERIZATION OF PORCINE CIRCOVIRUS 3 (PCV-3) IN WILD UNGULATES AND ASSOCIATED TICKS IN FRIULI VENEZIA GIULIA

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In the last decades, modern swine farming has been affected by the emergence of new pathogens, like Porcine reproductive and respiratory syndrome virus and Porcine circovirus 2, which had a remarkable impact on animal health, farm management and profits[1]. More recently, Porcine circovirus 3 (PCV-3) has emerged as a potential threat for swine industry, being consistently reported in presence of several clinical syndromes all around the world, although a clear causal nexus has not been established yet [2]. Despite a prolonged undetected circulation in the pig population is highly likely, the actual origin of this virus remains elusive. Recently, its presence in wild boar has been demonstrated at high prevalence [3]. This evidence is surprising since the lower density of wild populations might not be expected to sustain such efficient viral transmission. Porcine circoviruses exhibit a certain plasticity in their host tropism and have been detected in unrelated species, like mice, dogs and ruminants. However, it remains to be established if this scenario applies also to PCV-3 and wild animals. Therefore, the present study investigated the presence of PCV-3 in different wild ungulate species and related haematophagous ectoparasites. A hundred and nine animals were sampled from different mountain areas of Friuli Venezia Giulia, including 9 chamois (*Rupicapra rupicapra*), 17 red deer (*Cervus elaphus*), 4 mouflons (*Ovis musimon*), 50 roe deer (*Capreolus capreolus*) and 29 wild boar (*Sus scrofa*). Additionally, host matched ectoparasites were collected when present. PCV-3 was diagnosed using molecular techniques and sequencing. The results confirmed the high PCV-3 occurrence in wild boar (44.8%) and reported for the first time its presence, at lower prevalence, in chamois (12.5%) and roe deer (4%). Moreover, 2 ticks (Ixodes ricinus), one of which non-engorged, collected from PCV-3–negative roe deer, tested PCV-3 positive. The genetic uniqueness of the strains collected from non-swine hosts allowed to prove the absence of among-samples contamination, confirming the actual presence of PCV-3 genome in these new hosts. The actual vector competence of Ixodes ricinus and the relevance of other wild ungulates in PCV-3 transmission and maintenance remain to be investigated. Nevertheless, this study highlights an unexpected broad PCV-3 distribution and circulation in the wild, which raises further questions on porcine circoviruses infectious cycle, epidemiology and origin.

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A SEROLOGICAL SURVEY OF BRUCELLOSIS AND TUBERCULOSIS IN EURASIAN WILD BOAR (*SUS SCROFA*) IN CAMPANIA REGION

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Bovine tuberculosis (bTB), caused by *Mycobacterium bovis* and related members of the Mycobacterium tuberculosis complex (MTBC), as well Brucellosis are zoonotic diseases [1,2] undergoing to specific eradication plans worldwide. In Italy the eradication process has led to the eradication of bTB and Brucellosis in several regions, however, it is still present in livestock in Campania region. Both diseases are frequently reported among wildlife populations in Europe and particularly in the Eurasian wild boar which population is in a progressive expansions throughout Europe in the last decades, raising concerns regarding the control of diseases in this species [3]. In this study we evaluated the presence of antibodies against Brucella spp. and *Mycobacterium bovis* (or cross-reacting members of the MTBC) in wild boar hunted in the Campania Region during the hunting season 2016-2017. Serum samples collected from 434 wild boar (*Sus scrofa*) were tested for antibody against *Brucella* spp. and *Mycobacterium bovis* or cross-reacting members of the MTBC. For the diagnosis of swine brucellosis, tuberculosis and MTBC, Rose Bengal Test (RBT), Blocking ELISA and an ELISA with MPB83- antigen assays were performed, rispectively, according to the manufacturer's instructions. Of 434 serum samples, twenty-two sera (5.07%, 95%CI: 3,02 – 7.12), tested by RBT, and fifty-eight (13.36%, 95%IC: 10.2 – 26.72) serum samples tested using Blocking ELISA assay, were positive to Brucella. Gender and age of hunted wild boar have been recorded. Fortysix out of 434 (10.6%, 95%CI: 7.71 – 13.49%) sampled animals presented antibodies against M. bovis in MPB83-ELISA. The seroprevalences of *Brucella* spp. do not differ between the gender and/or age classes, while the prevalence of infected animals was positively correlated with Avellino province. Statistical analysis indicated that the sero-positivity to *M. bovis* was not associated with age, gender or location of sampling. Our data show that wild boar in the Campania region are exposed to Brucella and MTBC infection, and that the prevalence of the diseases has increased in recent years [4].

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INVESTIGATION ON THE EFFECT ON 1,3-1,6 B-GLUCANS ORAL ADMINISTRATION IN DEFORMED WING VIRUS (DWV) NATURALLY INFECTED HONEYBEES (*APIS MELLIFERA* L.)

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Honeybees have recently suffered extensive losses mainly due to exposure to pesticides, malnutrition and pathogens. A possible approach to counteract the effect of honeybee's death could be represented by feeding them with a diet supplemented with molecules able to stimulate their natural immune defences. A previous investigation demonstrated that a diet supplemented with different concentrations of 1,3-1,6 β-glucans, positively affected survival rate and immune defences; activation of phenoloxidase (PO) and restraining of viral replication were also observed (1). In the present work, the DWV viral load kinetic was studied by collecting faecal samples of honeybees fed with different 1,3-1,6 β-glucans diet levels. Data on feed consumption, survival rate and phenoloxidase activity were monitored. A total of 390 newly emerged worker honeybees naturally infected with Deformed Wing Virus (DWV) were collected. Thirty honeybees were sampled to constitute T0 group while the remaining insects were grouped in 12 glass jars (30 individuals each). Honeybees were reared for 24 days in laboratory condition and fed *ad libitum* with two dosages (0.5 and 2%, w/w) of 1,3-1,6 β-glucans in syrup-based diet. Water was constantly available. Dead honeybees were removed twice a day, counted and stored at -80°C. Honeybees' faeces were collected by a piece of absorbent paper settled in each glass jar and replaced every three days. Twenty punches for each paper were pooled and processed for RNA extraction. Dead honeybees collected each day were pooled at the same time point of faecal samples. At day 24th, all surviving bees were pooled within each group and tested accordingly. The viral load was assessed by One-step TaqMan™ RT-qPCR assay. The PO activity was measured on six honeybees collected from each group at day 0 and at 24th day of the experimental period. Results indicate that the 2% β-glucans diet resulted in a decrease of survival rate, while the administration of 0.5% β-glucans showed a survival rate increase in respect to the control group. Interestingly, the faecal viral load was significantly different among groups only at the end of experiment: control group (G0) reached the highest viral load compared to the other groups fed with either 0,5% or 2% β-glucans. Moreover, honeybees fed 2% 1,3-1,6 β-glucans and collected at the end of the test showed the lowest viral load value associated with the highest level of phenoloxidase. In conclusion, our results suggest that 1,3-1,6 β-glucans represent a molecule able to modulate honeybees defence pathways. At the best of our knowledge, this is the first work where the kinetic of DWV viral load in honeybees has been monitored on faecal samples demonstrating that this could represent a valid method to quantify DWV.

Mazzei M, Fronte B, Sagona S, Carrozza ML, Forzan M, Pizzurro F, et al. (2016) Effect of 1,3-1,6 β-Glucan on Natural and Experimental Deformed Wing Virus Infection in Newly Emerged Honeybees (*Apis mellifera ligustica*). PLoS ONE (11): e0166297.

SEROSURVEY OF SCHMALLENBERG VIRUS IN SEVERAL WILD RUMINANT POPULATIONS IN NORTH EASTERN ITALY

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Schmallenberg virus (SBV), a novel teratogenic, insect-transmitted Orthobunyavirus, emerged in 2011 at the German-Dutch border and then spread quickly throughout ruminants across Europe. Several studies reveal a huge SBV circulation in wild ruminants, especially in roe deer and red deer [1]. In Italy SBV infection is reported in red deer and chamois from Lombardy hunted from 2007 to 2013 [2]. To get information on the evolution of SBV infection in wild ruminants in North Eastern Italy and to add some insight into their role in maintaining SBV infection, serosurveys were carried out in 2014, 2015, 2017 and 2018 in some alpine and sub-alpine areas. Blood samples from hunted roe deer, red deer, fallow deer and chamois were submitted to virus neutralization test [3]. The animals were classified into 2 age groups based on tooth replacement: yearlings (<1 year) and sub-adults and adults (>1 year). Overall prevalences were 41% in fallow deer from the Colli Euganei Park in 2014 (59 animals tested); 54% in roe deer, red deer and chamois from the Province of Trento in 2015 (333 animals tested); 19% in roe deer, red deer and chamois from the Province of Udine in 2017 (54 animals tested); 37% in roe deer and red deer from the Vicenza and Belluno Provinces in 2018 (67 animals tested). The detection of antibodies in yearlings, sometimes at very high titre, over the whole study period suggests an endemic low SBV circulation in wild ruminants and their potential role as SBV reservoir.

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ANTIBIOTIC SUSCEPTIBILITY AND VIRULENCE FACTORS OF *ESCHERICHIA COLI* ISOLATED FROM WILD BOAR (*SUS SCROFA*) IN TUSCANY

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Antibiotic resistance is currently one of the most important problem concerning public health at global level and the role of wildlife is under investigation [1,2]. Wild boar is the most diffused wild mammal in Europe and this species could act as reservoir of different disease [3]. The aim of this study was to evaluate the role of wild boar, ranging in Tuscany region (Italy), as carrier of antibiotic resistant and pathogenic Escherichia coli. During last hunting season (October 2018-January 2019), 200 rectal swabs were collected from hunted wild boar. E. coli were isolated on TBX agar. Isolates from 175 different animals (99 males and 76 females) were then subjected to antibiograms and PCR for the detection of genes encoding virulence factors. E. coli isolates were grouped based on animal geographic provenience: North (63), Center (45) and South (67) Tuscany. The highest resistance rates were against cefalotin (94.3%) and amoxicillin-clavulanic acid (87.4%), followed by ampicillin (68.6%), tetracycline (44.6%), cefoxitin (29.7%), cefotaxime (27.4%), aztreonam (20.6%), streptomycin (20.0%), enrofloxacin (13.7%), gentamicin (13.7%), trimethoprim-sulfomethoxazole (1.7%) and chloramphenicol (0.6%). No resistance was found for imipenem. Moderate susceptibility rates were recorded against tetracycline (33.1%), streptomycin (30.9%), gentamicin (30.3%), cefotaxime (18.9%), enrofloxacin (15.4%) and imipenem (3.4%). No significative differences were found between male and female resistance rates. The chloramphenicol resistance was recorded only in samples from the South, while no trimethoprim- sulfomethoxazole resistance was detected in samples from the Center. No significative differences were found for the other antibiotic. Concerning genes encoding virulence factors, 55 out of 175 isolates (31.43%) were negative for all tested genes. The most detected gene was hlya (83), followed by astA (47), stx2 (43), eaeA (30), stx1 (20), pic (12), aggR (6), saa (3) and escV (2). Among the 120 isolates, 35 different genetic profiles were detected. Based on genetic profile, 31 E. coli were classified as EHEC (17.71%), 18 as STEC (10.29%), 9 as EAEC (5.14%), 6 as aEPEC (3.43%). Other 56 isolates (32.00%) cannot be classified as one specific pathovar, since they showed genes specific for more than one class of virulence. No ETEC, EIEC and tEPEC were detected. The results show how wildlife could resent of antimicrobial resistance phenomena, carrying not only virulence genes, but also antibiotic resistance factors, that could represent a problem for the transmission to domestic animals and humans.

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LEPTOSPIRA SPP. SURVEILLANCE IN WILD BOAR (*SUS SCROFA*) IN LIGURIA (ITALY)

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Leptospirosis is a re-emerging and widespread zoonosis, caused by pathogenic serovars of *Leptospira* spp. This bacterium is worldwide distributed, due to a large variety of wild and domestic animal species that can play a role as natural or accidental hosts [1]. Wild boar (*Sus scrofa*) population is increased everywhere in Europe, as in Italy, during last years; this animal could represent a *reservoir* host for different etiological agents, such as *Leptospira* [2]. The aim of this investigation was to evaluate the prevalence of *Leptospira* spp. in the kidneys of wild boar hunted in Liguria region, Italy, during two year hunting seasons (2017/2018, 2018/2019). From 611 hunted wild boar, kidneys were collected. DNA was extracted from each organ and different target was used to detect *Leptospira* spp. genus (*16S rRNA* gene), pathogenic (*lipL32* gene), intermediate *(16S rRNA* gene) and saprophytic (*23S rRNA* gene) *Leptospira* with Taqman-based RealTime-PCR assay [3,4]. Samples with Ct<35 were considered positive. Overall, kidneys were sampled from 282 adult, 155 subadult and 174 young wild boar (in total 314 males and 298 females). By RealTime PCR 77 kidney were positive to *Leptospira* spp. genus (12.60%). Among these, 74 resulted positive for pathogenic *Leptospira* (96.10%) and 3 (3.90%) for intermediate. No positivity for saprophytic *Leptospira* have been detected. Intermediate *Leptospira* were detected in two young animals, one male and one female, and in one adult female. No significant differences of pathogenic *Leptospira* infection ratio were detected from male (11.50%) and female (12.75%). Moreover, only 13 subadult animal (8.39%) resulted infected by pathogenic *Leptospira*; 23 young animals (13.22%) and 38 adult animals (13.47%) were positive. The results of this study confirmed that wild boar are a potential *reservoir* of pathogenic *Leptospira* spp., which can infect other animal species (domestic and wild) and humans. Rarely, intermediate *Leptospira* could be able to infect wild boar with a renal localization that can contribute with their shedding and circulation.

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ITALIAN AND GLOBAL PERSPECTIVES ON MOLECULAR EPIDEMIOLOGY, EVOLUTIONARY AND POPULATION DYNAMICS OF CANINE PARVOVIRUS (CPV)

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Canine parvovirus (CPV) is a common pathogen among puppies causing severe clinical signs [1]. Its phenotypic variability led to an antigenic-based classification into different variants (2a,2b,2c) reported worldwide[2]. However, this classification does not reflect the phylogenetic relationship among genomic sequences [3] and can disguise CPV complex evolutionary patterns. This study evaluated the local and global variability of the virus and CPV epidemiology and evolution were described using phylodynamic and phylogeographic approaches. Italian CPV positive samples were obtained from the clinical and diagnostic activity of San Marco Veterinary Clinic. Samples were processed using a VP1-targeted PCR [4] and then Sanger-sequenced. Sequences were aligned and trimmed to the 1755 bp-long VP2 coding region. An international database was created by downloading all available full-VP2 sequences. An overall Maximum likelihood phylogenetic tree was reconstructed and Median Joining-based haplotypes were reconstructed separately on the Italian and international database. CPV Time to the Most Common Recent Ancestor (TMRCA), substitution rate, population dynamics and phylogeography were estimated on the whole database. One hundred Italian sequences were obtained from 16 regions between 2008-2015 and 727 international full-VP2 sequences from 18 countries from 1979 to 2015. Among the Italian sequences, all antigenic variants were detected and CPV-2a was prevalent. CPV showed a wide distribution and great genetic heterogeneity both globally and within-country. CPV variability was clear also within-variant, whereas many strains were phylogenetically related although belonging to different antigenic variants, thus stressing once more the incongruence between genotypic and phenotypic classification. CPV origin was estimated in 1970 and the substitution rate was around 10-4 s*s-1*y-1. A complex viral exchange among countries was proven both over short and long distances and the expanding trend of the viral population size retraced the most crucial events along CPV historical timeline. CPV epidemiology is characterized by a dense network of dissemination pathways, which enhance the local and global viral heterogeneity and could predict the introduction of new strains. The expansion of CPV population size should alert about the relentless variability acquisition that can lead to new variant emergence showing new clinicopathological and immune-escaping features.

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MOLECULAR ANALYSIS OF FELINE CALICIVIRUS IDENTIFIED IN CATS AFFECTED BY POLYARTHRITIS

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Feline calicivirus (FCV) is one of the most important infectious agents of respiratory disease in cats and in spite of vaccination it continues to be widespread in the feline population. The limping syndrome, a particular arthritic form characterized by fever and lameness was reported both during acute infections and as consequence of vaccination [1]. Aims of this retrospective study were to assess the frequency of polyarthritis in cats infected by FCV and to investigate the genetic characteristics of the identified FCV. All cats referred to a veterinary hospital between January 2016 and December 2018 reporting clinical signs of FCV infection and tested positive for the presence of FCV RNA in conjunctival and/or oropharyngeal swabs and/or synovial fluid samples using a SYBR Green real-time RT-PCR assay [2] were included in the study. On the basis of the concomitant diagnosis of polyarthritis (synovial cytology and/or radiological examination) the cats were grouped as: (A) cats with polyarthritis and (B) cats without polyarthritis. The hypervariable E region of the ORF2 was amplified from FCV identified in cats in group A and sequenced [3]. The obtained nucleotide sequences were aligned with FCV reference sequences from GenBank and translated into amino acid sequences using BioEdit 7.2.5. Phylogenetic relationships were evaluated using MEGA X version 10.0.5. Signalment data and vaccination status of each cat included in the study were retrieved from medical records. Data were evaluated using standard descriptive statistics and analysed using the Chi-squared test considering significant a P value <0.05. Twenty-eight cats were included in the study: 6/28 (21.4%) showed polyarthritis and were included in the group A and 22/28 (78.6%) were included in the group B. For 4/6 cats with polyarthritis the hypervariable E region of the FCV genome was sequenced and analysed. No distinctive genetic features were identified in FCV detected in cats with polyarthritis and phylogeny did not allow to cluster the FCV sequences on geographical, temporal or clinical basis. Two viruses identified in two cohabitant cats with polyarthritis showed a nucleotide identity >95% between them and with the vaccine strain F9 (M86379). No significant association was evidenced among the development of polyarthritis and signalment data and vaccination status but 14/28 (50%) enrolled cats and 5/6 (83.3%) cats of group A were correctly vaccinated. The study provides new data on the frequency of polyarthritic forms during FCV infection in cats and supports two theories: polyarthritis may be associated to FCV vaccine and this clinical form is not correlated to peculiar FCV genetic characteristics.

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"GREEN SYNTHESIS" OF SILVER NANOPARTICLES: CHARACTERIZATION AND IN VITRO ANTIBACTERIAL ACTIVITY AGAINST *P. AERUGINOSA* AND *S. PSEUDINTERMEDIUS* ISOLATES

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Over the last few years metal nanoparticles (NPs), especially the noble metals (e.g. silver and gold), have been studied as alternative methods to fight against infectious diseases. "Green synthesis" of silver nanoparticles (AgNPs) is a fast-growing area of nanoscience research [1,2]. AgNPs can be used for multifunctional bio-application such as an antibacterial, antifungal, antiviral and anticancer agent [1]. The aims of this study were the production of AgNPs using green synthesis methods and the determination of their antibacterial ability against *P. aeruginosa* and *S. pseudintermedius* isolates. AgNPs were biosynthesized using two eco-friendly methods: an infusion of Curcuma longa and the culture supernatant of E. coli. The reduction of Ag+ to Ag0 was monitored using UV-vis spectra analysis and the distribution of the nanoparticles was determined by TEM [2,3]. Ten isolates of P. aeruginosa and ten of multidrug resistant *S. pseudintermedius*, isolated from clinical cases, were enrolled in this study to determine the antibacterial activity of AgNPs. Purified nanoparticles from *C. longa* (ClAgNPs) and E. coli (EcAgNPs) were used alone and in combination with carbenicillin and ampicillin in the Kirby-Bauer disk diffusion assay. The minimum inhibitory concentration (MIC) of both ClAgNPs and EcAgNPs was determined using microdilution method. The ultraviolet-visible spectrum analysis revealed a maximum absorption peak at around 440 nm for both ClAgNPs and EcAgNPs confirming the synthesis of metal nanoparticles. TEM showed mean diameter and standard deviation of 11.107±2.701 nm and 27.282±2.48 nm for ClAgNPs and EcAgNPs respectively. All the Pseudomonas strains were resistant to carbenicillin, ClAgNPs and EcAgNPs alone showed a mean inhibition halo of 9.5 and 14.4 mm respectively resulting in statistically difference if compared to carbenicillin+ClAgNPs (14 mm) and carbenicillin+EcAgNPs (17.45 mm). Staphylococcal strains were resistant to ampicillin and significant differences were found between ClAgNPs and EcAgNPs alone (9.75 mm and 16.40 mm) and ampicillin+ClAgNPs (16.21 mm) and ampicillin EcAgNPs (21.38 mm) halos. The MIC of the ClAgNPs against *P. aeruginosa* and *S. pseudintermedius* was 71 nM and 140 nM respectively. Nanoparticles from E. coli showed lower MIC of 0.39 nM and 3.73 nM for *Pseudomonas* and S*taphylococcu*s respectively. These results confirmed the potential antibacterial ability of AgNPs used alone or in combination with antibiotics, suggesting their potential application for the treatment of infectious diseases caused by MDR bacteria.

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QAC GENE-MEDIATED DISINFECTANT RESISTANCE IN COAGULASE NEGATIVE STAPHYLOCOCCI FROM OVINE MILK AND EFFECTS OF EXPOSURE TO SUB-INHIBITORY CONCENTRATIONS

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Disinfectant resistance in *Staphylococcus* spp. is often associated with the presence of plasmids hosting qac genes which encode for efflux pumps for disinfectants, antibiotics and other antimicrobials [1]. Due to qac genes presence, repeated exposure to sub-lethal/inhibitory concentrations of an antimicrobial could select for a microbial population with a reduced susceptibility to antibiotics and disinfectants [2]. The aims of this study were: i) assess the resistance of 74 coagulase negative staphylococci (CNS) from ovine milk against disinfectants commonly used in teat dip; ii) evaluate a possible phenotypic variation in disinfectant/antibiotic resistance after a disinfectant sub-inhibitory stress. A screening was performed to detect qac genes, then qac(+) isolates and some *S. epidermidis* presumptively biofilm producers (icaA- D(+)) were selected to determine their MIC and MBC for chlorhexidine digluconate (CHDG) and benzalkonium chloride (BC). Isolates with a MIC ≥ 2 µg/ml for CHDG or BC were chosen to be exposed at a sub-inhibitory concentration of the respective disinfectant. BC and CHDG resistance was then assessed again. Cross-resistance to antibiotics was evaluated by disc diffusion method. Only 9 isolates were positive for qac genes: smr (1), qacH (1) and smr-qacC' (7). Twenty isolates were subjected to MIC and MBC determinations. For CHDG as well as for BC, a MIC ≥ 2 µg/ml was observed for 12 isolates. Qac(+) isolates did not show a mode higher than the other isolates. After CHDG stress, most of the isolates doubled their MIC for BC and CHDG (67% and 83%, respectively). Two isolates (both *S. epidermidis*) showed a four-fold increase of their MBCs for CHDG, even though they were qac(-). After BC stress, MICs for BC and CHDG doubled in 54% and 77% isolates, respectively, while one smr-qacC'(+) isolate (*S. simulans*) showed a four-fold increase of its MIC for BC. One smr-qacC'(+) isolate (*S. simulans*) reached the highest MBC value (32 µg/ml) for BC. As for antibiotic resistance, the variations occurred almost exclusively for qac(+) isolates. After BC and CHDG stress, one qac(-) isolate (*S. epidermidis*) hosting blaZ gene expressed resistance against ampicillin. Moreover, after BC stress, 2 qac(+) isolates (*S. simulans* and *S. caprae*) expressed resistance against cefoxitin, although they were negative for the genes commonly involved in this resistotype. In the light of our results, in milking routine it would be important to employ disinfectants at recommended concentrations. Indeed, while on the one hand mastitogens resistant to disinfectant working concentrations still have not been identified *in vitro*, an inappropriate use of these products could lead to a reduction of bacterial susceptibility to biocides and antibiotics.

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COULD HONEY BEES SIGNAL THE SPREAD OF ANTIMICROBIAL RESISTANCE IN THE ENVIRONMENT?

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Antimicrobial resistance (AMR) is an emerging problem throughout the world with antimicrobial-resistant organisms found in people, food, animals, plants and the environment and moving between ecosystems. The honey bee has long been known to be a bioindicator of environmental pollution considering that honey bees forage over many square kilometres collecting thousands of pollen samples per day from different environmental matrices [2]. This work aims to evaluate, for the first time, the role of Apis mellifera as a possible indicator of environmental AMR *Enterobacteriaceae* were isolated from gut's pool of honey bees collected in five different environmental sites, where different antimicrobial selective pressures were hypothesised, named ES (ES1: hilly area, as a poorly anthropized environment; ES2: hilly area, with reported cases of American foulbrood in the flying range; ES3: urban area; ES4: area near an intensive livestock farm; ES5: area near an organic livestock farm). Isolates were analysed to assess the resistance patterns. Resistance against ampicillin, amoxicillin/clavulanic acid, cefazolin, ceftazidime, tetracycline, imipenem, enrofloxacin, amikacin and trimethoprim/sulfamethoxazole was tested. Forty-eight isolates were identified: twenty four different species of *Enterobacteriaceae* were isolated from bee guts, many of which are currently considered an emerging public health concern. *Klebsiella oxytoca* was the species most frequently isolated (8 isolates) and the only bacterium isolated in all the environmental sites, followed by *Pantoea agglomerans* (7 isolates) and *Serratia marcescens* (6 isolates). Twelve isolates out of 48 (25%) showed resistance to at least one antimicrobial drug. There were no significant differences between the resistance rate observed in the different environmental sites, even if the highest percentage of resistance was found in ES4 (area near an intensive livestock farm). Resistances against amoxicillin/clavulanic acid resulted significantly higher than those detected towards the other antimicrobials. The results of this study show that honey bees harbour different environmental bacteria belonging to *Enterobacteriaceae*, many of which are responsible for nosocomial infections. The found resistance to amoxicillin/clavulanic acid, antimicrobial not used in beekeeping but the most utilized for humans and animals, suggest a possible role of honey bee as a useful indicator of the spread of AMR resistance in the environment.

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STAPHYLOCOCCUS AUREUS FROM GOAT TO VETS!

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Staphylococcus aureus is an important opportunistic pathogen that causes infections among human, domestic and wild animals [1]. Here, we report the first case of transmission of S. aureus from a goat to two veterinarians during the calving assistance. Seventy-two hours after the calving, that ended with abortion and goat death, the vet who assisted the calving and the vet who performed the necropsy showed the presence of multiple, isolated, painful lesions of 1 to 5 mm in diameter, clearly looking like pustules, located all along forearms and knees. Other clinical signs included a mild fever of 37.3°C for one day. Headaches or myalgia, and lymphadenopathy was not observed. By 3 to 5 days the skin lesions disappeared. Infectious dermatitis related to the goat abortion was suspected. The initial differential diagnosis included pyogenic coccal infection, cutaneous listeriosis or salmonellosis and Orf virus infection. Specimens from human pustules, goat placenta and uterus, foetus organs (brain, thymus gland, abomasum, liver and spleen), as well as scrotum and eyes swabs of the buck and mammary pustules of a goat present in the same herd, were collected for bacteriological examinations. All the samples were inoculated onto Blood Agar and Mannitol Salt Agar plates and the colonies were identified by MALDI-TOF (Bruker Daltonics). The clonality among the isolates was analysed by IR Biotyper (Bruker Daltonics). Histological examinations were carried out on samples taken from placenta, uterus and foetus organs. From all the samples, a pure culture of gram positive, catalase and coagulase positive cocci was shown after 24 hours of incubation. One isolate from each sample was identified by MALDI-TOF with score values ranging from 1.83 to 2.33 for *S. aureus*. The typing of isolates, performed to highlight the clonality among isolates, identified two different clusters: the first included *S. aureus* isolated from humans, goat, foetus and buck; the second included S. aureus isolated from the mammary pustules of a different goat within the same herd. The histologic examination revealed numerous coccoid bacteria on the placental surface and the uterine mucosa; the uterine mucosa showed also a purulent inflammation. The results of this study clearly support the hypothesis of an episode of professional zoonosis caused by *S. aureus* occurring during an abortion case. S. aureus associated with abortion in ruminant is reported with low prevalence [2, 3] but this report highlights the need for accurate epidemiological surveillance and the advocacy of best practices to avoid its transmission.

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HEPATITIS E VIRUS IN RAW SEWAGE IN ABRUZZO REGION

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Hepatitis E virus (HEV) infection is a major health problem worldwide. Serological studies suggest that HEV infection is common in particular geographical regions of Italy [1], with 48.9% anti-HEV IgG positivity amongst blood donors in Abruzzo region (Southern Italy); HEV Gt3c has been detected in two of these donors [1]. Untreated sewage represents a valuable source for viral disease surveillance reflecting the pattern of infections in the population. In order to investigate in more detail, the epidemiology of HEV in Abruzzo Region, we assessed sewage samples collected at four wastewater treatment plants located in the province of Teramo. A total of 56 influent sewage specimens were collected during 2016-2017, by swabbing different surface points of the separation grids used for primary wastewater treatment. Total RNA was extracted from each sample by using the TRIzol LS (Invitrogen, Ltd, Paisley, UK) procedure and analysed by HEV-specific qRT-PCR [2]. Amplification of RNA for sequencing was attempted on all the samples containing quantifiable HEV RNA by using three nested RT-PCR strategies (A, B, C) [3,4,5]. Molecular screening by qRT-PCR detected HEV RNA in 13/56 (23.2%) samples with viral loads ranging from 6.1x102 to 5.8x105 copies/ml sample. The 13 positive specimens were distributed over the four wastewater treatment plants tested. By using nested RT-PCR targeting a 146 bp portion at the 3' of ORF2 gene (strategy B) [4], viral sequences were obtained from 2 specimens (AF1/2, AG3/4). For the sample AF1/2, a 187 bp region was amplified using the primer sets targeting the partial ORF1 (strategy A) [3]. A sequence of 348 bp of ORF2 was obtained for two additional samples (E1, PF1) by using the strategy C [5]. After phylogenetic analyses based on the partial ORF2 regions, all the sequences segregated within the Gt3 subtype c, together with other HEVs previously identified in humans and animals in Abruzzo Region [1,6]. These findings support previous suspicious of a wide distribution of this virus in the geographical area assessed. Furthermore, the identification also in raw sewage of HEV Gt3 subtype c adds further evidence that multiple inter-species transmission might be occurring in the surveyed area.

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ORAL COMMUNICATIONS

ARNA

BDELLOVIBRIO AND LIKE-ORGANISMS (BALOs) AS POTENTIAL CANDIDATES TO IMPROVE THE SHELF-LIFE AND SAFETY OF FOODS

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Bdellovibrio and like organisms (BALOs) are Gram-negative, ubiquitous, aerobic microorganisms which are predatory towards other Gram-negative bacteria [1, 2]. The potential use in foods of microorganisms that are parasitic on other bacteria has only been explored to a limited degree [3]. The aim of this study was to test by double-layered agar plaque assay the ability of *Bdellovibrio bacteriovorus* 109J to prey *in vitro* a variety of foodborne and spoilage bacteria belonging to *Escherichia coli,* VTEC *E. coli*, *Enterobacter* spp., *Salmonella enterica* serovars. Moreover, we tested *Bdellovibrio bacteriovorus* 109J in challenge experiments at different prey/predator ratios and temperatures using *E. coli* as a prey, and monitored their respective abundance at 6 hrs and 24 hrs. *Bdellovibrio bacteriovorus* 109J showed predatory activity towards several strains of *E. coli*, VTEC *E. coli*, *Enterobacter* and *Salmonella*. In challenge experiments at different temperatures and predator/prey ratios, the predator determined a decrease of *E. coli*. In general, independently of the initial level of the predator, by lowering the initial level of the prey, the time required for its maximum reduction was extended. The highest prey reduction, equal to 5 log, was obtained at 30°C after 6 hrs, in challenge with 10^9 PFU/ml/ 10^7 CFU/ml and 10:1 predator/prey ratio. Because *Bdellovibrio bacteriovorus* 109J exhibits predatory activity under a moderately wide range of temperature conditions and prey/predator ratios, these results suggest that it may be used for biological control of pathogenic and spoilage microorganisms in diverse types of foods. It is currently under investigation the ability of this predator to attack the preys on meat and other food model systems.

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EFFECTS OF RIBES PET SYMBIO ON THE CANINE INTESTINAL MICROFLORA AND PRODUCTION OF SHORT-CHAIN FATTY ACIDS

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Intestinal microbes play a crucial role in maintaining the health of the host. For this reason, the interest in regulating the intestinal microflora to improve the health of the individual is growing. The present study evaluates *in vitro* the capacity of a specific product to modulate the composition of the canine intestinal microflora and the production of short-chain fatty acids (SCFAs). Ribes Pet Symbio is a product containing the probiotic *L. reuteri*, zinc oxide and blackcurrant seed oil.

Fecal specimens used as inoculum were obtained from 3 healthy adult dogs. A pilot fermenter, stirred, pH and temperature controlled, was used to perform the anaerobic batch culture. It has been equipped with a 2 liters vessel and with Bio-controller ADI 1010 and Bio-console ADI 1025 for setting parameters up. From each fermentation sample bacterial DNA extraction and Real-Time PCR were performed to enumerate the bacterial group selected as *Bifidobacterium* spp., *Bacteroides-Prevotella-Porphyromonas* spp., *Lactobacillus* spp., *Enterobacteriaceae*, *Staphylococcus* spp. and *Clostridium coccoides – Eubacterium rectale*. Samples were taken from the batch culture vessel at three specific time points (T0, T6 and T24hrs) and were analysed by GC to quantify the SCFAs content.

The results of the *in vitro* fermentation showed a significant increase of Lactobacilli ($p<0.05$) immediately after 6hrs, displaying a concentration of $9.22\pm0.04 \times 10^7$UFC/ml, reaching a final value of $1.89\pm0.3 \times 10^9$UFC/ml after 24hrs, while, Bifidobacteria increased significantly ($p<0.005$) especially after 24hrs ($6.85\pm0.1 \times 10^4$UFC/ml); these two bacterial groups are important to protect the host from infections exerting positive effects on the organism. The fermentation of Ribes Pet Symbio promoted an expected increase ($p<0.0005$) of Enterobacteria ($2.78\pm0.03 \times 10^7$UFC/ml after 24hrs) as normally present in the intestinal tract. Bacteroides increased gradually ($p<0.0005$) reaching a final amount of $7.50\pm0.04 \times 10^5$UFC/ml; it is not easy to predict whether the increase obtained in this study is completely positive as to this bacterial group belong both beneficial and pathogenic microorganisms so, more thorough studies on bacterial strain-specificity would be needed. Interesting was the ability of Ribes Pet Symbio to avoid a great increase in Clostridia: these bacterial species remained quite stable after 6hrs of fermentation showing a modest increase ($p<0.05$) only after 24hrs ($1.10\pm0.2 \times 10^6$UFC/ml).

Finally, the fermentation of Ribes Pet Symbio was able to increase acetic acid (21.75±0.3 mmol/Kg) and propionic acid even if in lesser amount (11.01±0.2 mmol/Kg); butyric acid showed a modest increase (5.30±0.04 mmol/Kg).

ORAL COMMUNICATIONS

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PARAMETERS OF PROTECTIVE IMMUNITY IN SWINE INDUCED BY PCV2 VACCINES WITH DIFFERENT ANTIGEN PAYLOAD

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Porcine Circovirus associated disease complex (PCVAD) is caused by co-infections of PCV2 and other common pathogens and/or environmental stressors. Different vaccines have been developed to reduce PCV2 infections and PCVAD. Such vaccines are poorly standardized in terms of antigen payload and recognized correlates of protection.
A) To define the relationship betwwen PCV2 Ag mass and immunizing efficacy. B) To define reliable correlates of protection. We selected twenty, 40-day old piglets, and allocated them to 4 groups (5 animals each) with uniform levels of maternally-derived antibody to PCV2. Animals were vaccinated with 450/150/50/0 nanograms of an inactivated PCV2b strain, formulated in the same adjuvant of the commercial Circovac vaccine. Twenty-seven days later, all pigs were challenged intranasally with the homologous PCV2b strain. The main findings can be summarized as follows: 1) No clinical signs were observed in the pigs under study. 2) Viremia was observed in all the control pigs, as well as in 3 pigs of the 150 and 50-ng groups, respectively. No pigs of the 450-ng group developed viremia. 3) There was no correlation between protection and ELISA Ab titers in the single animals, even though the 450-ng group developed on average a stronger Ab response. 4) All the pigs with a PCV2-specific IFN-gamma response at 3 weeks after vaccination were fully protected against viremia. The IFN-gamma response at this time point was peculiar to CD4+, single positive T cells, whereas both CD8alpha and CD8 beta+ T cells were also positive after challenge infection. 5) In tissues (mainly tonsils and ileum) the presence of sparse reactive hystiocytes and multinucleated giant cells was the only PCV2-associated feature and, by immunohistochemistry, only 3 out of 20 subjects (still viremic at PID 35) had a low viral load (grade 1 in 10 samples and grade 2 in 2 samples). 6) Weight gain was very different among PCV2-infected pigs, with no significant correlation with Ag payload in the vaccine. 7) All the pigs were IFN gamma-negative during viremia. 8) Most viremic pigs were instead positive in an Interleukin (IL)-10 release assay. Our data point at the IFN-gamma release assay as a useful tool for monitoring the efficacy of PCV2 vaccines.

Project license for animal testing 230/2018-PR of Italian Ministry of Health as laid down in article 31 of D.lgs 26/2014.

AFRICAN SWINE FEVER VIRUS DIFFERENT VIRULENCE: MODULATION OF IFNS TYPE-I GENE EXPRESSION

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African swine fever (ASF) is a devastating disease which poses a threat to the swine industry worldwide and currently there is no licensed vaccine available [1]. African swine fever virus (ASFV) has a tropism for cells of the myeloid lineage, including macrophages [2]. In order to generate information to underpin ASFV vaccine development, we conducted an in vitro characterization of the interaction of un activated porcine monocyte-derived macrophages (moMΦ) with two different ASFV: 22653/14 (high virulence strain) and NH/P68 (low virulence strain). Blood monocytes from 5 different pigs were differentiated using 50 ng of hM-CSF and were then infected with using a multiplicity of infection (MOI) of 1 of 22653/14 or NH/P68 strains, alongside mock infected control. At different time-point (0, 3, 6, 9, 12, 21 hours post infection, hpi) the gene expression of IFN-β and 17 different IFN-α subtypes was determined by RT qPCR. In each sample, the relative expression of the selected genes was calculated using the formula 2-ΔΔCt where ΔΔCt = ΔCt (mock) - ΔCt (target gene after infection). After calculation of 2-ΔΔCt and the Kolmogorov-Smirnov test, data sets were checked for statistically significant differences by the Friedman test, followed by Dunn's test as implemented in PRISM software [3]. These ASFV strains of diverse virulence induced a different panel of IFN genes expression. Infection with virulent 22653/14 caused up-regulation of gene expression for IFN-α3 at 21 hpi and IFN-α9 both at 9hpi and 21hpi. NH/P68 strain determined up-regulation of gene expression for IFN α5/6, - α8, - α10, - α12, - α13, - α15, - α16, - α17 and IFN-β at 21hpi. All reported variations in up and down regulated genes resulted statistically significant ($p<0.05$). In a recent study we demonstrated a different anti-inflammatory effects and antiviral activity by IFNA subtypes. In particular, IFN−α2, - α5, - α9, and - α10 showed high level of antiviral activity [4]. In this study we observed up regulation of IFN-β and several IFN-α after NH/P68 infection. On the other hand, 22653/14 infection up regulated IFN- α9. These data suggest that virulent isolates developed mechanisms to evade host immune response and promote their survival in infected pigs.

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FURTHER INSIGHTS INTO INTERACTION OF MACROPHAGE SUBSETS WITH AFRICAN SWINE FEVER STRAINS OF DIVERSE VIRULENCE

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African swine fever (ASF) is a devastating disease for which there is no vaccine [1]. The aetiological agent ASF virus (ASFV) has a predilection for cells of the myeloid lineage and macrophages are its main target, nevertheless little is known about the interaction of the virus with polarised macrophages [1, 2]. This study focused on the *in vitro* interactions of porcine un-activated (moMΦ), classically (moM1), alternatively (moM2), and IFN-ϒ activated monocyte-derived macrophages with a virulent (22653/14) and an attenuated (NH/P68) ASFV strains. Virus-interaction was analysed usign multi-parametric flow cytometry, qPCR, multiplex ELISA. Using a multiplicity of infection (MOI) of 1, both viruses infected all macrophage subsets, but NH/P68 presented a reduced ability to infect both moM1 and IFN- activated moM compared to 22653/14. Both viruses grew efficiently in all the subsets (MOI 0,01), with initially lowest in moM1 and IFN-ϒ activated moM, but in the latter just for the attenuated NH/P68. Infection with a MOI of 1 of NH/P68 but not 22653/14 resulted in a reduced expression of MHC class I in all the subsets and higher levels of IL-18 and IL1-β were released by NH/P68-infected moM1 compared to 22653/14 or mock-infected control. Effect of both isolates on moM1 polarization in under investigation. The differences observed between these strains suggest that virulent ASFV strains developed mechanisms to covertly replicate in macrophages, independently on their activation status, and impairment of their responses might affect the development of a protective immune response.

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IN VITRO EVALUATION OF POTENTIAL PATHOGENICITY OF *YERSINIA ENTEROCOLITICA* 1A

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Yersinia enterocolitica (YE) are zoonotic bacteria able to infect humans and animals, recognized as the third cause of foodborne disease in Europe (1). Many studies highlighted the molecular basis of pathogenesis of YE 1B infection, few data are available about 1A biotypes, often isolated in cases of human disease. The aim of our work was to verify the ability of different YE strains to adhere and invade enterocytes, to express chromosomal genes of pathogenicity and to modulate innate immunity of swine enterocytes (IPEC-J2). In our study, overnight cultures of 6 different YE strains (S): YE 1B (S1: O:8, ail+, ystA+, inv+, myfA+, ymoA+); 1A (S2: O:9, inv+, ymoA+); 1A (S 3: O:5, ystB+, inv+, ymoA+); 1A (S4: O:8, ystB+, inv+, ymoA+); 1A (S5: O:5, ystA+, ystB+, inv+, ymoA+); 1A (S6: O:9, ystB+, inv+, myfA+, ymoA+) were sub-cultured for 1 h at 37 °C in BHI medium. Each bacterial strain was used to infect IPEC-J2; untreated cells were employed as negative control. Invasivity, adhesion, innate immune responses and expression of genes of pathogenicity were evaluated as previously described (2,3,4). Differences between data were checked for significant differences by ANOVA (significance threshold set at $P< 0.05$). Our results showed different expression of pathogenicity genes at 4°C and 37°C. In particular, myfA was expressed at 4°C by S1 and S6 whereas no pathogenicity genes were expressed at 37°C. All strains were able to adhere and penetrate into IPEC-J2 cells; however, YE 1B strains showed greater ability to adhere to enterocytes compared to YE 1A strains ($P<0.05$). Concerning the invasion assay, YE S1 and S6 showed greater ability to invade IPEC-J2 cells compared to other strains under study. Moreover, different strains showed different ability to modulate IL-8 gene expression and protein release. In particular, YE strains 2, 3 and 5 caused significant increase of IL-8 release ($P<0.05$) associated with an increase of IL-8 gene expression ($P<0.05$). YE 1 B strains determined a pro-inflammatory effect characterized by up-regulation of IL-8 and TNF-α, and by a decrease of antimicrobial peptide gene expression: bD3, bD4. At the same time we observed down-regulation of CD14, MD2, TLR1, TLR4 and TLR5; all reported variations were statistically significant. YE 1A S4 showed a gene expression profile similar to that of S1. Our data suggest a potential pathogenic role of YE 1A strains and a different interaction with the host's innate immune response.

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CYTOKINE EXPRESSION IN WATER BUFFALOES EXUDATE AFFECTED BY TUBERCULOSIS

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Tuberculosis (TBC) is one of the most relevant diseases affecting animals and humans. The immune response to TBC has been investigated in peripheral blood mononuclear cells and tissue in cattle and humans, demonstrating a different cytokines expression among TBC-positive and TBC-negative individuals [1-2]. Few studies have been carried out on water buffalo (*Bubalus bubalis*) immune response to TBC. We aimed to investigate for the first time the development of cytokine network within the exudate lump after the intra-dermal tuberculin test (IDT) reaction of TBC-infected animals from TBC-free herds with positive (group A; n=6) or negative (group B; n=18) microbiological culture for *Mycobacterium bovis* and of uninfected animals with positive IDT for *Mycobacterium avium* (group C; n=12). The total RNA was extracted from the intra-dermal reaction swellings after IDT procedure; then, cytokine mRNA abundance was measured by means of quantitative RT-PCR, focusing on targets involved in the polarization towards Th1 (TBET, STAT4, IFNG, IL1β), Th2 (STAT5B, GATA3, IL4), Th17 (RORGT, STAT3, IL17A) and Treg (RORGT, FOXP3, TGFβ, IL10) response. Preliminary results showed that no differences in mRNA expression were detected among groups, even if an increase of the Th1-related IFNγ in Group A and of the Th2-related transcription factor STAT5B in Group B was appreciable. Further investigation is needed to better understand the cell switching within the exudate after IDT procedure and the expression of miRNA will be also evaluated.

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POST-CALVING LEUKOCYTE IMMUNE-RELATED GENES ARE UPREGULATED IN SIMMENTAL COMPARED WITH HOLSTEIN COWS

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The calving event and the consequent adaptation to the onset of lactation leads high yielding dairy cows to an altered inflammatory response gene expression in leukocytes, which confirms the evidence of changes in the innate and adaptive immune response [1,2]. Simmental is a cattle breed selected for meat and milk production. However, in the last decades this cattle type underwent to increased high milk yield selection for intensive production systems, but still maintaining the dual-purpose. The aim of this study was to investigate changes in the expression of genes involved in leukocytes function between cows highly specialized for milk production, Holstein, and cows selected for meat and milk production, Simmental. The study involved 13 Simmental and 12 Holstein cows enrolled in two different farms, with similar management procedures according to the protocol set up for this study. Blood was collected on d 3 after calving in PAXgene tubes (Preanalytix) to measure mRNA expression of 33 genes. The final data were normalized using the geometric mean of 3 internal control genes: *ACTB*, *SDHA*, and *YWHAZ*. Normalized data were subjected to MIXED model of SAS. Compared with Holstein, overall Simmental cows had greater ($P \leq 0.05$) transcript abundance of proinflammatory cytokines and receptors genes (*IL1B*, *TNF*, *IL1R*, *TNFRSF1A*), cell migration- and adhesion-related genes (*CX3CR1*, *ITGB2*, *CD44*, *LGALS8*), and antimicrobial *IDO1* gene. In addition, Simmental cows tended to have higher gene expression of *CD16* ($P=0.10$), *MYD88* ($P=0.07$), *RPL13A* ($P=0.10$), *MPO* ($P=0.07$), and *ALOX5* ($P=0.10$) compared with Holstein. In contrast, compared with Simmental, Holstein cows had greater ($P<0.05$) abundance of *TLR2*, *MMP9*, *LTF*, and *S100A8* genes. Gene expression profiling has shown that leukocytes of Simmental cows were characterized by an increased function of chemotaxis, cell-cell interaction, pathogen-recognition pathway, and inflammatory mediators, suggesting an enhanced capacity of transepithelial migration of leukocytes (mainly neutrophils and monocytes) and adhesion to microvascular endothelial cells. Furthermore, compared with Holstein, the higher expression of inflammatory mediators in Simmental cows is supportive of a greater capacity from leukocytes to recruit and activate mainly neutrophils and monocytes. Taken together, the data support the hypothesis that Simmental cows are prone for mounting a better immune response to the homeorhetic adaptation of the new lactation, compared with Holstein. In conclusion, these results reveal a breed-specific immune response between Simmental and Holstein cows in the first days post-calving under intensive dairy management, accounting for important biological insights and functional information into the immune-function differentiation among cattle breeds.

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MODULATION OF CIRCULATING MIRNA IN RESPONSE TO THE METABOLIC IMBALANCE DUE TO LONG-TERM EXERCISE IN THE ATHLETE HORSE.

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Animal welfare in equine field acquire particular importance due to the nature of "product" delivered by this animal: the sport performance. Avoiding cross the blurred line that exists between an superior athletic performance and animal abuse is a major concern. Therefore, scientific interest in genetics, physiology and sports medicine tend towards animal welfare manteinance. Endurance discipline represent a good model to test metabolic responses to high intensity prolonged effort where animals compete in 30 to 160 km per day races. Physical exercise was recently associated with the modulation of micro RNAs (miRNA), that act as post-transcriptionally regulators of gene expression [1]. Actively released in the body fluids miRNA are recognized as accurate biomarkers with respect to classical serum/plasma marker proteins [2].

The aim of this study was to monitor the genomic response towards the restoration or destruction of cellular homeostasis in horses that finished a 90 km endurance race in excellent metabolic conditions, compared to those eliminated due to metabolic imbalance. Serum semples were obtained from 19 Arabian horses participating to a 90 km endurance race: 9 were eliminated from the competition (M) and 10 that succesfully ended the competition (BC). Circulating miRNAs were analyzed with Illumina NGS approach. After cleaning procedures, reads were mapped against miRBase database and the latest reference genome (equcab 3.0). Differential gene expression analysis was assessed comparing BC versus M samples. Protein-Protein Interaction (PPI) network and significant enriched pathways of target genes were explored with Cytoscape 3.7.1 suite creating clusters of related targets from which Gene Ontology (GO) enrichment was calculated.

Our results reveal the modulation of a set of miRNAs (up regulation of eca-mir-145, eca-mir-18, eca-mir-133b, eca-mir-374a, down regulation of eca-mir-122, eca-mir-450b,eca-mir-1839) arising from tissues involved in exercise response such as muscle, heart, liver, vassels, brain and activation of correlated processes like inflammatory response, immunity, cell comunication.

In conclusion, this study reports putative biomarkers for prediction of disease risks related to prolonged activity and metabolic adaptations monitoring, to ultimately establish efficient training programs, and validate the accuracy of clinical evaluations in eliminating animals from competition.

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IN VITRO PATHOGENICITY MARKERS OF TYPE I PRRS VIRUS

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Porcine Reproductive and Respiratory Syndrome (PRRS) is an elusive model of host/virus relationship in which disease is determined by virus pathogenicity, pig breed susceptibility and phenotype, microbial infectious pressure and environmental conditions. Outbreaks with high morbidity and mortality have been described, but no reliable pathogenicity markers *in vitro* of the involved PRRS virus (PRRSV) strains have been identified so far. To define *in vitro* assays differentiating reputedly virulent and attenuated Type I PRRSV strains.

We isolated several PRRSV strains from both respiratory and reproductive disease cases using Pulmonary Alveolar Macrophages (PAM) and monocyte-derived pig macrophages. After measuring PRRSV concentrantion by quantitative RT Real time PCR, each PRRSV strain was reacted with Peripheral Blood Mononuclear Cells (PBMC) of PRRS-naïve, Specific Pathogen Free (SPF) pigs. After 18 hours at 37°C in 5% CO2, cells and supernatants were collected to measure IL-1beta, IL-8, TNF-alpha, IL-10 and Caspase 1 responses. These assays were repeated on a reputedly attenuated (BS114) and a pathogenic (BS773) PRRSV strains as established by a previous *in vivo* study.

Four strains were selected as representatives of distinct patterns of response: A) 270433/5 (Caspase+, IL-8+, TNF-alfa+, IL-10-); B) 271009/6 (IL-10+); C) 271009/8 (IL-8+, TNF-alpha+); D) 21377 (negative for all responses). BS773 also tested negative in all the assays but for a slight Caspase 1 response. On the contrary, the attenuated BS114 strain tested positive in both IL-1beta and IL-8 assays. Interestingly, the BS773 strain had been shown to cause *in vivo* early IFN-alpha and IFN-gamma responses, and the early IFN-alpha response *in vivo* is known as a correlate of poor clinical outcome of PRRSV infection (Harding JCS et al., 2017, Vet. Microbiol. 209, 114-123).

Type I pathogenic PRRSV strains inhibit to a different extent *in vitro* the development of a robust innate immune response in PBMC, which are not competent for PRRSV replication. In this respect, the IL-1beta response clearly discriminated the attenuated BS114 strain from all the pathogenic ones under study. Interestingly, amino acid differences were demonstrated among our PRRSV strains in the small gE glycoprotein, affecting inflammosome formation and the IL-1beta response (Zhang K. et al., Virology 2013, 442: 156-162). On the basis of previous research data (Singleton et al., 2016, Frontiers Microbiology, 7, 832), we believe that the lack of a robust primary inflammatory response to PRRSV could be conducive to the development of highly susceptible pig macrophages, competent for high-titered PRRSV replication. This might in turn underlie long-lasting viremia and serious clinical symptoms, as shown with some Type I , subtype III PRRSV strains (Morgan SB et al., 2013, Vet. Microbiol. 163, 13-22).

All the experiments were carried out *in vitro* and were based on previous *in vivo* studies with a regular project license for animal testing.

ORAL COMMUNICATIONS

SICLIMVET

SYNDROME IN SADDLEBRED HORSES IN CENTRAL ITALY: PRELIMINARY RESULTS

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Equine Gastric Ulcer Syndrome (EGUS) is a worldwide disease, that has been described in horses of different breeds and exercise levels. It is characterized by the presence of gastric hyperkeratosis, hyperaemia, erosions and ulcers in either the squamous (Equine Squamous Gastric Disease, ESGD) or the glandular (Equine Glandular Gastric Disease, EGGD) mucosa [1]. Aim of this study was to evaluate the presence of EGUS, ESGD and EGGD in a population of registered Saddlebred horses living in Central Italy and to identify risk factors for each disease. Based on the recommendations of the referring vets, horses registered as Italian, German, French or Dutch Saddlebred were submitted for gastroscopic examination. Information about signalment and activity of the animals were registered and used to identify possible risk factors. Presence and severity of the different diseases were graded according to Sykes et al, 2015 [1]. Statistical analysis was performed using generalized linear models (R project, USA). One-hundred-thirty-one horses aged between 1 and 26 years were evaluated. Females were 71/131 (54%), geldings were 40/131, (31%) and males 20/131, (15%). Italian Saddlebred composed more than half of the horses evaluated (75/131, 57%), followed by German (33/131, 25%) and Dutch Saddlebred (20/131, 15%). Most of the horses were used for low level jumping (74/131, 56%), with lower numbers used as breeding animals (31/131, 25%) or retired because of old age or injury (19/131, 15%). EGUS was found in 55/131 (42%) equids, with ESGD (grades 2-4 [3]) present in 53/131 (40%) and EGGD in 19/131 (15%) animals. Possible risk factors evaluated were age group, breed, gender and type of activity performed. Being male was considered risk factor for both EGUS and EGGD, while no specific risk factors were found for ESGD. This population of horses did not have risk factors commonly associated with EGUS, ESGD and EGGD [1,2], but the prevalence of the disease can be considered quite high. Further studies are needed to confirm the data collected and to better define the pathophysiology of EGUS in populations of horses not considered at risk for gastric ulcerations.

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METABOLIC CHANGES INDUCED BY LONG TERM THOROUGHBRED RACEHORSES

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Thoroughbred racehorse is a valid animal model to investigate those changes for training schedule fine tuning and enhance animal welfare. The aim of this study was to investigate long term metabolic modifications on serum biochemical parameters, serum protein electrophoresis (SPE) and expression of related genes that could inducing these modifications in a 4 month time frame. Twenty-nine clinically healthy, never trained, 2 years old thoroughbred racehorses were followed during their incremental sprint exercise schedule. Blood collection, obtained from jugular vein, was performed once a month, for 5 times (T-30; T0; T30; T60; T90) before training and feeding. For each sample, whole blood lactate concentration (Accutrend Lactate, Roche Diagnostic) and microhematocrit (PCV, Microhematocrit centrifuge, Gima) evaluation were immediately determined; then, within 4 h after blood collection, serum biochemical parameters (urea, creatinine, AST, GGT, ALP, BIL-T, LDH, CK, glucose, cholesterol, triglycerides, Alb, TPs, P, Ca2+, Mg, Na+, K-, Cl) were determined (Hitachi 904; Boehringer). For T-30 and T90, SPE (AGE system (Hydragel-Hydrasis; Sebia) was performed and RNA was isolated from buffy coat (whole blood, EDTA) for gene expression assays. qRT- PCR was performed on all samples to evaluate the expression of IL4, IL10, IL6, BCL11A and Oct-1 transcripts normalizing with two reference genes (SDHA and HPRT) previously determined as optimal housekeeping for blood cells in horses [1]. For each serum biochemical parameter, significant modifications were identified between T-30 vs all other time points (ANOVA test) for PCV, glucose, triglycerides, cholesterol, urea, creatinine, BIL-T, ALP, LDH, Na+, K-, Ca2+, lactate, and TPs. T-test was applied for SPE parameters revealing a significant increasing of all globulin fractions in particular γ- globulins, A/G ratio between T-30 and T90. To deeply investigate the reliability of γ- globulins increasing during training we analysed the expression of key genes and cytokines related to Th2 immunity response in T-30 and T90. As expected, IL4 and IL6 were up- regulated in T90 whereas IL10 was not modulated. The key inducer of γ-globulins expression, Oct-1 transcription factor, is strongly upregulated in T90 samples whereas BCL11A is down regulated. As described in human medicine, our results indicate several biochemical variations induced by exercise. Decreased glucose and cholesterol levels as well as increased triglycerides and lactate concentrations are identified. An improved glucose uptake by muscle cells and lipomobilization can be assumed. As in human medicine, the globulin fractions raise supported by molecular results, especially immunoglobulins, could be due by the increased secretion of cortisol, cathecholamine and neuropeptides in course of long term regular exercise [2].

[1] Jose-Cunilleras et al. Expression of equine glucose transporter type 4 in skeletal muscle after glycogen-depleting exercise. Am. J. Vet. Res. 66(3): 379-385,2005

Karacabey et al. The effects of exercise on the immune system and the stress hormones in sportswomen, Neuroendocrinology Letters, 26:361-366, 2005

RESPONSE TO ALLERGEN-SPECIFIC IMMUNOTHERAPY BASED ON INTRADERMAL TESTING IN 33 HORSES AFFECTED BY ALLERGIC SKIN DISEASES

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Allergic skin diseases (ASD) represent a challenge in equine practice. They include atopic dermatitis, recurrent urticaria, food allergy and insect byte hypersensitivity (IBH)[1,2]. Their chronicity, along with the adverse effects of systemic medications, often makes the allergen specific immunotherapy (ASIT) the treatment of choice [2,3]. Aim of the present study was to evaluate the responses to ASIT based on the results of intradermal testing (IDT), which represent the gold standard for ASD diagnosis [2,4]. Thirty three horses (mean age of 10±3 y.o.) with historical and clinical signs consistent with ASD were included in this study. All horses underwent a thorough diagnostic protocol before performing the IDT. 0.1 ml of 39 allergenic extracts, plus positive (saline) and negative (hystamine) controls, were administered in the neck region. Injection sites were evaluated 30min after administration for immediate reactions (IR), 4hrs for late phase reactions (LPR) [4,5,6], 24 and 48hrs for delayed reactions (DR) [1,2,7]. Reactions were evaluated on a scale of 0 to 4. Criteria for the scoring included wheal erythema, induration and diameter. ASIT based on the results of IDT was performed in all patients. For ASIT, an aqueous allergen extract was used with an incremental dosage on a weekly basis until it was reached the maintenance dose. A telephone survey was carried out with owners and referring veterinarians after the first immunotherapy vial to define whether the symptoms were under control, if the use of complementary treatments was needed (partial improvement), or if there was no response to ASIT. The largest number of positive reactions (PR) to IDT was observed for *Dog epithelium* (52%), *Candida albicans* (48%), *Dermatophagoides mix* (45%), *Culex* (33%) and *Culicoides* (30%). Twenty four out of 33 horses (75%) showed reactions at all observation times and to different allergens. Positive IR and LPR not followed by DR were recorded in 5 horses (15%). Positive DR not preceded by PR at an earlier time accounted for 4 horses (10%); *Trichoepidermophytes mix, Glyciphagus domesticus, Helmintosporium sativum* were detected exclusively in DR. Twenty three out of 33 owners (72%) reported that ASIT significantly reduced the clinical signs, including those cases that had only DR; partial resolution was reported in 7% and no improvement in 21% of cases. Of the latter, 66% were affected by IBH. Our results focus on the importance of IDT for correct identification of allergens, that is essential for the outcome of ASIT. In contrast to some reports [4,5,6], the evaluation at 24-48hrs post injection provided significant diagnostic information. The poor response to ASIT observed in patients affected by IBH agrees with the most recent literature [5].

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SELENIUM AND VITAMIN E CONCENTRATIONS IN A HEALTHY DONKEY POPULATION IN CENTRAL ITALY

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Selenium and vitamin E protect the body against oxidative stress [1,2]. Clinical manifestations of their deficiency in equids include neurologic and muscular symptoms [3]. Despite the importance of donkeys as production animals, no reference range exist for selenium and vitamin E. Therefore, the aim of this study was to investigate the plasma concentrations of selenium and vitamin E in healthy donkeys belonging to different ages, sexes and productive phases. Animals were divided into five groups including foals (Group A: n=7), weanlings and yearlings (Group B: n=7), non-pregnant non-lactating jennies (Group C: n=5), pregnant non- lactating jennies (Group D: n=9), and adult males (Group E: n=9). Plasma samples were tested for vitamin E, using high performance liquid chromatography (HPLC), while selenium concentrations were assayed in atomic absorption. One-way ANOVA showed significant differences in selenium concentrations (p=0.001), between Group A and Group E. In this study, we found the selenium range for donkeys to be 0.02-0.14 µg/ml which is lower than the recommended range for horses [4-6] suggesting that donkeys may have a lower selenium requirement than horses. A similar trend was observed by Shawaf et al. [7] who found lower selenium concentration in donkeys compared to horses, however this study investigated the level of trace minerals but not vitamins. Plasma vitamin E levels were 3.29-12.99 µmol/L, with foals having lower concentrations compared to adults [8]. Knowing specific reference ranges for vitamin E and selenium in healthy donkeys can help clinicians to prevent deficiencies that could compromise donkeys' overall health and wellbeing.

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ENDOSCOPIC ANATOMICAL FEATURES OF BROWN BEAR'S RESPIRATORY SYSTEM: PRELIMINARY FINDINGS

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In this report, the description of the endoscopic anatomical features of brown bear's respiratory system will be reported starting from the assessments of two cases observed by Endoscopy Service of the University Veterinary Teaching Hospital (OVUD) of Perugia.
It start with the description of their clinical signs and finish with the delineation during the endoscopic procedure of their respiratory system. Serious cough and in one of them severe dyspnoic crises were reported with regard to bear's clinical hystory. Both were treated with antibiotics, steroid and mucolytic drugs without effect. Broncoscopy, bronchoalveolar lavage and bronchial biopsy were planned for both[1]. In the first case the inflammatory process was classified as a chronic tracheobronchitis with a prevalence of macrophages and neutrophils cells with severe catarrhal exudate. In the second case the inflammatory process was classified as a chronic polypose tracheobronchitis with a prevalence of neutrophils and hemosiderophagi cells, which means a chronic pulmonary hemorrhage, with also severe catarrhal exudate. The endoscopic anatomical features of brown bear's respiratory system highlighted during the bronchoscopic examination will be described below. The corniculate cartilages of larinx have a very rudimentary form and the vocal cords are very thick. The trachea, the division between the two main bronchi and the right pulmonary lobes are similar to other conventional species [1]. The exploration of left pulmonary lobes in bears allowed identifying a third main branch that is supposed to be a third pulmonary lobe. Finally some of the further branches of the caudal left lobe were observed. The study of endoscopic images was particularly difficult because of severe exudate in bronchi, diffuse nodular lesions, exuberance of the mucosa such as determinate formation of real folds and because of collapsing of the deep airways. Although to confirm what has been described it would be useful to compare these data, but unfortunately in literature there is a total absence of anatomical endoscopic descriptions related to bear. This lack could also limits the possibility of studying the pathologies of this animal and the possibility of intervention by veterinarian.

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EAR MICROBIOTA IN HEALTHY DOGS

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The advent of next-generation sequencing (NGS) techniques allowed a better understanding of the human skin microbiota in healthy subjects and in people with skin disturbances. Few studies have investigated the canine skin and ear microbiota [1-2]. The aims of the present study are to characterize the ear canal microbiota in healthy dogs and to study the differences of its composition evaluated using the culture-dependent and the NGS approach. Twenty healthy dogs with no history of skin disease were enrolled. Clinical history and demographic data were recorded. Dogs treated with ear cleaners, antimycotics or antibiotics in the 2 and 4 weeks before sampling, respectively, were excluded. Ear cytology was performed in all dogs. Samples were obtained from the right vertical external ear canal of each dog by sterile swabs. Bacterial isolation was performed according to standard technique. Taxonomic identification was done by MALDI-TOF MS analysis. The DNA was extracted from each sample and quantified. After PCR amplification, V3-V4 16S-rDNA amplicon libraries were prepared and sequenced on Illumina MiSeq platform. The final feature table was obtained with QIIME2 pipeline [3] and the Greengenes database (http:// greengenes.lbl.gov) was used for taxonomic assignments. Alpha diversity and beta diversity were calculated after rarefaction, as part of the QIIME2 analysis workflow. Bacteria cultures identified *Bacillus* spp. and *Staphylococcus* spp. in 65% and 40% of dogs, respectively. Streptococcus spp., Clostridium spp., *Micrococcus* spp. and *Exiguobacterium* spp. were isolated in 10% of cases. *Enterobacter* spp., *Acinetobacter* spp., *Ureibacillus* spp., *Kocuria* spp. and *Curtobacterium* spp. were isolated in 5% of dogs. Bacteria from 31 phyla were identified with NGS approach, with Proteobacteria being the most abundant phylum in all samples. Photobacterium was the prevalent genus in 95% of dogs, whereas Bacillus spp. and Staphylococcus spp. were identified in 75% and 90% of dogs, respectively. Diversity analysis showed a strong individual variability in terms of alpha and beta diversity. The Richness index showed large differences ranging from 30 to 449 observed features (mean: 159, SD: 96). The Pielou index values highlighted a good equitability in the population composition in most of the samples, with the exception of three dogs whose microbiota showed to be driven by the presence of some dominant species. In conclusion the NGS approach allowed to identify a very complex canine ear microbiota with great individual variability and partial agreement with the culture-dependent approach. Further studies may provide a clearer understanding of the clinical significance of these results.

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KLOX FLUORESCENCE BIOMODULATION SYSTEM (KFBS), AN ALTERNATIVE ADJUNCT THERAPY FOR THE MANAGEMENT OF CLINICAL MANIFESTATION OF CANINE PYODERMAS

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Canine pyodermas are one of the most common compliant in small animal practice [1]. With the emergence of multi-drug resistant bacteria, alternative treatments that increase efficacy and reduce antibiotic use have gained popularity [2]. The aim of this study was to assess the potential of Klox Fluorescence Biophotonic System (KFBS) to accelerate the clinical resolution time in the treatment of both canine deep and superficial pyoderma. A total number of 45 dogs were enrolled in this study, eighteen with a dignosis of superficial pyoderma and twenty-seven affected by deep pyodermas. For superficial pyoderma a group received only oral antibiotic cefadroxil (n=8; 20 mg/kg, twice daily) while other groups involved received only KFBS at once (n=5) or twice weekly (n=5) frequency. For deep pyoderma, five and eight dogs received only KFBS or oral antibiotic cefadroxil (20 mg/kg, twice daily), respectively. Additional treatment groups involved oral cefadroxil (same dosage) and KFBS at once (n=5) or twice weekly (n=9) frequency. The KFBS treatment exhibited excellent safety profile and achieved complete resolution (CR) for superficial pyoderma in 2.4 ± 1.1 ($p=0.05$) and 2.3 ± 0.7 ($p<0.05$) weeks in once-KFBS and twice-KFBS dogs, respectively, whereas cefadroxil treated group required 3.75 ± 1.0 weeks for clinical resolution. KFBS achieved CR for deep pyoderma in 4.3 ± 1.3 weeks in all dogs, whereas cefadroxil treated group required 15.5 ± 3.5 weeks for CR. In deep pyoderma-affected dogs' skin biopsies obtained before and after KFBS treatment revealed a significant mRNA up- regulation of EGF, PDGF, TGF-beta VEGF as well as matrix metalloproteinase 1 ($p\leq0.01$) and down regulation of TNF-alpha, compared to cefadroxil group. KFBS has demonstrated to have an excellent safety profile and and it is effective as sole treatment for canine superficial pyoderma, reducing the time for CR, alongside avoiding the prescription of antibiotic administration. In deep pyodermas, KFBS significantly accelerate time to CR and reduce the duration of exposure to systemic antibiotics for deep pyoderma treatment. The rapid emergence of antimicrobial resistance makes the prolonged use of antibiotics difficult to justify; the choice of agents should be based on bacterial culture and antimicrobial sensitivity testing and prescribed only if there are no other options [3,4].

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TREATMENT OF CUTANEOUS SQUAMOUS CELL CARCINOMA ON THE HEAD REGION WITH ELECTROCHEMOTERAPY IN A GROUP OF 19 CATS

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Cutaneous squamous cell carcinoma (SCC) accounts for 10% of all feline cutaneous tumours, and it is the third most common malignancy in cats. [1] SCC is locally invasive with rare metastasis to the regional lymph nodes. [2] Standard treatment approaches include surgery and radiation therapy. [1,2] Electrochemotherapy (ECT) uses the application of electric pulses combined with chemotherapeutic drugs (bleomycin) causing cytotoxic effects on the treated area. ECT also influences the immune system and tumour blood flow. [2,3] The aim was to evaluate the feasibility and efficacy of ECT in the treatment of SCC on the head. Nineteen cats were retrospectively enrolled (December 2004-March 2019). SCCs were diagnosed with cytology and/or histology. ECT was combined with IV bleomycin (15000 UI/ m2) alone in 16/19 cases, post-surgery in 2/19, and before surgery in one case. Parameters considered were tumour site and size, electroporation parameters, response rate (response complete [CR] or partial [PR], stable disease [SD]), local recurrence rate (RR), disease-free interval (DFI), survival time, median survival time (MST), treatment outcome and local treatment toxicity (6-point scale). [4] Tumours were mostly located on the nasal planum (14/19). Median tumour size was 0.7 cm. Three different electroporators were used: Cytopulse Oncovet (12/19), Leroy Biotech Electrovet S13 (6/19) and Cliniporator, IGEA (1/19). Electroporation frequencies were 1 Hz or 5kHz and pulse amplitude to electric distance ratio was ranging 1000, 1200 or 1300 V/cm. Response rate was 94.7% (18/19; 13 CR and 5 PR). One cat had SD. Additional ECT was performed for 7 cats; 4 had a 2nd ECT, one a 3rd ECT and two a 4th ECT. For two cats with PR, RR was 10.5%, DFI was 44 and 694 days and survival time was 184 and 751 days respectively. MST for cats with recurrence was 467 days. At the end of the observation period 14 cats died and 5 were still alive. MST for cats dead without tumour (n=8) was 520 days and for cats dead with tumour (n=5) was 228 days. One cat with SD died with tumour 9 days after ECT. Treatment toxicity was ≤2 in 15/19 cases, two cats experienced toxicity score 3 and one each toxicity scores 4 and 5. Two cats were FIV+ and required multiple ECTs (PR), they had recurrence and no further response to treatment after the 4th ECT. All cats with tumours <1 cm achieved CR. A possible reduction of the treatment response in FIV + cats was noticed. However, ECT seems to be a good alternative to excisional surgery, especially in smaller tumours. Treatment toxicity was low and survival time considerably long.

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IMPROVING CASE–BASED LEARNING IN VETERINARY HEALTH SCIENCES EDUCATION: PRELIMINARY RESULTS FROM THE VET-HIN APPROACH

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Veterinary medicine students, called to achieve a difficult balance between owner-related factors and animal welfare, need to develop strong critical thinking skills [1]. The implementation of Case-Based Learning (CBL) as major educational method allows linking theory to practice through the application of theoretical knowledge to real cases and encourages the use of methods of inquiry-based learning [2]. In such scenario, Health Issue Network (HIN, originally developed by the National Research Council of Italy and the Italian Society for Medical Education) is presented as a CBL-based approach that introduces formalisms to represent how Health Issues (HIs) show up and evolve over time. This allows reconstructing the implicit knowledge that lies behind the doctor's way of thinking, turning it into an explicit knowledge. HIN is based on Petri Nets (PNs), but in an educational environment a lighter version, named f-HIN (friendly HIN), is implemented based on the same mathematical properties as PNs–based HIN for a generic patient [3]. A first attempt to decline HIN for veterinary medicine education (vet-HIN) is being conducted at the Veterinary Teaching Hospital (OVUD) of the Department of Veterinary Medicine and Animal Production (DMVPA) in Naples. Five cases were selected from OVUD database and proposed to five interns, each one of which had to translate own case into f-HIN and show it to their colleagues. To evaluate the level of perception and comprehension of vet-HIN a questionnaire was administrated to the interns. ANOVA tests were run to analyse the rating score of the questionnaires. The limited sample dimension led to not statistically significant results, nonetheless the novelty and usefulness of the approach were recognized by the all the interns. Such preliminary results, as well as the need of a larger study sample, suggest the deployment of vet-HIN as routine form of education for III, IV and V year's veterinary students. Vet-HIN stands then as an innovative way to "guide future veterinarians to become veterinarians" [1], as it makes possible to guide the students in the analysis of the evolution dynamics of a real/realistic clinical case for educational and decision-making skills development purposes, also setting the method within a unique "One Health" perspective in its overall. The next research steps will focus on the creation of specific software as formal tool to be connected to a veterinary EHR's database (overcoming the current semi- automatic process), in order to extract clinical data to figure out case studies to be used for veterinary health sciences education.

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DETERMINATION OF PLASMATIC DIMETHYLARGININES IN HEALTHY DOGS AND IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE, WITH OR WITHOUT PULMONARY HYPERTENSION

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Myxomatous mitral valve disease (MMVD) is the most common canine acquired cardiac disease that can eventually lead to congestive heart failure (CHF). Dimethylarginines are produced by protein metabolism: asymmetric dimethylarginine (ADMA) plays a role in vascular remodeling and it is a recognized cardiovascular risk factor in humans, while symmetric dimethylarginine (SDMA) is eliminated by the kidneys and it is considered a biomarker of early renal damage. Renal dysfunction is a well-documented complication of CHF in humans while this association is poorly documented in the dog [1]. The aim of this study was to assess plasma concentration of SDMA, ADMA and its precursor L-arginine in healthy dogs and in dogs with MMVD at different stages, with or without pulmonary hypertension (PH). The study protocol was approved by the ethical committee of University of Padua, OPBA authorization 26/2017. Dogs were prospectively recruited among animals visited at the Veterinary Teaching Hospitals of the University of Padua and Bologna. Each dog underwent a complete clinical exam, arterial blood pressure measurement, CBC, biochemical profile, urinalysis, thoracic radiography, 6-lead standard electrocardiogram and trans-thoracic echocardiography. Disease stage was assessed according to the ACVIM classification [2]. A control group of clinically healthy dogs was included. Plasma concentration of ADMA and SDMA were determined through high–performance liquid chromatography [3]. Statistical differences among ACVIM groups and dogs with or without PH were analyzed with a Kruskal- Wallis or Mann-Whitney test, respectively. P value<0.05 was considered statistically significant. A total of 70 dogs were recruited, 7 control dogs and 63 dogs with MMVD, including 22, 24 and 17 dogs classified in ACVIM stage B1, B2 and C+D, respectively. PH was diagnosed in 14 dogs with MMVD. Plasma concentration of SDMA and ADMA was significantly higher in dogs of group of C+D compared to that of dogs of group B1 (P=0.005 and P=0.002, respectively). SDMA was significantly higher in dogs with PH (P=0.044). These preliminary results suggest the possible role of ADMA and SDMA as biomarkers of disease severity in dogs with MMVD. Further studies with a larger number of dogs are needed to confirm this hypothesis.

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ULTRASONOGRAPHIC ASSESSMENT OF ABDOMINAL AORTIC STIFFNESS IN HYPERENSIVE DOGS

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Systemic hypertension (SH) refers to a persistent and pathological increase of arterial blood pressure (BP). In clinical practice, Doppler and oscillometric devices are commonly used to non-invasively and indirectly estimate BP[1]. Excessive movements or tremors can make difficult or even impossible to measure BP by those devices, moreover, animal's anxiety or excitement can induce situational hypertension, leading to an erroneous diagnosis of pathologic SH [1]. Chronic SH leads to increase in aortic (Ao) stiffness [2,3], therefore, non- invasive measurements of Ao elastic properties could help clinicians to differentiate pathological from situational hypertension and to overcome measurement difficulties related to animal's movements and tremors. The objective of our study was to compare the abdominal Ao stiffness, assessed by 2D ultrasonography (US), between hypertensive (HT) and normotensive (NT) dogs. Forty-seven dogs who presented clinical signs or conditions potentially associated with SH were prospectively included in the study. The local ethical committee (OPBSA) approved the study protocol (50675/18) and all owners signed an informed consent form before enrolment of their dogs. BP was assessed by an oscillometric device. Dogs were considered HT if systolic BP (SBP) values ≥160 mm/Hg were measured in at least 3 consecutive occasions. Ao stiffness was estimated by calculating the Ao strain (AoSt), which is the percentage change of the Ao diameter, by the following formula: AoSt=(AoDs−AoDd/AoDd)x100 [4,5] where AoDs and AoDd are the Ao diameter in systole and in diastole respectively. AoSt was calculated from 2 different abdominal Ao transverse sections, the first just caudally to the left renal artery emergence (K_AoSt) and the second just cranially to the external iliac arteries emergence (I_AoSt). Twenty-seven dogs were included in the HT group and 20 in the NT group. Both mean (SD) K_AoSt and I_AoSt of HT dogs, 7.37 (3.53) and 5.64 (2.47) respectively, were significantly lowers ($P<0.05$) than those calculated in NT dogs, 9.97 (3.5) and 8.16 (3.72) respectively. The K_AoSt and I_AoSt indices can provide complementary information in the diagnosis of SH in dogs especially when clinicians must differentiate true chronic hypertension from situational hypertension and when animal's movements and tremors can make the indirect BP measurements inaccurate.

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TRANSVERSE RIGHT VENTRICLE STRAIN AND STRAIN RATE ASSESSED BY 2-DIMENSIONAL SPECKLE TRACKING ECHOCARDIOGRAPHY IN DOGS WITH PULMONARY HYPERTENSION

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Right ventricular (RV) strain analysis using 2-dimensional speckle tracking echocardiography has focused on assessing longitudinal strain and strain rate variables in dogs [1-3]. However, RV contraction is also characterized by transverse deformation; this strain component has not been investigated in dogs. Therefore, we evaluated the ability of transverse RV strain and strain rate, obtained by 2-dimensional speckle tracking echocardiography in healthy dogs and dogs with pulmonary hypertension, to identify dogs with pulmonary hypertension. Additionally, we examined relationships of transverse strain and strain rate variables with heart rate, age and bodyweight in healthy dogs, and with tricuspid regurgitation (TR) velocity and left atrial size in dogs with pulmonary hypertension by univariable linear regression. We acquired 2D echocardiographic cineloops from the left apical 4-chamber view optimized for the right ventricle and analyzed transverse RV free wall strain and strain rate in 74 dogs (40 healthy dogs and 34 dogs with pre-capillary and post-capillary pulmonary hypertension) using XstrainTM software. Dogs were classified as having pulmonary hypertension based on the TR jet velocity (> 3 m/sec) [4]. We classified dogs as having moderate pulmonary hypertension if TR velocity >3.5 m/sec, and severe pulmonary hypertension if TR velocity >4.5 m/sec. Seven dogs (3 healthy and 4 dogs with pulmonary hypertension) were excluded during the analysis for low quality images. In healthy dogs, strain and strain rate showed no relationship with heart rate, body weight or age (all P>0.05). In dogs with pulmonary hypertension (TR velocity: median 3.68 mmHg; range, 3.11-6.25 mmHg), strain and strain rate showed weak negative relationships with TR velocity (r2=0.25, P=0.006 for both variables), but no relationship with left atrial size (r2=0.05, P=0.2 for both variables). Although transverse RV strain (but not strain rate) showed a negative relationship with class of pulmonary hypertension, it was not useful in identifying dogs with pulmonary hypertension. Transverse RV strain and strain rate using 2- dimensional speckle tracking echocardiography can be obtained in most dogs, but does not help in identifying dogs with pulmonary hypertension.

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ANALYSIS OF THE CORRELATION BETWEEN RENAL PATHOLOGY AND RENAL ULTRASONOGRAPHIC ABNORMALITIES

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Ultrasonographic examination of the kidneys is a fundamental procedure in renal pathology staging. A limited number of published studies 1,2 is focused only on the correlation between renal cortical echogenicity and renal pathology. To analyse the correlation between renal ultrasonographic abnormalities and renal histopathology 53 dogs, referred to the University Veterinary Teaching Hospital of the Università degli Studi di Padova within January 2015 – December 2018, and death for different reasons have been included in the study. A complete ultrasonographic examination of the abdomen was performed as part of the routine clinical evaluation of the patients and all the ultrasonographic images of the kidneys have been reviewed. The presence of different abnormalities, such as: irregularity of the margins, abnormal shape, lack of cortico-medullary definition, hyperechogenicity of the renal cortex, hyperechogenicity of the medulla, perirenal fluid, cysts, scars, mineralizations, and hydronephrosis has been recorded. A novel parameter, called renal ultrasonographic score (RUS), based on the total amount of ultrasonographic abnormalities counted for each kidney, is proposed. The renal degeneration and inflammation degrees, as determined by histopathology, have been graded from zero to three 1. The correlation between the RUS and the degeneration score was moderate ($R=0.67$, $p<0.001$) whereas the correlation between the RUS and the inflammation score was only low ($R=0.33$, $p<0.001$). Among the ultrasonographic abnormalities the presence of cysts ($R=0.679$, $p<0.001$), and the lack of cortico-medullary distinction ($R=0.534$, $p<0.001$) showed the highest correlation with renal degeneration. According to literature 1 the correlation between renal cortical echogenicity and renal degeneration was only low ($r=0.288$, $p=<0.001$). Renal ultrasonographic score resulted positively correlated with degeneration degree but not with inflammation degree. The presence of cysts and the lack of cortico-medullary distinction were the ultrasonographic abnormalities showing the highest correlation with renal degeneration.

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CHARACTERIZATION OF 127 URINARY SAMPLES IN DOGS WITH URINARY TRACT INFECTIONS: A RETROSPECTIVE STUDY (2015-2018)

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Uncomplicated canine bacterial urinary tract infection (UTI) commonly occurring in approximately 14 % of dogs over its lifetime. Persistent or recurrent infections, including refractory bacteria to conventional antimicrobial therapy, are also reported in 4.5% of dogs with UTI, with pet regarded as potential reservoirs of drug-resistant uropathogens [1]. The aim of this study was to describe clinical, bacterial culture and urinalysis findings of dogs with suspected UTI, with emphasis on incidence of multi-drug resistant (MDR) infections and factor affecting their occurrence. From 2015 to 2018, 127 urine samples of 79 dogs were retrospectively evaluated. Among dogs (mean age 8.5 ± 3.5 years), 51/79 were males (8 neutered) and 28/79 females (8 sterilized). Mixed breed (6/79), Pinscher (5/79) and German Shepherd (5/79) were the more represented. Urine samples were obtained by cystocentesis (66/127), midstream free flow (56/127) and catheterization (5/127). Positivity on bacterial culture was recorded in 25/127 urine samples (Group A), while 102/127 samples scored negative (Group B), without predisposition of sex (p=0.6) and age (p=0.2). The most frequently bacterial isolates were E. coli (12/25), Staphylococcus spp. (4/25), Pseudomonas spp. (3/25), Enterococcus spp. (2/25), Proteus spp. (2/25) and others (2/25). Lower urinary tract signs (e.g. dysuria, hematuria, pollakiuria) ($p<0.05$), bacteriuria ($p<0.001$) and active sediment ($p<0.001$) were significantly increased in Group A, regardless the collection methods (cystocentesis vs midstream free flow) (p= 0.75). No statistical differences between Group A and B were observed in regards of urine pH (p=0.56), urine specific gravity (p=0.69) and urine protein to creatinine ratio (p= 0.89). An history of antibiotic treatment within 6 weeks before the sampling was recorded in 20/79 dogs and 6/20 had urine positive to urinalry culture. Antibiotic-resistance was detected in 20/25 bacterial isolates and, of them, 9/20 were identified as MDR; a significant increase of detection of MDR bacteria was observed in dogs underwent antibiotic therapy (amoxi-clav. and enrofloxacin) within 6 weeks before the sampling ($p<0.05$). The infections were resolved in 21 dogs, while a chronic cystitis developed in 4 dogs. This study confirms that the presence of bacteriuria, active sediment and clinical signs are associated with UTI in dogs [2], demontrates that the urinalysis is not dependent to the collecting method, and midstream free flow can be an alternative method in dogs. The high prevalence of antibiotic-resistance in dogs with UTI and the increased incidence of MDR in dogs previously treated with antibiotic, confirm the clinical utility of bacterial culture to decrease the prevalence of persistent or recurrent UTIs often difficult to treat using conventional antimicrobial therapy.

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BACTERIAL COLONIZATION OF NON-PERMANENT CENTRAL VENOUS CATHETERS IN HEMODIALYSIS DOGS

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Non-permanent central venous catheters (CVCs), are the most commonly used vascular access in veterinary patients undergoing hemodialysis. In human dialysis patients, CVC infection represents a common cause of morbidity and mortality (50-70% hospitalization rate), which may lead to CVC-related infection (CVC-RI). CVC contamination may occur for a break in aseptic technique during placement and maintenance of the catheter, or for hematogenous colonization from other infection sites. The aim of this retrospective observational study was to evaluate the prevalence of bacterial colonization of CVCs in dogs submitted to hemodialysis treatment at time of CVC removal. The CVCs of all dogs submitted to hemodialysis (n=23) at the VTH Pisa University between January 2015 and December 2016 were considered. Signalment, reason for hemodialysis treatment, duration of catheterization, CVC complications, and 30-day survival were considered. Dogs were divided in young (0-1 years old), adult (2-7 years old), and aged (≥8 years old). According to duration of catheterization, dogs were divided in two groups: ≤15 days and >15 days. Dogs were classified in survivors (S) and non-survivors (NS). Survivors were still alive 30 days after discontinuation of hemodialysis, or replacement of CVC. NS included died or were euthanized in the intra- or inter-dialysis time. Reasons for CVC removal were: 1) discontinuation of hemodialysis, 2) CVC replacement due to malfunction or infection, 3) death or euthanasia. Dogs with positive CVCs for bacterial growth were classified as (CVC +), while dogs with negative CVCs were classified as (CVC -). Statistical analysis was performed using Graph Pad Prism™.

Five over 23 dogs (22%) showed positive bacterial culture, and 18/23 dogs (78%) negative culture of CVC. The most prevalent microorganism was Staphylococcus spp. (3/5; 60%). No significant difference was found in the prevalence of CVC infection according to age (p=0.64), gender (p=0.99), reason for hemodialysis (p=0.84), CVC complications (p=0.99), duration of catheterization (p=0.99), and outcome (p=0.99). No statistically significant difference (p=0.64) in survival curves was reported at log rank analysis between dogs with CVC – and CVC +.

In conclusion, the prevalence of bacterial CVC contamination in our canine dialysis population showed relatively low, and only rarely associated with clinical and laboratory signs of CVC-RI. Exclusive use of CVC for hemodialysis, good hygiene practice during CVC management, and use of chlorhexidine as an antiseptic seemed to sufficiently protect against bacterial contamination.

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REMOVAL OF ENDOGENOUS CORTISOL IN DOGS UNDERGOING INTERMITTENT HEMODIALYSIS

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Human patients on hemodialysis (HD) showed elevated, basal, serum levels of endogenous cortisol, although its removal during the dialysis treatment seemed to be trivial (1,2). Beside its therapeutic effects, HD stimulates cortisol release, due to its stressing effect. The aim of the present study was to evaluate the removal of endogenous serum cortisol (C) in dogs submitted to HD. We prospectively included 17 uremic dogs of different breed, gender, age and body weight, with diagnosis of AKI (n=8), AKI on CKD (n=9), referred for HD between September 2017 and September 2018. Four dogs on steroid supplementation at time of dialysis were excluded (n=4). Pre-HD blood sample was collected immediately before starting HD, post-HD was collected at the end of HD (15"bypass, at 50 ml/min of blood flow). C analysis (immunofluorimetric method; TOSOH AIA 360, Futurlab®) was added to routine pre- and post-HD blood work. For each patient, C reduction ratio (sCRR) was calculated by the following formula: sCRR=[(pre-HD serum C – post-HD serum C)/pre-HD serum C]x100. According to the reference range of our laboratory for C (1-5 mcg/dL) dogs were divided in: normal (1-5 mcg/dL), elevated (> 5mcg/dL), and reduced (<1 mcg/dL). Data distribution was tested, and median pre- and post-HD C levels were compared. For pre- HD samples, correlation analysis between C and serum creatinine, and C and serum urea were performed. The number of dogs with normal, elevated and reduced C was compared between pre- and post-HD. For all dogs, correlation analysis was run between sCRR and URR (urea reduction ratio), sCRR and CrRR (creatinine reduction ratio). Data were statistically analyzed with GraphPad Prism™ ($p<0.05$). No significant difference ($p=0.43$) was found between median pre-HD C (2.82 mcg/dL; 0.01-17.5 mcg/dL) and post-HD C (3.9 mcg/dL; 0.01-9.85 mcg/dL). In pre-HD serum samples, no correlation was found between C and creatinine ($p=0.77$), and between C and urea ($p=0.48$). No difference in the number of dogs with normal, elevated, and reduced C levels was found between pre- and post-HD ($p=0.49$). Finally, no correlation was found between sCRR and URR ($p= 0.80$), and sCRR and CrRR ($p=0.62$). Similarly to human medicine, C levels did not change significantly between pre- and post-HD treatment. This finding seemes to reflect a scarce HD clearance of middle size molecules, C included. However, although a trivial HD removal of C was documented, no significant increase in post-HD C levels was found. Therefore, we may hypothesize that HD clearance is able to compensate for increased C release.

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FAILURE OF PASSIVE TRANSFER OF IMMUNITY: PREVALENCE AND RISK FACTORS IN CHIANINA COW-CALF OPERATION. PRELIMINARY DATA

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Failure of passive transfer of immunity (FPTI) has been related to increased morbidity and mortality in calves [1], particularly in beef production, where it might impair profitability due to additional costs of treatment and reduced weight gain [2]. FPTI can be assessed by several methods. Serum total protein (STP) measured with a refractometer provides an indirect measure that is highly correlated with blood IgG concentration and is more practical for use on-farm [3]. The aims of this study were to estimate the proportion of FPTI among a selected population of Chianina calves and to identify calf- and herd-level variables associated with FPTI. Managers of study herds completed a questionnaire on herd-level variables and colostrum management practices that were in use at the time each calf was sampled. The authors collected for each calf information about sex and identification number. A blood sample was collected from 60 clinically healthy Chianina calves between 1 and 7 days of age. The calves were selected from 5 different Chianina-farms, which raised between 50 to 190 beef cattle and heifers. STP levels were determined by the use of digital refractometer (MISCO Palm Abbe no. PA201, Misco, Solon, OH). Calves were classified as having FPTI when the STP concentration resulted less than 5.2 g/dl [4]. The estimated prevalence of FPTI in Chianina calves was 36.6%. However, these preliminary data must be subsequently investigated by directly assessing of serum IgG concentrations. Preliminary risk analysis performed on the preliminary data, shows that the percentage of calves affected by FPTI might be higher in the Chianina-farm with more than 150 cattle and heifers and for calves born from a twin birth or dystocia. Moreover, preliminary data obtained by in farm study reported the possibility for cows older than 10 years old or with 8 parity or greater not to guarantee adequate passive immunity to the calf. The same condition seems to involve calves born from the cows with a body condition score lower than 2.75. However, the use of colostrum supplements or replacement products within the first 24 hours in beef calves could insure a lower rate of FPTI. The present study is the first survey performed in Italy and aims to improve the welfare of the calf and the entire chain in the cow-calf operation. The transfer of passive immunity could be improved in Chianina calves, considering that the prevalence of FPTI is likely to be reduced by at least hand-feeding the calf with colostrum supplements or replacement products immediately after calving in particular in animals that show risk factors mentioned above.

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THE RELATIONSHIP BETWEEN COLOSTRUM QUALITY, PASSIVE TRANSFER OF IMMUNITY AND BIRTH WEIGHT IN NEONATAL CALVES

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Calves born almost agammaglobulinemic so they need an adequate amount of good quality colostrum to avoid failure of passive transfer (FPT) [1]. The quality of colostrum is influenced by different factors such as the volume of colostrum produced, concentration of immunoglobulins (IgG), breed, the age of dam and mastitis events in the previous lactation [2]. The main important risk factors for FPT in calves include feeding calves with a poor quality of colostrum or with an inadequate volume of colostrum in the first 24 hrs of life and feeding calves with colostrum contaminated by bacteria [2]. A simple method to estimate the quantity of IgG in the colostrum is the use of refractometer. The break point for a high- quality colostrum is 21% Brix [3]. Serum Total Protein (TP) evaluation by refractometer has been used for field FPT diagnosis in calves, with a threshold >5.5 g/dL [1]. The aim of the present study was to evaluate the relationship among colostrum quality (CQ), serum TP, birth and weaning weight of the calf. Inclusion criteria were easy calving and the complete assumption of 6 L of dam colostrum in the first 24 hrs of life [2]. Ready after calving and at 60 days of life each calf has been weighed. Colostrum has been evaluated after calving by a qualified operator using an optical Brix refractometer. Twenty-four hours after birth, 10 mL of blood were collected from each calf in order to evaluate the concentration of immunoglobulins using a digital refractometer. The relationship between CQ, serum TP, birth and weaning weight was analyzed by using a mixed linear model. Colostrum quality increased with parity, serum TP increased along with the increasing of CQ administered to the calves. Serum TP levels were higher in calves born with a lower birth weight compared to those with a higher birth weight. Both CQ and serum TP did not significantly affect the calves' weaning weight. Colostrum quality has been related to parity in Holstein Friesian cows [4]. Studies confirmed the relationship between serum TP and IgG in calves [1,2]. Study found no significant differences in growth rate between calves fed with different IgG level colostrum during the first 6 months of life [5]. Birth weight might influence the passive transfer of immunity in calves thus it might be taking into account by the farmer. Further studies evaluating the volume of colostrum as a percentage of the calf's birth weight are recommended.

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EVALUATION OF ORAL ADMINISTRATION OF CHESTNUT TANNINS IN PREVENTING CALVES' DIARRHEA

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Calf diarrhea is generally caused by infectious agents and is a very common disease in bovine practice, leading to substantial economic losses [1]. Tannins are known for their astringent and anti-parasitic properties in the gastro-enteric tract [2]. The aims of this study were to evaluate the preventive effect on calves' diarrhea of the oral administration of chestnut tannins (Castanea sativa) and its potential toxicity. Forty Italian Friesian female calves were included and divided into a Control Group (C-G) and a Tannin Group (T-G). Since the 3rd day of life (T0) to the next 60 days (T60), calves from the C-G received 2 L of warm water, while calves from T-G received 2 L of warm water plus 10 g of extract of chestnut tannins powder (750 g/kg of dry matter equivalent of tannic acid). Calves were weighted at birth (T0) and after two months (T60). The age at diarrhea onset, the duration of diarrheic episode and the frequency of diarrheic episodes were recorded. Blood methemoglobin, plasmatic Albumin (ALB), Gamma-Glutamyl Transferase (GGT) and Aspartate aminotransferase (AST) were weekly evaluated starting from T0. A t-Student test was performed to verify differences for the age at diarrhea onset, duration of diarrheic episode, frequency of diarrheic episode and daily weight gain between the two groups. ANOVA test for repeated measures has been performed to evaluate the differences of Albumin, GGT, AST and methemoglobin during time in both groups. The age at diarrhea onset was 7.7±3.8 days for C-G and 12.0±8.2 days for C-T with a statistically significant difference ($p=0.04$). There were no differences concerning duration and frequency of diarrheic episode and daily weight gain. Blood methemoglobin, plasmatic Albumin, GGT and AST were never over the physiological range values reported in literature [3,4], confirming the absence of hepatic toxicity for chestnut tannins. Phytotherapic treatments for various diseases have become more common both in human and in veterinary medicine, in order to reduce the presence of antibiotic molecules in the food chain and in the environment [2]. Administration of tannins in calves since the 3rd day of life seemed to delay the onset of diarrhea by almost 4 days, suggesting an effective preventive action of chestnut tannins in the calf. Literature showed that later the diarrhea onset, lesser would be the economic losses due to the disease [1]. The use of chestnut tannins in calves could represent an effective, low-impact preventive strategy for neonatal diarrhea.

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EFFECT OF INTRAMAMMARY ADMINISTRATION OF BENZATHINE CLOXACILLIN AGAINST *STAPHYLOCOCCUS AUREUS* MASTITIS WITH DRY PERIOD ORIGIN IN DAIRY WATER BUFFALO (*BUBALUS BUBALIS*)

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Mastitis is one of the most costly diseases in dairy buffaloes, but at present, just a few specific antibiotics registered for this species are synthesized. The administration of antibiotics ad-hoc created for cow can be considered a common practice in buffalo [1]. The current investigation evaluates clinical outcomes of an antibiotic dry buffalo therapy (aDBT) against Staphylococcus aureus (S. aureus) mastitis with dry period origin, based on intra- quarter administration of 600mg of benzathine cloxacillin. One hundred sixty quarters originating from 40 pluriparous Mediterranean buffaloes (MBs) were divided in aDBT group (receiving therapy at drying-off) and no-aDBT group (left untreated) of 80 quarters each one. All quarters were sampled at drying-off and at the first sampling of the new lactation [<30 days in milk (DIM)]. Clinical efficacy was verified calculating: prevalence of animals/quarters affected by intramammary infections (IMI), subclinical mastitis (SCM) and clinical mastitis (CM) due to S. aureus as well as fresh calver infection rate, dry period new infection rate, dry period cure rate and failure of cure rate. Effects on somatic cell count (SCC) values and milk yield were also considered. An intra-group (drying-off vs. <30 DIM) statistically significant difference was detected regarding prevalence of positive MBs ($P<0.05$) and positive-cultured quarters in the aDBT ($P<0.01$); concerning the latter, an inter-groups difference within 30 DIM was also recorded (aDBT vs. no-aDBT, $P<0.01$). No CM due to the bacterium were observed, while for SCM a higher intra-group significant difference (drying-off vs. <30 DIM) was observed in treated ($P<0.01$) than untreated group ($P<0.05$), as well as between two groups within 30 DIM (aDBT vs. no-aDBT, $P<0.001$). Regarding IMI, none protective effect was observed, while concerning the clinical indexes the results within 30 DIM showed inter-groups differences (aDBT vs. no- aDBT) for fresh calver infection rate ($P<0.001$), dry period new infection rate ($P<0.01$), dry period cure rate ($P<0.0001$) and failure of cure rate ($P<0.05$). No significant inter- and intra- groups differences were observed about daily mean production, while profitable results were detected for SCC since an inter-groups difference was found within 30 DIM (aDBT vs. no-aDBT, $P<0.001$). The direct effects against S. aureus in-udder infections of an administration of benzathine cloxacillin at drying-off were demonstrated for the first time in MBs. Its use shows encouraging results in reducing prevalence of positive animals and mastitis at the resumption of the lactation, associated with lower values of SCC. Nevertheless, the effects on the entire dairy herd under field conditions should be assessed by further scientific surveys to understand fully its efficacy.

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A RETROSPECTIVE STUDY ON TRANSABDOMINAL ULTRASOUND MEASUREMENTS OF THE RUMEN WALL THICKNESS TO EVALUATE RUMINAL ACIDOSIS IN BEEF CATTLE

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Rumen acidosis is a severe metabolic disease of cattle. Until today the diagnosis is performed by detection of ruminal pH [1]. The aim of this study was to determine cut-off measurements of the rumen wall (RW) by transabdominal ultrasound for the diagnosis of acidosis in beef cattle. From a large data set of patients subjected to measurement of the ruminal pH, 478 beef cattle of Charolaise breed were selected on the base of age (10.8±0.7 months), weight (434.05±30.44 kg) and ruminal pH (sick animals = pH≤5.8; healthy animals = pH≥5.9). Ultrasonographic examination of the RW thickness was conducted before rumenocentesis and on the same area. RW was evaluated by the same operator with a portable ultrasound scanner equipped with a multi-frequency convex probe (2.2-4.3-6.6 MHz). Ruminal pH was measured ready after sampling using a portable pH-meter. The quantitative determination of the Volatile Fatty Acid (VFA) was performed using High Performance Liquid Chromatography. One-way Analysis of variance was used to detect differences in the measured parameters. Regression analysis (R2) and Pearson's correlations were performed with thickness of the RW, rumen mucosa (RM) as dependent and ruminal pH as independent variables. Receiver Operating Characteristics (ROC) analysis were conducted to identify suitable cut-off for thickness of the RW and RM ultrasonography. The regression analysis conducted on ruminal fluid pH and total ultrasound thickness of RW and RM showed respectively R2=0.5637 and R2=0.5895. Pearson's analysis showed interaction between pH and ultrasound thickness of RW (-0.700; P<0.0001) and RM (-0.7921; P<0.0001). A significant Pearson's correlations was found between Propionic Acid and thickness of RW (+0.543; P<0.0001) and RM (+0.553; P<0.0001); Acetic Acid and thickness of RW (+0.407; P<0.0001) and RM (+0.442; P=0.005), n-Butirric Acid and thickness of RW (+0.471; P<0.0001) and RM (+0.301; P<0.0001). The difference RM layer thickness in healthy and affected animals by acidosis were confirmed. The area under the receiver operator curve (AUROC) was 0.970 (p<0.0001; 95% CI:0.935-0.989) and the cut-off value was of 5.4 mm (96.30% of sensitivity; 91.60% of specificity) in sick animals. Similar results were found in RW thickness between healthy and affected animals by acidosis. AUROC of the RW thickness was 0.956 (p<0.0001; 95%; CI:0.918-0.980) and the cut- off value was 8.2 mm (91.36% of sensitivity; 91.60 % of specificity) in sick animals. In conclusion, the thickening of the RW and RM were correlated with the changes of ruminal pH. Morphological changes in RW and RM are well established in response to VFA concentration. The increased concentrations of VFA in the rumen are the main trigger for ruminal papillar growth wherever the mucosa is directly exposed to these acids [2]. Transabdominal ultrasonography of the RW and RM have the potential to be a suitable as a diagnostic tool useful to identify fattening bulls affected by rumen acidosis.

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FAST LUNG ULTRASONOGRAPHY (FLUS) AS A RAPID DIAGNOSTIC METHOD FOR BOVINE RESPIRATORY DISEASE

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Clinical scores and lung auscultation are commonly used for the diagnosis of bovine respiratory disease (BRD) in field conditions, despite low sensibility and specificity (Calf Respiratory Scoring Chart - CRSC: sensibility (Se) 55%, specificity (Sp) 58%, thoracic auscultation: Se 3-17%) [1]. Thoracic ultrasonography (TUS), on the other hand, represents the "gold standard" for the evaluation of lung damages (Se 79.4%, Sp 93.9%) [2]. However, TUS is often demanding, especially for untrained veterinarians and it is not suitable to investigate a large number of animals. The aim of this study is to evaluate the accuracy of a fast lung ultrasonography (FLUS) for the diagnosis of BRD, compared to TUS. One hundred Holstein Friesian calves, aged between 1 to 6 months, belonging to different dairy herds with different BRD prevalence, were enrolled. All calves were examined by a single operator using CRSC [3], thoracic auscultation and complete TUS [2]. Between lung lobes those are more frequently affected by BRD, for fast lung ultrasonography we scanned only those are easier to detect: the caudal aspect of cranial lobe of the left lung (IV and V left intercostal spaces), the caudal aspect of cranial lobe of the right lung (IV right intercostal space) and the middle lobe of the right lung (V right intercostal space). Both TUS and FLUS were scored using the ultrasonography scoring system proposed by Ollivett and Buczinski [2] and animals were considered affect by BRD if the score was ≥ 2. Sensibility (Se), specificity (Sp), positive predicting value (PPV), negative predicting value (NPV), accuracy (Acc) and Coehn' Kappa concordance test (K) of FLUS were calculated using TUS as a gold standard. A total of 55 out of 100 calves had a TUS score ≥2 and therefore were considered affected by BRD. FLUS presented a Se=84.62%, Sp=100%, PPV 100%, NPV=77,78% Acc 90% and K=0.577 (P value<0.001). Our results suggest that FLUS is a practical and speedy method that can provide a good representation of pulmonary lesions and can be easily performed by a single operator. FLUS can be considered an additional tool to reach an early and accurate diagnosis, especially during examination of a herd. Furthermore, FLUS can be used to apply a selective therapeutic protocol with reduction of unnecessary antibiotic treatments.

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FOCUSED ULTRASONOGRAPHY ASSESSMENT OF HEPATIC HYDATID CYST IN SHEEP

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Cystic echinococcosis (CE) is a well-known widespread zoonotic disease of sheep and other intermediate hosts caused by the larval stages of Echinococcus granulosus which lead to important health and economic issues [1]. Some studies showed that ultrasonographic (US) examination of liver is a non-invasive, reliable, intra-vitam diagnostic tool for hydatid lesions [2,3]. The aim of this study was to perform a focused assessment of the standard scanning liver technique in order to develop a fast evaluation of CE based on anatomical distribution of hydatid cysts in the liver of naturally infected sheep. A total of 136 female sheep were submitted to US examination of the liver within 24 hours from the scheduled slaughtering. The US study was conducted by longitudinal and transverse scan in three contiguous zones on the right side: Z1 (from the right hypochondrium to the 11th IS), Z2 (from the 10th IS to the 8th IS), Z3 (form the 7th IS to 6th-5th IS). Each suspected hydatid lesion was recorded, classified according the WHO standards, localized in one of the zones and confirmed by post mortem examination. Each zone was considered positive if at least one cyst was present. A comparison was made between single zones (e.g. Z1 vs. Z2), the single zones and combination of them (e.g. Z1 vs. Z1+Z2) and between each combination (e.g. Z1+Z2 vs. Z1+Z2+Z3). The results of this study showed a CE prevalence value of 49% (66/136) detected by US, whereas the diagnostic sensitivity of US was 91% and specificity 81% considering post mortem examination as gold standard. The fast ultrasound scan developed on zone cyst positivity failed to reach an acceptable result. Neither the distribution in each single zone, nor the distribution in the combination of two of them was statistically significant. Thus, the complete scanning of the liver still represents the elected method able to detect the higher number of sheep positive to CE compared to any other scanning zones.

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EVALUATION OF RADIOGRAPHIC METHOD TO DETECT GROWTH PLATE LESIONS IN METATARSUS OF FATTENING BULLS

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The growth plate lesions are frequently detected in young beef cattle. These pathologies are usually associated with the presence of physitis or osteochondrosis, which are probably due to the rapid growth and high weight increase, traumatic injuries, inadequate environmental conditions, excesses or deficiencies in diet (alteration Ca/P ratio, deficiency in microelements, hypoparatyroidism), genetic causes [1]. Early radiographic diagnosis of physitis is pivotal to evaluate the severity of inflammation, to detect these pathological changes and to provide well-timed management [2]. The aim of the study was to evaluate and to compare radiographic changes on these sites to establish a possible classification based on lesions severity observed in the physis of fattening bulls and to reduce mis-interpretation of the radiographic findings. Seventy-six lame young bulls of different beef breeds, were included. The average age and body weight were 14.76±1.76 months and 674.37±90.56 kg respectively. All the bulls were housed on a hard slatted floor. Diet was provided daily as a total mixed ration (TMR) for ad libitum intake based on 10% feed refusal (as-fed basis) once a day. On clinical examination, the animals presented swelling of the metatarsophalangeal joint that was not painful. Animals treated with antibiotic and/or antinflammatory drugs were not included in the study. Radiographic examinations of the distal metaphysis in the metatarsophalangeal joint were performed using a portable X-ray unit AJEX 140H (Meditec©). One-way ANOVA and Bonferroni t-test were carried out to calculate statistically differences and interactions between different grades of physitis and metric surveys and body weight. Significance was set at $P<0.05$. In this study, a physitis score was established. Four grades of physis alterations (from G0 to G4) were found using radiographic measurement. The different grades of epiphysitis or osteochondrosis had different metric features in the growth plate. Statistical differences ($P<0.001$) were found in mean values on every grade of physitis (G0<1mm; G1=5.9±2.4mm; G2=8.5±1.2mm; G3=11.4±1.05mm; G4=14.7±3.7mm). The metatarsal width in G0 and in G1 were statistically different ($P<0.05$) compared to other degrees of physeal lesion. The body weight mean values showed the effects on physitis score ($P<0.001$). According to Reiland et al. [3], the prevalence of lesions in the growth plate were 78.9%. Radiographic measurement of physeal thickness represents a new method to detect different types of lesions in the growth plate based on their score. Classification of lesions according to radiographic measurements has determined that it mighy be possible to make a diagnosis in relation to the worsening conditions of the growth plate following this physitis score.

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SERUM AMINO ACID CONCENTRATION IN HEALTHY DOGS AND IN DOGS WITH IMMUNOSUPPRESSANT-RESPONSIVE ENTEROPATHY (IRE)

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Low tryptophan concentration has been identified in humans with inflammatory bowel disease (IBD) and in dogs with protein-losing enteropathy (PLE) [1,2]. At present, no data on serum AA are available in dogs with immunosuppressant responsive enteropathy (IRE). The aims of the study were to compare serum AA between healthy (HD) and IRE dogs, and to compare AA according to clinicopathological variables and follow-up. Twenty-six dogs (HD) were included with owner's consent. Fifty-one IRE dogs were included and fed with hydrolysed diet for at least one month prior the inclusion. The Canine Chronic Enteropathy Clinical Activity Index (CCECAI) and serum AA were assessed for each dog at presentation. The following serum AA were evaluated in HD and IRE,: SER, ASP, GLU, GLY, HIS, ARG, THR, ALA, PRO, CYS, TYR, VAL, MET, LYS, ILE, LEU, PHE, TRP. IRE were classified as responders (decreased CCECAI >75% at 1 month compared to T0), partial responders (decreased CCECAI from 25-75%) and non-responders (decreased CCECAI <25%); then divided in PLE (albumin <2.7 gr/dL) and non-PLE (albumin ≥2.7 gr/dL) [3]. AA were compared between HD and IRE dogs. Serum AA were compared according to gender, age (<2, 2-7 and >7 years), BCS, PLE/non- PLE, CCECAI categories (0-3, 4-5, 6-8, 9-11 and >12) and clinical response categories. AA were correlated with total protein and albumin. Variables were compared using Mann-Whitney U-test or unpaired t-test, Kruskal-Wallis or one-way ANOVA, Pearson's or Spearman's correlation depending on normality test (GraphPad Prism 6). IRE showed significantly higher levels of SER ($p=0.02$), GLU and ARG ($p<0.001$), THR ($p=0.013$), PRO ($p=0.044$), CYS ($p=0.003$), VAL ($p=0.018$), LYS ($p=0.01$) and ILE ($p=0.005$), and lower levels of TYR and PHE and TRP (all $p<0.001$). In PLE, HIS ($p=0.008$), PHE ($p=0.005$) and TRP ($p=0.005$) were significantly lower than non-PLE. In IRE no significant difference in AA was found according to age, gender and clinical response categories. In IRE total protein was positively correlated with GLU ($p=0.047$, $r=0.28$), PHE ($p=0.031$, $r=0.30$) and negatively correlated with SER ($p=0.049$, $r=-0.28$), TYR ($p=0.043$, $r=-0.28$), TRP ($p=0.032$, $r=0.30$). Albumin was positively correlated with HIS ($p=0.025$, $r=0.31$), PHE and TRP ($p=0.001$, $r=0.46$). As previously described [2], TRP is decreased in dogs with PLE, although in our cohort also PHE and HIS were decreased. In humans, HIS and PHE are considered potential biomarkers of IBD [4]. Although all dogs were fed with the same diet, GLU tend to be lower in IRE dogs with BCS 3/9. This preliminary study can create a prospective for studies on the possible therapeutic and prognostic relevance of AA in IRE dogs.

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LEGAL AND ETHICAL ISSUES IN VETERINARY CLINICAL RESEARCH

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Biomedical research is still widely based on the use of animals as a model for studying human diseases, but animals can represent themselves the target species of the research.
The use of animals for scientific purposes is regulated under the Directive 2010/63/EU, transposed into Italian law by Legislative Decree 26/2014. According to the current legislation, a project including any procedure which may cause the animal a level of pain, suffering or distress equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice requires an authorization by the Competent Authority, which performs a technical and scientific evaluation [1].
"Non-experimental clinic veterinary practices" are excluded from this regulatory framework. But the question is: “when does clinic become research”? In fact, routine clinical practice can lead to the collection of samples and information which could be used for research purposes at a later stage. Therefore, the acquisition of data from healthy subjects or the study of spontaneous diseases are often essential to the progress of veterinary science; consequently, animal care and the acquisition of new knowledge through research can be concurrent. In such cases, written informed consent should always be obtained from the owner of the animal for permission to use blood and tissue samples, as well as images obtained as part of diagnosis and treatment, for research purposes [2]. This is also relevant in case information of clinical importance is identified, in which case the owner should be informed, thus arising confidentiality issues. Furthermore, the fact that the research doesn’t fall within the Directive doesn’t exempt it from being subject to legal and ethical review, which is increasingly required by scientific journals [3]. In light of these considerations, the ethical review in animal research beyond law boundaries becomes a matter of great importance which cannot go unmentioned. A formal ethical and scientific evaluation, performed by an ethics committee prior to the research being conducted, would be a guarantee, both to the researchers and to the publishers, of the quality of the research design, the adequacy of the methodology, the accordance to the ethical standards and the protection of animal welfare. Moreover, the ethical and scientific review could lead to the conclusion that the proposed research project falls under the scope of the Legislative Decree 26/2014 and therefore requires an official authorization by the Competent Authority, thus avoiding potentially illegal situations.

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APPROACH TO FATAL DOG ATTACKS: A FORENSIC CASE STUDY

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A 62 years-old man died due to the fatal clinical complications occurred after a serious dog attack. Immediately after the attack, the victim pointed to two pitbull dogs as responsible for the attack. The dogs were found by the competent authorities few kilometers away from the site of the attack. Because of the lack of direct witnesses and since the dog owners refused any responsibility of the attack, a technical evaluation was necessary in order to establish the attack details and responsibility. Then, the casts of dental arches were collected with dogs under general anesthesia (with dedicated kit and alginate Hidrogum 5, Zhermack). The forensic investigation verified the perfect compatibility between the dogs' dental arches and the wounds present on the victim body. The investigation of places, type, diffusion and size of injuries on the victim led us to classify the aggression as "predatory typology with playful onset", moreover due to their breed behavioral features, pitbull dogs are genetically predisposed to chase and predate. For all these reasons the two dogs were considered responsible for the aggression.

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LIPIDOMIC ANALYSIS OF THE ERYTHROCYTES MEMBRANE IN STORED CANINE PACKED RED BLOOD CELLS UNITS WITH AND WITHOUT LEUKOREDUCTION

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The leukoreduction (LR) of packed red blood cells units (pRBC) significantly reduces the accumulation of pro-inflammatory mediators (PIM) deriving from platelets and leukocytes that appear to be modifiers of biological response. In human medicine, it has been shown that PIM accumulate in stored pRBC despite the LR [1]. Some of that PIM have been implicated in the pathogenesis of multi-organ failure and of Transfusion Related Acute Lung Injury, experimentally induced by administering lipid derived from RBCs stored at 28 and 42 days [2]. Recently, lipidomic analysis (LA) has become an interesting topic of study also in the evaluation of RBC storage lesions [3].
The aim of this study was to perform a LA of the RBCs membranes of canine pRBC stored for up to 42 days, with and without LR. Three donor dogs were used for collection of 450 ml of whole blood using a CPD-SAG- Mannitol transfusion bags with a LR filter in-process, to produce 2 pRBC for each donor, before (nLR pRBC) and after (LR pRBC) LR. The pRBC were stored in blood bank refrigerator at 4°C and one sample from each pRBC was removed aseptically at T0 and T42. LA evaluated a cluster of 10 fatty acids (FA), comprised of saturated (SFA), monounsaturated (MUFA) and polyunsaturated FA [PUFA (ω3 and ω6)] on each sample at 0 and 42 days, using Gas- Chromatography to obtain quantitative data as relative percentages of this cluster. All data were expressed as median (range) and compared by rank tests (MedCalc software, 12.6.1). Statistical significance was set at $P<0.05$. The % of SFA, MUFA and PUFA in nLR pRBC membranes were 33.2 (32.9-35.1), 11.9 (9.4-16.8), 53.0 (50.3-57.4) at T0 and 30.7 (29.5-34.6), 11.7 (11.4-13.4), 57.6 (52.0-59.1) at T42, respectively; whereas the % in LR pRBC were 39.6 (27.8-41.2), 13.1 (12.4-17.9), 53.2 (46.3-54.3) at T0 and 32.4 (31.8-34.7), 10.8 (10.5-13.0), 55.2 (54.5-57.1) at T42, respectively. There was no significant difference in FA composition both between pRBC at T0 and T42, and between LR and nLR pRBC in the same time of storage. Our preliminary data show that the LR does not modify the lipidomic profile of stored RBCs in dogs. In human blood, accumulation of ω6 PUFA in RBCs after 42 days of storage was reported [1] and it could indicate a potential increased susceptibility to oxidative damage due to the PUFA increase in RBCs membranes. A similar increase in canine blood was not detected probably due to the small sample size in our study. LA monitoring of the canine RBCs membranes could be a potentially useful tool for assessing storage lesions. An increase of the case studies is needed to confirm or not the trend observed in this first preliminary study in veterinary medicine.

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CONTROLLED FIELD STUDY EVALUATING THE CLINICAL EFFICACY OF THE SPOT-ON FORMULATION CONTAINING EMODEPSIDE 2.1% W/V/ PRAZIQUANTEL 8.6% (PROFENDER®, BAYER ANIMAL HEALTH) IN THE TREATMENT OF NATURAL CAT AELUROSTRONGYLOSIS

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Aelurostrongylus abstrusus is the most important nematode affecting the respiratory tract of cats in terms of spread and clinical relevance [1]. Few products are labelled to treat aelurostrongylosis and knowledge on clinical response to treatments in relation to the course and the length of clinical recovery needs to be implemented.
With the aim to evaluate the efficacy of emodepside/praziquantel (Profender®, Bayer Animal Health) in the treatment of aelurostrongylosis in randomized controlled field conditions, 8 cats received two doses of Profender two weeks apart (T group), while 9 cats (C group) remained untreated and received a rescue treatment at the study day 28. Each owner signed a consent form and accepted to participate in the study. All cats were evaluated every two weeks (physical examination, CBC, serum chemistry, thorax radiographs and Baermann test). A comparison between the two study groups in terms of presence/ absence of clinical signs, clinic-pathological abnormalities and radiographic patterns was done. Cats of the T group were followed-up every two weeks for 10 weeks and clinical response was assessed through a pre- and post-therapy evaluation of clinical and radiographic scores, as previously described [2]. Data were compared using RM one-way- ANOVA or a Friedman test, while Fisher's exact test was used to compare categorical variables. In the T group, a post-treatment reduction of clinical ($p < 0.01$) and radiographic scores ($p<0.05$) were observed 14 days after the second administration of Profender. However, in comparison to C group, already two weeks after the first application, abnormal lung sounds were recorded less frequently ($p<0.05$) and hyporexia, lethargy, oculo-nasal discharge and pallor of the mucosae disappeared. Two weeks after the second treatment clinical and parasitological recovery, the resolution of inflammatory leucogram pattern and a significant reduction of radiographic lesions ($p<0.01$) were observed. The complete regression of radiographic patterns was recorded after 8 weeks from the second application. In this study a partial improvement, was observed two weeks after the first treatment however only the second Profender application ensured the disappearance of clinical and radiographic signs and the parasitological negativization. Therefore, an apparent improvement of clinical picture should not encourage a single application regimen for treating cat aelurostrongylosis with this parasiticide. The safety and efficacy of Profender in treating aelurostrongylosis is here confirmed in controlled field conditions. Two applications of spot-on solution two weeks apart lead to a parasitological negativization and, importantly, to a general improvement of clinical picture, via the early reduction of clinical and radiographic signs, followed by a complete remission of clinic-pathological abnormalities.

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ASSESSMENT OF CIRCULATING IMMUNE COMPLEXES DURING NATURAL AND EXPERIMENTAL CANINE LEISHMANIASIS

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Canine Leishmaniasis (CanL) is a chronic systemic disease characterized by a large variety of clinical signs and clinicopathological alterations, the majority of which due to immune mediated mechanisms. The progression of the disease depends on the type of immune response that infected dogs develop [1]. Dogs that present clinical disease have high levels of Leishmania-specific immunoglobulins (IgG mainly, IgA and IgM) and a decreased or absent cellular immune response. This condition can create circulating immune complexes (CICs) that determine the reduction of the macrophage ability to kill the parasite and induce vasculitis that activates the complement cascade. Defective clearance of these CICs by scavenging macrophages leads to their deposition in specific organs [2]. The aim of this study was to assess the serum level of CICs in dogs exposed to CanL infection, in natural and in experimental conditions. Fifty-two sera were examined for the detection of CICs level. These sera belonged to 4 different untreated control groups of naïve beagles previously studied to evaluate the efficacy of Leishmania vaccines under natural (n=22 dogs) or experimental (n=30 dogs) transmission conditions. Natural studies were performed in Italy during the years 2010-2013 and were approved by Italian Ministry of Health; the experimental study was performed in Spain (2016-2017) and approved by Health Catalan Authorities. Sera were classified in 5 different groups (A; B; C; D; E) according to the IFAT value and the bone marrow nested (n)-PCR results. A: n=10 negative dogs before the experimental infection; B: n=10 asymptomatic dogs experimentally infected, IFAT negative - n-PCR positive; C: n=10 asymptomatic dogs naturally infected, IFAT ≤ 320 - n-PCR negative; D: n=10 sick dogs experimentally infected, IFAT > 320 - n-PCR positive; E: n=12 sick dogs naturally infected, IFAT > 320 - n-PCR positive. CICs levels (µg/ml) were assessed by ELISA method (canine CIC assay - Cloude-Clone Corporation, USA). Statistical analysis was performed with MedCalc software (Frank Shoonjans, V.7.2.1.0) by the Tukey's multiple comparison test. The two groups characterized by negative IFAT (A and B) had the lowest level of CICs (16.09 and 12.78 µg/ml respectively). CICs value increased progressively in the group C and reached the highest levels in the groups D and E, both characterized by high antibodies titre and severe disease, independently from the way of infection. Statistically significant differences ($p<0.0001$) were demonstrated between the groups A, B and C when compared with D and E. No differences were assessed among the first 3 groups, nor between the last two. The present study demonstrates that CICs increases during the progression of Leishmania infection, strictly related to the increasing of antibodies during the time. CICs level seems to be not predictive of the evolution to disease, however further studies are requested. High CICs levels detectable by commercial ELISA are specific to an established Leishmania infection in dogs without concomitant infections as demonstrated by the same trend assessed in both naïve experimentally and naturally infected dogs.

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MAPPING CANINE VECTOR-BORNE DISEASE RISKS IN GREECE

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The Greek islands are amongst the most visited touristic places around the world, attracting millions of people per year, many of whom travel with their own pets. Greece has been repeatedly reported as an area highly endemic for various canine vector-borne diseases (CVBDs) [1-3], however, comprehensive knowledge on the distribution and abundance of various CVBDs across different islands is missing, at least in a large scale. The aim of this study was to determine the distribution of canine vector-borne infections in populations of dogs living in Greek islands located in different geographical areas (Ionian, Aegean and Cretan seas). In total, 1154 dogs of all ages, breeds and of different lifestyles (shelter, stray, household) were selected according to their distribution on the different islands and examined for the presence of clinical signs suggestive of CVBDs. Blood and serum samples were collected from each animal. For the detection of antibodies against Leishmania infantum, the WITNESS® Leishmania test [Zoetis; Rapid Immuno Migration (RIM™)] was performed, whereas positive samples were further examined with indirect enzymatic immunoassay (indirect ELISA) [INGEZIM® LEISHMANIA (INGENASA, Spain)]. Antibodies against Borrelia burgdorferi, Ehrlichia canis/E. ewingii, as well as Anaplasma phagocytophilum/A. platys were investigated with Snap® 4Dx® Plus test (IDEXX Laboratories Inc.). Positive Ehrlichia spp. as well as Anaplasma spp. samples were further examined with indirect enzymatic immunoassay (indirect ELISA) [INGEZIM® EHRLICHIA (INGENASA, Spain) & ANAPLASMA-ELISA DOG (AFOSA Gmbh, Germany)]. In total, 25.6% of the animals resulted seropositive at least to one pathogen, also considering the possible limits due to the use of rapid tests. Of the infected dogs 27.4% displayed clinical signs suggestive of CVBDs, such as cutaneous lesions, enlarged lymph nodes, pale mucous membranes, onychogryphosis and weight loss. The overall infection rate detected using rapid tests was 15.2% for Leishmania spp., whereas 13.3% of examined dogs were found to be positive for Anaplasma spp. and 7.5% for Ehrlichia spp. None of the animals was positive for Borrelia spp. The infection rate as confirmed by ELISA (testing only samples positive in the rapid test) was 9.2% for Leishmania spp., whereas 13% of the examined dogs were found to be positive for Anaplasma spp. and 7.5% for Ehrlichia spp. In the current study, the exposure to different pathogens transmitted by arthropod vectors was identified in dogs from all studied islands, although regional differences in the prevalence of the different infections were observed. The results confirm that in Greek islands CVBDs represent a constant health risk for both native and visiting dogs. Prevention with repellents and vaccines, together with the owner education, have a pivotal role to reduce the risk of transmission and the spread to non- endemic regions.

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ORAL COMMUNICATIONS

SESSIONE INTERDICLIPLINARE EQUINI

MONITORING THE ANTIELMINTHIC RESISTANCE IN EQUINE PARASITES IN NORTHWESTERN ITALY

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Nematodes have been recognized as an important cause of disease and loss of performance in horses. Changes in the parasitic fauna of horses have occurred in the past decades making Cyathostomins become the major parasites in adult horses, while *Strongylus vulgaris* and other large Strongyles have become less prevalent. *Parascaris* spp. remains the most important parasite infecting foals and weanlings [1]. Anthelmintic resistance is highly prevalent in Cyathostomins and *Parascaris* spp. worldwide and it must be factored into treatment decisions [2]. In order to asses the degree of antiparasitic resistance in Northern Italy we sampled 222 horses from 18 sport and horse-breeding farms (mean=12 subjects/farm, sd=11). Faecal egg count reduction tests (FECRT) were used to assess anthelmintic resistance. Quantitative copromicroscopic analysis were performed using MiniFLOTAC before treatment with Fenbendazole (n=45 horses from 3 farms), Pyrantel pamoate (n=4 horses from 1 farm) or Avemectins (n=173 from 14 farms) and repeated 14 days post treatment. Cyathostomins were detected in 147/222 horses (P=66.22%, CI95% 59.77-72.12%), while *Parascaris* spp. was detected in 6/222 subjects (P=2.70%; CI95% 1.24-5.7%). No significant differences were detected in prevalence values among farms, age classes or sex of tested animals. Cyatosthomins resulted to be resistant to Fenbendazole in 55.56% of the treated animals (CI95% 41.18- 69.06%), while Pyrantel pamoate resistance was detected in 75% (30.06-95.44%). *Parascaris* spp. showed no resistance to the antiparasitic treatments used. Resistant Cyatosthomins were detected in 2 farms, a breeding center and a racecourse. Despite increasing awareness within the veterinary profession and equine industry of the potential implications of anthelmintic resistance, further detailed, ground-based actions must be set in place to provide veterinary surgeons with up to date information on worm control plans that will prevent clinical disease while minimizing selection pressure of resistant parasites.

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UPDATES ON INTESTINAL STRONGYLES OF HORSES IN ITALY

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Intestinal strongyles (IS) in horses represent a healthcare problem of relevant importance, for their worldwide distribution and the negative repercussions that they could determine on health and sports performances [1]. The aim of this study was to update the epidemiological scenario in Italy with an extensive survey on IS of horses in Italy. During 2018, 6,896 horses aged 6 months-36 years old, from 548 different stables were examined for the presence of IS. For each horse a form was filled reporting animals' data (age, sex, breed, stable). In Sassari Parasitological Lab, coprological examination was performed with McMaster technique, using a Sodium Chloride (NaCl) solution (1200 s.g.). Coprocultures were also performed for 68 positive farms on faecal pools to obtain third stage larvae (L3) [2]. Data were processed according to animals' provenance as follows: Zone 1, Northern Italy; Zone 2, Central Italy; Zone 3, Southern Italy. Data were processed with Epi-Info® 6.0 (CDC/WHO, Atlanta, GA, USA) and Chi square and Mann-Whitney test were performed. IS eggs were found in 39.5% (95% CI: 38.3-40.7) of the examined animals. Among the examined stables, the 86.5% (95% CI: 83.6-89.4) showed at least one horse positive for IS eggs, and the 68.4% (95% CI: 64.5-72.3) presented horses with more than 200 EPG. The seasonal prevalence rates differ significantly (x2 trend = 23.079, P<0.01), with higher prevalence in winter (43.4%). Males were found significantly more infected than females (40.2% *vs* 35.8; x2 = 10.66; P<0.01). The prevalence rates slightly differ among the three zone (Zone 1 = 37.5%; Zone 2 = 46.4%; Zone 3 = 35.3%), but no significant difference was found (x2 trend = 2.820, P=0.091). The Kruskal-Wallis test highlights significant differences among the averages of the IS EPGs in the three areas of Italy, with significantly higher EPG levels in Southern Italy (H = 51.82 ; P = P<0.01). Data about lifestyle (indoor, outdoor, indoor+outdoor, paddock) showed significant differences regarding prevalence for IS (x2 3df = 146.98, P<0.01), with the highest prevalence in horses raised in paddocks (60%) and outdoor (50.5%), as the comparison between the EPG means (Kruskal-Wallis test: H = 113.68; P<0.01). Prevalence rates for IS were found inversely proportional to animals age, with the highest values in 2-5 years old horses (51.7%) and the lowest (28.4%) in horses with more than 15 years (x2 trend = 152.467; P<0.01). The correlation between age and EPG levels was weakly negative (Pearson Correlation R = -0.110; P<0.01). Coprocultures performed on 68 positive stables allow to isolate 7 different genus/species, among which, the most detected species was *Cyatostomum* spp (98.5%), followed by *Strongylus vulgaris* (79.4%) and *Trichostrongylus axei* (61.8%). Our study shown as IS still represent an important health problem for horses in Italy, that requires a constant parasitological monitoring and more rational treatments.

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FIRST EVIDENCE OF CLINICAL BESNOITIOSIS IN DONKEYS IN ITALY

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Besnoitiosis in equids, caused by *Besnoitia 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]. In Europe, evidence of clinical disease was recently reported in two donkeys in Belgium [2]. Furthermore, the presence of *Besnoitia* spp. specific antibodies was detected in equids in Spain [3] and recently for the first time also in horses and donkeys reared in Italy [4], in areas where outbreaks of bovine besnoitiosis were previously reported.

This study reports a case of clinical besnoitiosis in donkeys, for the first time in Italy. Two donkeys reared in a small paddock in Brescia suburbs had suspected skin lesions and poor body condition. The owner purchased the animals from a herd located in the mountains nearby. The animals were clinically examined to detect lesions suggestive of besnoitiosis. Endoscopy of upper respiratory tract was performed. Blood samples and skin biopsies were collected. To detect anti-*Besnoitia* spp. antibodies, Western Blot was performed. On DNA extracted from skin biopsies, endpoint PCR targeting ITS-1 region and sequencing were performed. Furthermore, quantitative copromicroscopic examination (FLOTAC dual technique) was carried out.

Clinical examination revealed the presence of scleral pearls in both animals and also skin nodules in region of neck, hind leg and on the pinnae. Specimens of *Haematopinus asini* were found on the skin. No cysts were detected in the nares and in the upper respiratory tract. Both animals resulted seropositive according to Western Blot results. Skin biopsies collected from both donkeys resulted positive for the presence of parasitic DNA. Sequencing demonstrated a homology of 100% with *Besnoitia* spp. sequences deposited in GenBank. Both donkeys were also infested by strongyles (1716 and 916 UPG), ascarids (244 and 1012 UPG) and lungworms (16 and 60 UPG). This is the first evidence of clinical besnoitiosis in Italian donkeys.

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CORRELATION BETWEEN LYMPHOID HYPERPLASIA AND UPPER AIRWAY DYNAMIC OBSTRUCTIONS IN STANDARDBRED TROTTERS

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Upper airway dynamic obstructions (UADO) are important causes of poor performance in racehorses. A proper pharyngeal and laryngeal muscle function is mandatory to maintain a complete distension during exercise. There is clinical evidence that regional inflammation may cause neuromuscular dysfunction of the upper airways predisposing to UADO, such as nasopharyngeal collapse (PC), and axial deviation of the aryepiglottic folds (ADAF) [1]. Pharyngeal and/or guttural pouch lymphoid hyperplasia (LH) represents a common endoscopic finding associated with inflammation of the upper airways.

Aim of the present study was to evaluate the role of LH in the pathogenesis of ADAF, PC or an association between the them.

The retrospective study included a large cohort of 295 Standardbred trotters presented for poor performance. All horses underwent a thorough diagnostic protocol including endoscopy of the respiratory tract at rest and during strenuous exercise on a high-speed treadmill. For each patient, LH was identified endoscopically and the severity was graded from 1 to 4 [2]; in case of guttural pouch LH we decided to add 1 more score to the severity grading. The diagnosis of PC, ADAF, or both was obtained by means of upper airways endoscopy during exercise as previously reported [3]. The different disease groups were compared with a randomly selected control group, matched for sex and age. Data were analyzed statistically by means of non-parametric ANOVA test ($P \leq 0.05$).

Endoscopy during exercise identified 10/295 (3.4%) horses with PC, 11/295 (3.7%) with ADAF and 15/295 (5%) with PC + ADAF. The statistical analysis showed a highly significant association ($P=0.0031$) between LH and PC, and between LH and PC + ADAF, whereas no association was observed between LH and ADAF alone.

The results obtained suggest a possible effect of LH on the functionality of the pharyngeal muscles, promoting the occurrence of PC and/or PC + ADAF.

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IN VITRO INHIBITING EFFECTS OF FOUR FUNGAL SPECIES ON EGGS OF DONKEY GASTROINTESTINAL NEMATODES

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The interest in the welfare and diseases of donkeys is considerably increased in Italy, mainly due to the popularity gained by donkey milk for human consumption and for the cosmetic industry [1]. Gastrointestinal nematodes (GIN) are considered a potential cause of disease and reduced productive performances in infected donkeys [2]. The use of anthelmintic drugs for the control of GIN is limited in dairy donkey farms, thus the need to develop new and alternative control methods. The aim of this study was to test *in vitro* the inhibiting effects on donkey GIN eggs of different fungal species able to degrade chitin [3]. More specifically, the ability of reference strains of *Pochonia chlamydosporia*, *Scopulariopsis brevicaulis*, *Metarhizium anisopliae* and *Beauveria bassiana* to inhibit GIN eggs was tested by using the egg hatch test (EHT) [4]. After concentration and purification of GIN eggs from positive donkey faecal samples, about 150 purified eggs in 0.5 ml distilled water and 0.5 ml of fungal inoculum, were placed in each well of 24-wells plates. Wells containing 150 purified eggs in 0.5 ml distilled water and 0.5 ml of the medium used for fungal cultures, were used as controls. After incubation for 48 hours in the dark, at 24°C and 90% relative humidity, plates were microscopically observed, and the number of eggs and larvae was counted in each well. Egg morphological alterations were also recorded. All experiment was repeated in triplicate and all data were statistically analysed ($P<0.05$). Obtained results showed that *M. anisopliae*, *B. bassiana* and *P. chlamydosporia* were able to significantly reduce (of about 60%) the hatch of GIN eggs respect to the untreated controls ($P<0.05$). Although statistically not significant, *S. brevicaulis* showed a lower efficacy than the former tested fungi, since it caused a percentage of GIN egg hatch reduction of about 52%. However, significant negative effects of *S. brevicaulis* on GIN egg hatch emerged from the comparison with the untreated controls ($P<0.05$). In some cases, egg morphological alterations were observed in treated wells. In conclusion, all fungal strains tested in this study showed inhibiting effects on the hatch and viability of donkey GIN eggs. It would be very interesting in the future to investigate the ability of examined fungi in reducing the number of donkey GIN eggs in contaminated environments.

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EFFECT OF LUNG INFLAMMATION ON LACTATE THRESHOLD IN STANDARDBRED RACEHORSES WITH MODERATE EQUINE ASTHMA

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Moderate Equine Asthma (MEA) is a common disease of young racehorses characterized by coughing, mucus accumulation and inflammation of the airways and diagnosed by cytological examination of bronchoalveolar lavage fluid (BALF) [1]. Despite the large evidence concerning the role of MEA as a cause of poor performance [2], it is difficult to quantify the impact of lung inflammation on the racing fitness. The most common parameter for equine athletic performance is the speed at 4 mmol/L of lactate (VLA4). Aim of the present work was to evaluate the effect of the different BALF inflammatory cells on VLA4 in racehorses affected by MEA.

Data from a cohort of 258 Standardbred racehorses in training investigated for poor performance were retrospectively evaluated. All horses underwent an accurate diagnostic protocol, which included an incremental treadmill test, with plasma lactate analysis and calculation of VLA4, and BALF collection. Horses with any other alteration potentially influencing performance were excluded, considering only those subjects with BALF cytology consistent with MEA. Of the 258, a sample of 30 horses (average age 3.4±1.0 y.o., median age 3.5 y.o.) was selected. The association between BALF inflammatory cells differential count and VLA4 was evaluated by means of linear regression.

Statistical analysis showed a significant association ($p=0.015$, $r2$ 0.19) between the increase in neutrophils differential count in BALF and the decrease in VLA4.

The results obtained suggest that neutrophils accumulation in the airways of MEA horses may have a direct impact on athletic capacity, possibly due to impaired alveolar-blood gas exchanges during strenuous exercise [3], confirming the role of neutrophilic MEA as a cause of poor performance in Standardbred racehorses.

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PROTEIN CARBONYL AS BIOMARKER OF OXIDATIVE STRESS IN SIRS HORSES

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The term Systemic Inflammatory Response Syndrome (SIRS), rather than endotoxemia, is used to describe the clinical status of endotoxemic horses [1,2]. Lipopolysaccharides (LPS) induce the cells involved in the endotoxemic event to produce reactive oxygen species (ROS). Neutrophils, with their high oxidant-generating capacity, are considered to play a vital part in this process. Protein carbonylation can be generated by a variety of oxidative processes [3,4]. The aim of the present study was to evaluate the protein carbonyl content (PCC) in healthy horses and horses affected by SIRS. A total of 48 horses were included in the present prospective study. An owner's written consent was obtained for collection of plasma for all the horses included in this study. Twenty-four were healthy horses, while 24 were sick horses referred to two different veterinary teaching hospitals (VTHs) providing secondary health care. The following data were recorded both for healthy and sick horses for classification in healthy and SIRS positive groups [5]: presence of abnormal leukocyte count or distribution as leukopenia, leukocytosis or >10% band neutrophils, hyperthermia or hypothermia, tachycardia, tachypnea. Blood samples were collected in LH-heparin tubes only once in healthy horses, while in sick horses blood was collected at admission in VTHs (T0), then 24 (T1), 48 (T2), 72 (T3), 96 (T4) hours after admission. Plasma was used to evaluate PCC using Levine et al. [5] method. Data were analyzed for distribution using the Kolmogorov-Smirnov test and results were expressed as median±standard error. Kruskal-wallis and Dunn's multiple comparisons tests were used to verify differences in PCC between healthy and SIRS positive horses at different sampling time. Statistical significance was set at $p<0.05$. All the 24 healthy horses presented a normal physical examination and laboratory data within reference ranges, thus they were included in the control group. Three out of 24 (12.5%) sick horses were not positive for SIRS score, thus they were excluded. The PCC (nmol/mL/mg) was 0.052±0.008 in healthy horses, while in SIRS positive subjects PCC was 0.312±0.007 at T0, 0.079±0.032 at T1, 0.093±0.036 at T2, 0.081±0.034 at T3 and 0.084±0.027 at T4. Differences were obtained between healthy and SIRS positive horses at T0, T1 and T2, and in SIRS positive horses between T0 vs T72 and T96. PCC seems to be a marker of SIRS positivity in horses. Moreover, OCC concentration varies in relation to sampling time.

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ULTRASONOGRAPHIC APPEARANCE OF ELBOW JOINT IN A POPULATION OF HEALTHY HORSES AND DONKEYS

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Ultrasound examination is a well established technique in musculoskeletal diagnostic imaging of the horse. It allows to accurately image articular and periarticular soft tissues and to assess bone and joint surfaces. It is therefore considered complementary to radiography in the investigation of joint disease in the horse.Few studies [1-3] were performed on the ultrasonographic aspect of this joint in the horse and no reports are present on donkeys. The aim of the study was to evaluate ultrasonographically the elbow joint in healthy horses and donkeys to assess the appearance of anatomical structures. A total of 70 elbow joints were evaluated. Inclusion criteria: no lameness or muscle-skeletal diseases on the basis of a clinical exam. Ultrasonography was performed in the weight-bearing position as previously reported [1-2] with a portable ultrasound machine using linear and convex probes (7.5-5 MHz). The joint was prepared by clipping and shaving the area delimitated by the lateral humeral condyle, the olecranon and the radial tuberosity. Alcohol coupled with ultrasound gel was applied to provide appropriate contact.]. The following structures were detected and evaluated: lateral and medial collateral ligaments; proximal *ulnaris lateralis*, distal *biceps brachii* and *triceps brachii* tendons; lateral aspect of the articular space at the level of the lateral collateral ligaments; bone surface [1,3-4]. For each structure, at least one good quality image was recorded. The echogenicity, fibres orientation and the presence of bone modelling were assessed based on species, age and activity; the evaluation was performed off line using a dedicated software. The prevalence for each structure was calculated, on horse and donkey groups. The lateral collateral ligaments and the proximal *ulnaris lateralis* tendon were visualized in 69/70 (98.6%) elbows (57/58, horses and 12/12 donkeys); the medial collateral ligament in 36/70 (51.4%) (no donkeys); the *triceps brachii* tendon in 70/70 (100%); the distal *biceps brachii* tendon in 41/70 (58.6%) (36/58 horses and 5/12 donkeys); the joint space in 62/70 (88.6%) (57/58 horses and 5/12 donkeys). Horses were divided in 3 groups based on: 1) age: A) <9 yo (n=13/29); B) aged 10-19 yo (n=14/29); C) >20 yo (n=2/29); 2) activity: a) n=9/29 athlete horses; b) n=20/29 pleasure horses. Donkeys were divided in 2 groups based on age: D) <9 yo (n=2/6); E) 10-20 yo (n=4/6). Donkeys were not divided for breed or activity because all Amiata donkeys used for reproduction. A different echogenicity and fibres orientation of the lateral collateral ligament and the *ulnaris lateralis* tendon were found between donkey and horses and between sport horses and pleasure horses. No qualitative differences or joint space or bone surface alterations were observed in horses or donkeys of different age. To the authors' knowledge, this is the first report on the ultrasonographic evaluation of the elbow joint in donkeys. Our results support the hypothesis that age does not influence the ultrasonographic aspects of the elbow joint both in horses and donkeys, while the activity seems to do in horses. Differences in the echogenicity of the lateral collateral ligament and *ulnaris lateralis* tendon were detected between donkeys and horses. Further studies on lame equids are needed to assess the ultrasonographic aspect of pathologic elbow joints.

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SMART TEXTILE TECHNOLOGY FOR ECG MONITORING IN HORSES: VALIDATION TESTS

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Electrocardiography (ECG) is a widely used technique which enables the acquisition of important electro-physiological information for both welfare and clinical purpose. High ECG signal quality is crucial for an accurate estimation of the autonomic nervous system's activity, such as that required during clinical investigation of athletic horses.
However, the ECG signal monitoring is still difficult; moreover its quality is affected by several factors generating the well-known "Motion Artifacts" (MAs) [1]. The MAs are electrical abnormalities of the waves resulting from the movement of electrodes against the skin. Recently, several bioengineering solutions have been adopted to collect physiological parameters in a comfortable and robust manner in human field. Among these, "smart textile" electrodes have been developed by combining conductive yarn with elastane [2] and have been hypothesized also for use in several fields of equine science [3]. The aim of this study was to evaluate the functionality and performance of "smart textile" electrodes compared to "classic" silver/silver chloride electrodes (Ag/AgCl) in horses. The assessments and validation tests were performed with horse standing quite in stall (REST) and during standardized exercise test (SET) on treadmill (which comprised 5 steps: Walk 1, Trot 1, Trot 2, Gallop and Walk 2). The tests were made on healthy and not pregnant mares (property of the Department of Veterinary Sciences, University of Pisa). Twelve mares were involved: 7 were used for REST and 5 for SET. The recording of ECG signal was obtained simultaneously from textile electrodes and Ag/AgCl ones. Both electrodes were placed in a modified base-apex configuration. Each horse was monitored individually for 1 hour in stall (REST) and for 18 minutes (SET) on treadmill. Collected ECGs were visually examined for labeling those segments that were corrupted by MAs. For REST, the percentage of MAs (MA%) was computed as the number of samples of corrupted segments over the whole length of the signal. Instead, for the analysis of the obtained ECGs during the SET phase, the Kurtosis (k) value (good ECG signal quality shows a k-value greater than 5) was calculated on the whole raw ECG signal collected in each step of the SET. Finally, the kurtosis Signal Quality Index (kSQI) was than calculated.The REST tests showed that the total MA% was lower for the smart textiles than for Ag/AgCl electrodes (Friedman test; x^2=55.4, df=1, P=9.8513e-14). Regarding the SET, the kSQI of textile electrodes was higher than the one related to Ag/AgCl signals, and it was very close to the optimum value of 1 (all 10 secs windows with a k-value>5), for all subjects and all steps of the SET.
The obtained results suggest that smart textile electrodes are more effective and accurate in collecting ECG traces than Ag/AgCl ones in both types of tests.

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EVALUATION OF INDIRECT BLOOD PRESSURE IN HORSES WITH AORTIC REGURGITATION

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Aortic regurgitation (AR) is common in middle-aged horses. Although, usually, AR degenerates slowly with minimal impact on athletic activity, horses with moderate/severe AR are at increased risk of sudden cardiac death due to ventricular arrhythmias. Therefore, when a horse is engaged in high-intensity competitive sports, it is important to evaluate the impact of AR on performance and to assess whether the horse is safe to ride. Moreover, it is mandatory to provide a correct prognosis about the progression of the disease [1]. It is reported that diastolic pressure in horses with severe AR results as low as 50 mmHg, and the pulse pressure is 60 mmHg greater than in horses with mild AR [2].

The aim of the present study is to evaluate whether systolic, diastolic and pulse pressure change accordingly to the severity of AR (mild, moderate and severe) or to the presence/absence of associated cardiac dilation. Indirect blood pressure was measured in 17 horses with AR, using an ultrasonic blood-flow technique [3]. In all subjects, standard 2D, M-mode and color flow Doppler echocardiography was performed. According to the echocardiographic findings, AR was classified as mild in 4 horses, as moderate in 9 horses and as severe in 4 horses. Moreover, echocardiographic dimensional changes associated with AR were found in 10 out of 17 horses. For statistical evaluation, one way and linear regression analyses were performed. Significant level was set at $P<0.05$. Statistical analysis did not show any difference in blood pressure related to the severity of AR. However, the mean systolic (P-value=0.0065) and diastolic pressures (P-value=0.0036) were significantly higher in horses with cardiac dimensional changes. Our results suggest that the indirect measurement of blood pressure in horses with AR may provide useful information concerning the progression of the disease and the onset of cardiac dilation.

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ORAL COMMUNICATIONS

SICV

ENGRAFMENT AND NEURO-LIKE TRANSDIFFERENTIATION OF OVINE MESENCHYMAL STEM CELLS IN SPINAL CORD TRASECTION MODEL: A STEP FARWARD TOWARD THE FUNCTIONAL RECOVERY

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Neurodegenerative medicine had seen recently a growing interest related to mesenchymal stem cells MSCs transplantation to cure the sequelae of the spinal cord injury (SCI). However, literature reports few data about the safety and efficacy of a cross-species xenogeneic MSCs transplant that, potentially, may extend the spectrum of possible grafts for neuroregeneration. The goal of the study is to infer the potential for engraftment and neurodifferentiation of the xenogeneic ovine bone marrow MSCs in a murine spinal cord transection model.
Twenty rats were undergone to spinal cord transection after being subjected to Basso-Beattie-Bresnahan (BBB) locomotor testing. A solution of fibrin glue and ovine bone marrow MSCs retrovirally transfected with red fluorescent protein was released at the injury site in 10 rats assumed to be the study group (MSCG). MSCs were subjected to no in vitro induction of neuro-glial differentiation before the transplant. In the control group (FGG), a solution containing only fibrin glue was injected. All the rats were weakly evaluated trough BBB rating scale until the 70th day, when the spinal cords were harvested, sectioned and analyzed in light microscopy and immunohistochemistry. BBB score data collected in both groups were statistically analyzed. Significance was set at $p<0.05$. CD34, CD44, CD45, CD54, CD73, CD90, CD166, Nestin, fibroblast grow factor-1, glial fibrillary acidic protein, tubulin βIII (b-tub-III), NG2 glia (NG2), neuron specific enolase (NSE), nerve grow factor receptor (NGFR), choline acetyltransferase, vimentin and 200 kD Neurofilament (NF-01) were tested. Fluorescence microscopy was also performed in MSCG to track the labeled transplanted MSCs. Immunocytochemical study of neuro-glial markers expression in MSCs showed positivity for nestin. MSCG had a significant and durable recovery of motor functions ($p<0.001$). Red fluorescence and CD44, CD54, CD73, CD90 and CD166 positivity was found at the injury sites. Positivity for nestin, b-tub-III, NG2, NSE, vimentin and NF-01 were also found in MSCG. Xenogeneic ovine bone marrow MSCs engraft into the injured spinal cord and start to differentiate into a neuro-glial phenotype at an early stage. They also affect positively the injured microenvironment thus leading to the assumptions for a not negligible functional recovery.

USE OF PIGLET GRIMACE SCALE TO ASSESS PAIN AFTER CRIPTORCHIDECTOMY: PRELIMINARY RESULTS

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Some husbandry procedures in pigs are invasive and potentially painful [1]. In pig farming pain assessment is characterized by low specificity and practical limitations, highlighting the lack of reliable and feasible tool [2]. The recently developed Piglet Grimace Scale (PGS), a facial-expression-based pain coding system, may represent a promising tool of assessing pain in piglets undergoing tail docking and surgical castration [2,3]. This preliminary study aimed to determine whether PGS can be use in growing pigs undergoing criptorchidectomy on farm. Fourteen healthy mixed-breed (Large White x Duroc) male pigs (weight: 25÷40 kg; age 55÷80 days) affected by unilateral abdominal cryptorchidism, were recruited and housed in an indoor commercial pig farm. The animals were handled according to European and national regulations on the protection of experimental animals (Directive 2010/63/UE and DL 26/2014) and the study was approved by the Institutional Animal Ethics Committee (protocol number E81AC.8/A). The pigs were pre-medicated with a combination of azaperone (2 mg/kg) and ketamine (10 mg/kg), administered IM by injection into the neck behind the base of the ear by using a 19 gauge needle connected to a line extension. The lateral auricular vein was catheterized as soon as the animals achieved lateral recumbency. Anaesthesia was induced and maintained using IV thiopental sodium based on assessment of anaesthetic depth using the following criteria: eye position, degree of palpebral reflex, HR, RR and spontaneous movement. When induced, a pain relief was administered (IM ketoprofene; 3 mg/kg). The eutopic testis and the inguinal incision site were infiltrated with lidocaine 2%. The pigs were submitted to surgery to remove the retained testicle and the normally descended testes. Pigs were filmed the day before the procedure and 6 hours after surgery. Behavior of pigs was evaluated (scan sampling every minute) both pre- (n=60 scan) and post-surgery (n=60 scan). Thirty six pictures of the faces were collected (18 pre- and 18 6 hrs post-surgery) and scored with PGS by 3 treatment-blind observers. The surgery was completed in all the animals, and there were no anaesthetic or surgical complications. Pre-surgery pigs spent 14% of time eating and/or drinking, while 6 hrs post-surgery they spent 94% of time resting. The inter-observer reliability was good with an overall Interclass Correlation Coefficient value of 0.78. The mean PGS score before procedure was 1.22 ± 1.16 and increased to 2.11 ± 1.42 after procedure. Although preliminary, these results confirm that the PGS can be applied to growing pigs to evaluate pain after criptorchidectomy on farm condition.

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COMPARISON BETWEEN TWO TOTAL LAPAROSCOPIC GASTROPEXY TECHNIQUES IN DOG USING PDS STRAPS AND SIMPLE BARBED SUTURE

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Gastric dilatation-volvulus (GDV) syndrome is one the most mortal disease in large and giant-breed dogs. Prophylactic gastropexy is performed to avoid the occurrence and to reduce the recurrence of GDV syndrome in predisposed breeds. In the last years minimally-invasive laparoscopic techniques have been successful for the decrease of pain and morbidity associated to more invasive open surgical approach. However, laparoscopic techniques require surgeon training and experience and longer procedure. To solve these problems, several variations of the original technique have been proposed using staples, barbed sutures, etc. In our study we have compared, in terms of duration of procedure, surgeon learning curve, complication and gastropexy adhesion, two techniques using PDS straps and simple barbed suture. Also ex vivo tests were performed. Ten giant dogs were enrolled in the study (6 Great Danes and 4 Neapolitan Mastiffs); the surgical procedures were performed by same surgeon. The dogs underwent ultrasound examination of the right flank region to monitor the integrity of the gastropexy in follow-ups at a distance of 7 15 and 30 days after surgery. In both surgical procedures no subject showed intra and post operative complications and recovery was excellent. The mini-invasiveness of the procedures allowed minimal post-operative care and the early return of normal activities. The ultrasound scans showed the presence of complete adhesions at gastropexy site and the lack of mobility of the stomach in all follow ups. The morbidity of GDV, in treated subjects, was absent until the last examination. However PDS staples technique showed shortest surgical time and a faster learning curve of the surgeon. The aim of ex vivo tests was to compare the tensile force required to distrupt the pexy adhesion. In a first phase we compared the hold of the pexy carried out using differents inclinations of the PDS straps device (30°, 60° and 90°). In a second phase we compared PDS straps gastropexy (without a precise inclination of device) and simple barbed suture gastropexy. The procedure were carried out using 15 different canine stomachs and a dedicated instrumentation. The results , analyzed with a one-way variance analysis, showed that there aren't statistically significative differences between the different inclination of the devices and between the two gastropexy technique.

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NEUTROPHIL-TO LYMPHOCYTE RATIO AS A PREDICTOR OF LOCAL RECURRENCE OF FELINE INJECTION SITE SARCOMA

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Feline Injection Site Sarcoma (FISS) is a highly aggressive neoplasm and local recurrence (LR) represents a major concern. Although achieving histologically tumor-free surgical margins is crucial for long term control of the disease, LR has been reported in 19-40% of cases with clean margins.1 Thus, it is desirable to identify further variables that can aid in prognostication and treatment planning. Pretreatment Neutrophil-to-lymphocyte ratio (NLR) is a marker of systemic inflammatory response that has been reported as a prognostic tool for several tumors in humans, and for canine mastocytoma.2,3 The aim of this study is to explore the prognostic impact on LR of NLR in cats with surgically excised FISS. Eighty-two cats with surgically excised, histologically confirmed FISS at first presentation, without distant metastasis were retrospectively enrolled from Veterinary Teaching Hospitals of Milan and Turin. We included cats with available preoperative hematological analysis (within 45 days before surgery). Statistical analysis explored the relationship between NLR and: age, glycemia, tumor dimension, ulceration, concomitant diseases, necrosis, histotype. The impact of NLR on LR and survival time (ST) was then estimated with Cox regression model. Receiver operating characteristic curves (ROC) were made; the area under the curve (AUC) was calculated and Youden index was used to determine the best cut-off values. At the end of the study, 58 cats were death of which 24 for tumor related causes; of these cats, 23/24 experienced LR. Median ST was 975 days. The only variables that were significantly correlated with NLR were tumor size (p=0,004), and histotype (p=0,029), with cats with bigger tumors and histotype other than fibrosarcoma having a higher NLR. No correlation was found between NLR and potential causes of alterations in white blood cell populations, such as concomitant diseases, glycemia (as an indicator of stress), ulceration, necrosis, age. Higher NLR was significantly associated with higher risk of LR (p=0.015; HR=1.066) and shorter ST (p=0.02; HR=1.045). The optimal estimated cut-off for LC was 1.82 at 1 year (Se=95%; Sp=30%) and 3.65 at 2-3 years (Se=52% ; Sp=66%). Preoperative NLR is a readily available variable that may aid the clinician in identifying cats at higher risk of tumor recurrence and poorer outcome; this variable does not seem to be influenced by confounding factors that can alter white blood cell counts. The mechanism underlying this finding remains unclear, although in human and canine medicine it has been hypothesized that a predominant neutrophil response and relative lymphopenia may promote tumor growth and dissemination.

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USE OF ELASTOSONOGRAPHY IN THE CHARACTERISATION OF SUBCUTANEOUS SOFT TISSUE LESIONS IN DOGS

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Elastosonography is an imaging technique based on ultrasound, which measures mechanical properties of tissues. The only study on elastosonography of subcutaneous lesions in dogs compares lipomas and malignant subcutaneous neoplasm.[1] Aim of the study: to characterize superficial soft tissue lesions in dogs with elastosonography. Materials and methods: Medical records were searched for dogs with subcutaneous soft tissue lesions, which underwent elastosonography and cytology or histology from January 2017 to January 2019. All examinations were performed by the same operator with the same equipment (Esaote MyLab Class C with ElaXto software), using a linear array (8-18 MHz). Images were considered only when the visual indicator provided by the software determined an adequate degree of correlation of the relative hardness over time (green ElaXto spring function) and the distribution of colours in the elastogram compared with the underlying B-mode image was coherent. Two values were measured for each region of interest (ROI): Elax-t%SFT (the percentage of tissue softness) and Elax-t%HRD (the percentage of tissue hardness). Semi-quantitative analysis were performed to create an elasticity score on the basis of the Elax-t%SFT values: 1) >70%: soft; 2) <70% >50%: mostly soft; 3) <50% >30%: intermediate; 4) <30% >20%: mostly hard; 5) <20%: hard. Elax-t%SFT and Elax-t%HRD values were compared between benign and malignant lesions with Student t test. Elax-t%SFT and Elax-t%HRD values were then compared with ANOVA and post-hoc Tukey test, considering 3 groups: tumours, non-tumours, lipomas. Receiver operating characteristic (ROC) curves of the sensitivity and specificity were obtained. Wilcoxon rank sum test was used to compare the semi-quantitative score with the nature of lesion (benign vs. malignant). A P value <0.05 was considered significant. Results: Eighty dogs of a variety of breeds (53 females, 27 males), aged between 3 months and 18 years (mean 8.75±3.41 years), met the inclusion criteria. Overall, 85 lesions were considered, 53 benign and 32 malignant (14 mast cell tumours, 14 sarcomas, 4 carcinomas). Among benign lesions, 12 were benign neoplasms, 20 were lipomas and 21 were non-neoplastic lesions. Mean Elax-t%HRD value was higher in malignant lesions. Mean Elax-t%SFT value was higher in benign lesions. There were significant differences in Elax-t%SFT and Elax-t%HRD between tumours and non-tumours, tumours and lipomas. No significant difference was found between lipomas and non-tumours. ROC curves identified a hardness cut-off of 52.2% (sensitivity: 83%, specificity 75%), and an elasticity score cut-off of 2.5 (sensitivity 79.2%, specificity 75%). Conclusion: malignant lesions were harder than benign lesions. Non-neoplastic lesions and lipomas were softer than neoplastic lesions. Elastosonography may be useful in the diagnosis of subcutaneous soft tissue lesions in dogs.

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EVALUATION OF INTRA- AND INTER-OBSERVER MEASURAMENT VARIABILITY OF A RADIOGRAPHIC FETLOCK OSTEOARTHRITIS SCORING SYSTEM IN THE HORSE

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Osteoarthritis is one of the most common diseases found in the fetlock joint [1]. The process is degenerative, so it is essential to perform an accurate and timely diagnosis to limit the evolutionary joint changes. Among the typical clinical signs of osteoarthritis there are the articular effusion and the mild to moderate lameness, depending on the severity, which compromise the athletic performance of the affected cases [2]. The most common diagnostic imaging technique currently used in equine medicine for the diagnosis of osteoarthritis is the radiology [3]. The aim of the study was to purpose a score grading system to evaluate the fetlock osteoarthritis, through the variability inter- and intra-observer [4]. The orthopedic files and radiographs (LM and DPa / P1) of ten adult horses of any breed investigated for lameness localized to the fetlock region were examined. Radiographs were examined on nine anatomical points from two groups (ROOKIE AND EXPERT). In intra-observer measurements the variability between the two measurements was -2 + 3.5 for the ROOKIE group; 0 + 1.4 for the EXPERT group. In inter-observer measurements the correlation was high, ($r=0.85$ for ROOKIE and $r=0.98$ for EXPERT; $p \leq 0.05$). The analysis of the results shows that there were no significant differences between the observations of the two groups and re-observations, showing that this scoring method of osteoarthritis of the fetlock can be of support for the diagnosis and staging of the osteoarthritic process.

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A PRELIMINARY STUDY ON THE USE OF PULSEOXYMETRY AS A GUIDE TO ALVEOLAR RECRUITMENT IN DOGS UNDER GENERAL ANESTHESIA DURING LAPAROSCOPIC SURGERY

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The aim of this study was to evaluate the effects of a stepwise alveolar recruiting manoeuvre (ARM) in dogs undergoing laparoscopic surgery, using pulseoxymetry as a guide to find the optimal level of PEEP. Our hypothesis was that SpO_2 could be a valid support or an alternative to the monitoring of the dynamic compliance (Cdyn) in the titration of PEEP in an open lung strategy. To test this hypothesis 8 female dogs scheduled for laparoscopic ovariectomy underwent to a stepwise ARM which consisted of the following phases: 1) reduction of FiO2 from 1 to 0.21; 2) set the ventilator to a volume controlled mode with a tidal volume (Vt) of 15 ml/kg and a respiratory rate (RR) of 12 breath/minute with a I:E of 1:1; 3) a gradual stepwise increase of the PEEP level with intervals of 5 cmH_2O up to reach 40 cmH_2O of plateau pressure (Pplat), this condition was maintained for 1 minute; 4) a gradual stepwise decrement of PEEP with intervals of 3 cmH_2O up to baseline conditions. During the decremental phase, at each step, Cdyn and SpO_2 were evaluated. The clip type probe of pulseoxymetry was positioned on the tongue for the entire duration of the procedure. The best PEEP was indicated as the one with the higher Cdyn. At this point the incremental phase for the ARM was repeated and thereafter the level of PEEP was adjusted at the BEST peep and maintained for the entire procedure. For the propose of the study the following parameters were monitored: airway peak and plateau pressures (Ppeak and Pplat, cmH_2O), Cdyn (ml/cmH_2O), driving pressure, Vt (mL), level of PEEP (cmH_2O), RR (breath/minute), room air SpO_2 (%), and the main hemodynamic parameters. The time points of the study were: 5 minutes before the induction of pneumoperitoneum (BASELINE); 10 minutes after the induction of pneumoperitoneum (PP); 5 minutes after the ARM (ARM); 20 minutes from the discontinuation of pneumoperitoneum (POST-PP). The monitored parameters were compared at each time of the study with the 2 ways ANOVA for repeated measures ($P<0.05$). The role of SpO_2 to detect the open lung condition during the PEEP titration phase and the cut off value of room air SpO_2 able to indicate the opening of the lung was evaluated with the receiver operating characteristic (ROC) curve. The mean value of Cdyn was reduced after the pneumoperitoneum from 1.76±0.43 to 1.07±0.36 $mL/cmH_2O/kg$ and increased to 1.33±0.29 $mL/cmH_2O/kg$ after the ARM. After the discontinuation of pneumoperitoneum Cdyn further increased to 1.64±0.44 ml/cmH_2O. The mean values of best PEEP determined according to the analysis of the Cdyn was 5.5±2.2 cmH_2O. Based on the ROC curve analysis the SpO_2 can be considered a valid tool to detect the open lung condition, indicating as cut-off value >95 % with a specificity of 59.46% and a sensibility of 72.73% with an area under the curve of 0.672 and P of 0.049. The results of this study prove that SpO_2 could be a tool with a discrete sensitivity and specificity, but that its applicability to detect the best PEEP during an open lung approach in clinical practice should be confirmed with further studies, on a larger number of cases.

ANIMAL MODEL FOR MINIMALLY INVASIVE NEUROSURGERY IN EDEN2020

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Minimally invasive surgery has been considered a major revolutionary method in brain surgery [1]. Neurosurgery has witnessed an accelerated growth in the advancement and clinical adoption of imaging as Computer Tomography (CT) and Magnetic Resonance Imaging (MRI). The major benefit of sheep as animal model for neuroscience research is the brain size, comparable to human in terms of vascular anatomy [2] to perform neurosurgeries and conventional imaging in living animals [3]. The aim of the study is to validate neurosurgical minimally invasive protocol in sheep model. We considered surgical safety, feasibility and catheter insertion accuracy. A total of 5 female adult sheep were used in this study. Sheep under general anaesthesia was placed in an ad *hoc head* frame system helmet. In vivo brain imaging was performed with initial CT scan followed by an MRI protocol optimized on a 1.5T MRI scanner. Diffusion Tensor Imaging (DTI) and vascular images (SWI and MR Angiography) were acquired. After the imaging study the target area has been defined as corticospinal tract. The neurosurgical trajectory has been planned with Renishaw® surgical planning software Neuroinspire™ for a stereotactic approach with Cosman-Roberts-Wells (CRW) system. A skin flap was created until the calvarium bone was identify. The bone surface was cleaned from periosteum tissue and the CRW was arranged with the right coordinates. One burr holes (14 mm of diameter) have been created with Anspach ® drill and *dura mater* was incised. The port was located and fixed and the rigid catheter has been inserted. A CT scan was performed to analyse any complication during the insertion procedure and to validate catheter matching with target point previously planned. The latter CT scan was upload to Renishaw® surgical planning software Neuroinspire™ to compare the real catheter trajectory obtained after the introduction with the trajectory planned. Post-processing analyses successfully enabled the reconstruction of the DTI-derived tensorial maps. The most important ovine white matter fiber bundles, including the corpus callosum, visual pathway, fornix, occipitofrontal fascicle and corticospinal tract, were identified and reconstructed. Two veterinary surgeons and one human neurosurgeon performed randomly the procedure. The surgery has been repeated two times for each sheep and turned out feasible and safe. No major or minor complications were found and the catheter insertion didn't showed criticisms. Major white matter tracts were identified in sheep. The entry points defined in our work allow to obtain a safe and accurate neurosurgical procedure avoiding vessels and functional/vital brain structures.

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BIOLOGICAL ACTIVITY OF FREEZE-DRYED HORSE PLATELET CONCENTRATES

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Platelet concentrates (PC) are a heterogeneous group of biologicals used in regenerative medicine mainly for the autologous treatment of spurious conditions. In horses, autologous PC have been used since many years for treating different orthopaedic lesions with consistent effectiveness [1]. Since the preparation of the PC from whole blood requires competences and instrumentation not readily available at veterinary practitioners, we have been preparing these biologicals at request of veterinary practitioners since many years. However, once prepared, the PC need to be sent back in tightly controlled cold chain. This dramatically increases the cost of the biologicals and greatly limits its applicability outside the research settings. The aims of this study were to evaluate the effect of freeze-drying in preserving intact the biological properties of PC derivatives for at least 45 days. Furthermore, the effect of different excipients was also evaluated. Materials and methods: The PC were obtained from 2 healthy horses and stored at -80°C at the DIMEVET. The study was approved by the University of Bologna Ethics Committee. PC were processed to obtain a derivative deprived of the fibrinogen/fibrin component, called Platelet lysate serum (Pl-serum) according to Mojica-Henshaw et al. (2013)[2] in order to confer a liquid appearance suitable for in vitro assays. The Pl-serum samples were aliquoted and each aliquot was kept as such or mixed in variable proportions with lyophilization excipients and either kept refrigerated or frozen or freeze-dried. Lyophilized biologicals were then evaluated using a MTT assay having as substrate Vascular Wall–Mesenchymal Stem Cells [3]. The results were analysed using the ANOVA test. Refrigerated, frozen and freeze-dried samples did not show differences in their biological activity ($p=0.65$). However, within the freeze-dried samples, a significant effect of the lyophilization mix has emerged ($p<0.01$); in particular the two lyophilization mixes containing the same sugar stabilizer performed slightly better than Pl-serum as such. However, the same freeze-dried samples showed collapsed cakes. Freeze-drying is a suitable method to preserve the biological activity of PC derivates at least for 45 days at room temperature allowing to abandon the cold-chain and, hence, to popularize the PC use among practitioners. Future, studies should be aimed to improve the cake stability and to evaluate the shelf-life beyond 45 days by refining the lyofilization excipients and freeze-drying protocols.

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RELIABILITY OF THREE DIFFERENT METHODS TO MEASURE THE AMOUNT OF TIBIAL TUBEROSITY ADVANCEMENT IN THE PREOPERATIVE PLANNING OF MODIFIED MAQUET PRECEDURE

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The most critical aspects of the preoperative planning for tibial tuberosity advancement (TTA) are the initial measurements needed to calculate the right amount of advancement of tibial tuberosity. These measurements directly determine the optimal wedge size needed to biomechanically counteract shear force in the stifle joint. A discrepancy exists between the desired advancement measured preoperatively and the trough advancement after wedge placement[1]. Several methods to measure the amount of advancement have been proposed[1,2]. Aims of this retrospective study were a) compare the reliability of three methods of measurement to calculate the right amount of advancement of tibial tuberosity; b) evaluate the differences between three methods. The preoperative radiographs of the same cohort of dogs with cranial cruciate ligament failure (CCrLf) were evaluate using three different measurement methods: Orthomed (A), common tangent (B) and a correction formula of common tangent developed by Bielecki 2014 (C). In a cohort of 40 dogs with CCrLf underwent to Modified Maquet Procedure, 34 preoperative radiographs in mediolateral projection were included. The amount of advancement of tibial tuberosity, the wedge size to achieved it, and the osteoarthrosis degree (OA)[3] were determined, by 3 different observers: expert surgeon (ob1), non-expert surgeon (ob2) and an intern (ob3). Data collected were statistically analysed using one-way ANOVA, paired T test and intraclass correlation coefficient ICC a p value <0,001 was considered significant. The reliability inter-observers were good for methods A(ICC 0,731), B(ICC 0,741) and very poor for C(ICC 0,248). The reliability intra-observers were very poor for ob1 (ICC 0,128) and ob3 (ICC 0,414), moderate for ob2(ICC 0,537). Intra-observer ANOVA and Paired T-test, applied to all combinations of measurement, shows differences between B and C methods. Several variables have been shown to affect the right advancement of TTA[1]. In our study the OA was the main obstacle to the accurate determination of the measurement landmarks. The method C give a very high amount for advancement (12,7±0,49) in a range of (4,6-19). This method presents a substantial margin of error due to the high variability in the landmark determination in stifles with a high OA. Given the very poor reliability inter and inta-observers with C methods, methods A and B seems to be more accurate with a good reliability. Further investigation needs to confirm our hypothesis and to assess the comparison between radiographs and real specimen.

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SINGLE-APPLICATION OF HEMOSTATIC CLIPS FOR VESSELS OCCLUSION IN SMALL INTESTINAL RESECTION AND ANASTOMOSIS

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Intestinal resection and anastomosis are techniques commonly performed in equine abdominal surgery [1]. Various methods of achieving hemostasis in equine mesenteric arteries during jejunal resection and anastomosis have been described, but are either costly, contraindicated in some cases or time consuming.

The aim of this study was to compare ligatures and metallic clips for mesenteric vessels occlusion both in a laboratory and clinical setting.

Twelve portions of jejunum with associated five mesenteric arteries each were assigned to two groups. In group A vessels were occluded with three sliding knots using monofilament suture material while in group B three metallic clips were applied. Time to perform ligatures or apply clips were recorded and compared between groups. Arteries were then cannulated and leaking pressure recorded and compared between groups.

Same hemostatic methods were used in ten horses subjected to small intestinal resection and anastomosis following strangulating obstructions. Intra and postoperative complications were compared.

Occlusion of mesenteric arteries was significantly faster to perform with clips than with sliding knots There were no statistically significant differences in leaking pressure between ligatures and clips. No complications were noted with both methods in a clinical setting.

Hemostatic clips are effective in providing hemostasis on equine mesenteric vessels when performing small intestinal resection and anastomosis, resisting pressure well above physiological values. Their application in clinical cases resulted effective in providing hemostasis on mesenteric vessels without intra-operative bleeding or postoperative hemostasis-related complications.

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COMPARISON OF CONTINUOUS POSITIVE AIRWAY PRESSURE AND TRADITIONAL OXYGEN THERAPY FOR TREATMENT OF POSTOPERATIVE HYPOXEMIA IN DOGS

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Postoperative hypoxemia (PH) may occur up to 10% of healthy dogs [1]. Oxygen supplementation should be provided to any patient with oxygen saturation (SaO_2) or pulse oximetry reading (SpO_2) of <95% or with an arterial partial pressure of oxygen (PaO_2) of <80 mm Hg. Helmet CPAP has been described as a feasible technique to non-invasively support the respiratory function in dogs [2]. The aim of this study was to compare face mask O_2 supplementation or 5 cmH_2O continuous positive airway pressure at room air ($CPAP_{air}$) administered with a helmet, to treat PH. Our hypothesis was that CPAPair could be superior in restoring normoxemia, compared to administration of O_2 by face mask. To test this hypothesis, SpO_2 at room air was monitored (Masimo Radical – 7) five minutes (T0) after extubation (EXT) and then every 15 minutes for one hour, in dogs recovering from general anesthesia. Dogs that showed hypoxemia at EXT (SpO_2 <95 %) were randomized to receive $CPAP_{air}$ or O_2 treatment. Of the 34 dogs included in the study, 21 were hypoxemic of which 10 were treated with $CPAP_{air}$ and 11 with O_2, the remaining 12 dogs were normoxemic, and served as the control group. For all data, the mean and standard deviation has been calculated. The mean SpO_2 values recorded at the different time of the study for each group were analyzed with ANOVA test ($P<0.05$). The SpO_2 values at T0 were similar in the $CPAP_{air}$ and O_2 group and lower compared to the CTR group. Dogs in $CPAP_{air}$ group showed significantly higher SpO_2 values at T15 and T30 (95.7±0.8 %; 96.7±0.9 %, $P<0.05$) when compared with dogs in the O_2 group at the same times (93.4±1.9%; 93.1±2.1%). At T45 and T60 there were no differences between the three groups. The $CPAP_{air}$ group had a value of SpO_2 at extubation significantly lower than the O_2 group. The results of this study confirmed that CPAP at room air is an effective treatment of PH in dogs ensuring a faster reestablishment of normoxemia, compared to face mask O_2.

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DIAGNOSTIC VALUE OF WHOLE BODY BONE SCAN IN HORSES

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Scintigraphy is widely used in the assessment of musculoskeletal disorders and often it is considered as a screening tool in lame or poor performing horses. It is proved that nuclear scintigraphy is useful in highlighting the presence of lesions undetectable by clinical examination, in horses that do not respond to local analgesic blocks or with intermittent lameness[1]. Despite the usefulness of bone scan is proven, in a recent report, Quiney et al. observed that false-negative results predominate and may lead to missed diagnosis[2]. The aim of this study is to analyze the diagnostic usefulness of whole body bone scan in horses referred for lameness or poor performance. For this retrospective study, bone scans acquired at the Ospedale Veterinario Universitario di Lodi between July 2014 and February 2019 were reviewed. In the study have been included only horses that had a whole body bone scan. On the basis of the history, horses were classified as poor performing, for localized lameness or non-localized lameness. Scintigraphic findings were organized in five categories: definitive diagnosis, localization of the lameness, no findings related to the present clinical signs, findings of unlikely clinical significance and findings that need further investigations. A contingency table and a chi-squared test were used for the statistical analysis. One hundred and eighty horses underwent scintigraphy and 102 were included in the study; twenty-one horses were referred for lameness localized using diagnostic analgesia while in 44 horses the source of lameness was not identified. Thirty-seven horses had an history of poor performance. Statistical analysis highlighted that the only correlation between clinical history and scintigraphic findings was between horse referred for poor performance and findings of unlikely clinical significance (59.5% of horses with a poor performance diagnosis). A final diagnosis or localization of the source of pain were observed respectively in the 5.9% and in the 29.4% of horses. In 11 subjects (10.8%) were found increased radiopharmaceutical uptakes (IRU) of uncertain clinical significant that needed further investigations using analgesic blocks. In the 20% of cases, all referred for lameness, no findings related to the present clinical signs were found. In order to increase the capability of bone scintigraphy, it is mandatory to consider that the sensitivity and specificity are higher in specific regions[2] and the interpretation of the relevance of IRU must be based on detailed clinical examination. In conclusion, we confirm that whole body bone scintigraphy should not be considered a diagnostic screening especially in poor performing horses and that localization of lameness can improve the possibility of a positive result.

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DETERMINATION OF RESISTIVE INDEX AND PULSATILITY INDEX IN HEALTHY ADULT SARDINIAN BREED SHEEP DURING DRY PERIOD

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Aim of the study: Determine physiologic values of Resistive Index and its related parameters in healthy adult, Sardinian breed sheep during dry period. Experimental protocol: Twenty five, healthy non pregnant female Sheep were recruited (CIBASA; protocol number 132/11). An ultrasohographic evaluation of kidneys (first the left) was performed twice by two different operators in a week period with the sheep in left lateral recumbency. Using a colour and pulsed wave Doppler, intrarenal blood flow of at least three segmental/interlobar arteries (e.g. cranial, middle and caudal) with three consecutive cardiac cycles was recorded for both kidneys. Images were recorded and processed subsequently by observers to obtain the Renal Resistive Index (RRI), Pulsatility Index (PI), Flow Velocity Integral (FVI), Peak Velocity (PV) Tele Diastolic Velocity (VTD) and Mean velocity (VM). Technique and measurement repeatability were evaluated. Further, any differences due to day or animals were analysed. All data were reported as mean±SD and difference evaluated by two-sample t-test and Wilcoxon rank-sum test, where $p<0.05$ was considered significant. Results: The mean (±SD) of RRI for right kidney was 0.436 ±0.151and 0.406±0.134 for the left, PI was 27.356±10.364 and 22.582±8.531 respectively, FVI was 14.208±4.940 and 13.831±5.085 respectively, PV was 30.748±8.107 and 29.778±8.006, VTD was 16.409±5.938, VM was 16.585±5.849. No significant differences were found between observers and between days. The images obtained were of good quality. Conclusions: This study could be considered the first report of the specific reference range of renal perfusion in Sardinian Breed sheep. Repeatability and reproducibility were good in term of difference between operators and day of evaluation. Left lateral recumbency provides good echographic window and better exposure of the kidneys but an extention of the examination time can negatively affect the evaluations due to the rise of ruminal gas content.

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EARLY EFFECT OF A SINGLE OR A REPEATED DOSE OF DEXMEDETOMIDINE AND ALFAXALONE ON CARDIO-RESPIRATORY VARIABLES IN HEDGEHOGS (*ERINACEUS EUROPEUS*)

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Clinical examination in hedgehogs requires chemical restraint because of their tendency to roll up as a defensive behavior. In captivity, this species is predisposed to obesity and this may cause the injection of sedatives to be administered into subcutaneous adipose tissue rather than intramuscularly. This condition delays the onset of immobilization leading to further administrations of sedatives [1]. Dexmedetomidine is used to restraint small mammals and its depressant properties are dose-dependent. Supplementary administration of such drug may cause detrimental cardiovascular and respiratory effects. The aim of this study was to describe the early effect of a single or repeated intramuscular administration of alfaxalone and dexmedetomidine on some physiologic variables in hedgehogs. This observational study enrolled 20 rescue European hedgehogs showing no evident signs of systemic diseases. Animals underwent routine pre-release health check after a rehabilitation period in a rescue centre. Alfaxalone 2 mg/kg and dexmedetomidine 0.05 mg/kg were injected into the quadriceps muscle. If the righting reflex did not disappear after 10 minutes both drugs were administered at half of the initial doses. Measurements included: sedation score obtained by a semi-quantitative scale, respiratory and pulse rate, haemoglobin oxygen saturation, end-tidal carbon dioxide ($EtCO_2$). All the variables were recorded every 5 minutes from the loss of righting reflex. Raw data included in the analysis represented the averages of the values measured in the first 20 minutes after the first injection. An ANCOVA compared differences between physiologic variables. The weight of the animal was a covariate. The results of this study indicated that the body weight affected the number of injections. Animals (n=8) that received more sedatives had a mean body mass of 0.802 ± 0.149 kg compared to 0.653 ± 0.106 kg of hedgehogs that received a single injection. Sedation score was higher in animal receiving a single injection (p=0.001) while pulse rate was lower (p=0.013). The respiratory rate did not change significantly between groups while $EtCO_2$ was statistically lower (p=0.027) in animals that had a single administration of sedatives. Haemoglobin oxygen saturation was not different between groups and was higher than 90%. In conclusion, this study shows that overweight animals may require an additional dose of sedatives to achieve adequate immobility. Physiologic variables remain within clinical acceptable limits reported in literature for alpha-2 agonist-based sedation in hedgehogs [2].

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EFFICACY OF INTRANASAL ADMINISTRATION OF TRAMADOL FOR PAIN MANAGEMENT IN DOGS AFTER OVARIOHYSTERECTOMY

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In humans, the analgesic delivery via intranasal route (IN) results easy to perform, well tolerated and with a rapid onset of pharmacological effects. As no studies have been conducted in dogs regarding IN administration of tramadol, the aim of this study was to explore the efficacy of such treatment in bitches after ovariohysterectomy (OVH). The study was approved by the Bioethical Committee of the University of Perugia (protocol no. 2017-02). Thirty bitches admitted for elective OVH were enrolled in the study. After surgery, they were randomly assigned to one of the following treatment groups (10 subjects/group): Group TIV: 4 mg/kg of Tramadol IV; Group TIN: 4 mg/kg of Tramadol IN; Group MIV: 0.2 mg/kg of Methadone IV. At baseline (before surgery) and every hour up to 8 hours after surgery, signs of pain using the Italian version of the Glasgow Composite Pain Score - Short Form (IGCPS-SF) [1] were monitored by one observer blinded to the treatment and trained on the use of IGCPS-SF. Any eventual side effects have been also recorded. At the first two observational times post-treatment, the degree of sedation was evaluated according to a composite simple descriptive sedation score [2] to point out any interferences of residual sedation from anaesthesia with the IGCPS scores. A rescue analgesia (0.2 mg/kg of IV methadone) was provided if dogs showed signs of pain according to the pain scores and the judgment of the observer. The three groups of treatment were homogeneous for age, weight, anaesthesia and surgery duration. No significant differences among treatments (significance was considered for a p value <0.05) regarding to the incidence of side effects and sedation were observed. Rescue analgesia was administered in 2 dogs of Group TIV and in 3 of both Group MIV and TIN, with no significant differences among treatments with regard to its occurrence and time of administration. Compared to baseline, pain scores (calculated excluding dogs that received rescue analgesia) were significantly increased for the first 4 and 6 hours post administration in TIN ($p<0.039$) and MIV ($p<0.046$) group, respectively, and for the entire observational period in group TIV ($p<0.024$). However, no significant differences were observed among groups. The lack of statistical differences among groups may suggest that the IN treatment with tramadol is as effective as the IV tramadol and methadone administration to manage pain after OVH in dogs.

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PROTEINASE ACTIVATED RECEPTOR 4 IN HEALTHY AND ISCHEMIC JEJUNUM OF THE HORSE

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In horses, epiploic hernia (EH) is a common cause of intestinal strangulation. In humans and in animal models proteinase activated receptors (PARs) have been identified to be involved in the pathophysiology of several intestinal diseases [1,2]. In particular, proteinase activated receptor 4 is considered to be involved in the regulation of inflammation and pain pathways [3,4]. In addition, in animal models, the activation of PAR4 by the single administration of a synthetic peptide, inhibited colonic hypersensitivity and decreased the nociceptive response to painful colorectal stimuli [4]. The aim of the present study was to evaluate the distribution and expression of PAR4 in the jejunum of healthy horses and in the ischemic tracts from horses undergoing surgery for EH. Eight healthy horses (Group H) and eight horses with epiploic hernia (Group EH) were included; the jejunum samples were collected at the slaughter or intraoperatively after enterectomy, respectively. The study was approved by the Ethical Scientific Committee for Experimental Animals of the University of Bologna (Prot. n 15-IX/, 08/05/2012).To evaluate PAR4 expression in sections of the jejunum, immunofluorescence, western blot and quantitative polymerase chain reaction (PCR) were performed. Immunohistochemistry of PAR4 in the jejunum in group H horses showed a weak receptor distribution, mainly in the cellular infiltrates especially leukocytes, scattered throughout the lamina propria of the mucosa and in the submucosa. Quantitative PCR data demonstrated that PAR4 mRNA was detectable in all of the samples analysed without any difference between the H and the EH groups; however the PAR4 protein level was significantly lower in the jejunums of the group EH horses. In the Group EH horses, PAR4 immunoreactivity was weak and was mainly expressed in the mast cells distributed in the sierosa. In this study, the distribution and expression of PAR4 in the jejunums of the healthy horses and in those with spontaneous occurring epiploic hernia was confirmed. Further studies are needed to elucidate the role of PAR4 in the modulation of visceral nociception in equine intestine. In addition, as previously described in experimental studies in animal models [4], the local administration of the PAR4 activating peptide intraoperatively could be taken into consideration as a novel therapeutic approach for the treatment of post-operative inflammation and pain in horses submitted to surgery for gastrointestinal disease involving the small intestine.

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USE OF PRF, PLATELET-RICH FIBRIN, INSIDE EXTRACTION SOCKETS: PRELIMINARY STUDY IN DOGS

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Platelet-rich fibrin (PRF) belongs to a new generation of platelet concentrate containing on a fibrin membrane all the constituents of a blood sample favourable to healing and immunity. In human dental surgery, the socket preservation concept was recently introduced to minimize alveolar bone resorption, which often occurs after tooth extraction, by means of bone graft materials filled into the socket immediately after extraction. The aim of the study was to evaluate the ability of autologous PRF to stimulate the healing process and tissue regeneration in dogs when it is applied inside dental postextraction sockets. Seven crossbreed dogs, showing grade III/IV periodontitis, underwent extraction surgery for a total of 89 dental sockets. In each patient, some dental sockets were filled with PRF after extraction (Group PRF), whilst other sockets were left to heal without treatment (Group C). At T0, clinical and radiographic studies were carried out in each dog before and after surgery and gingival bone biopsies were taken both in PRF-treated and control site areas. All assessments were repeated after three weeks (time 1, T1). Radiographic optical densities (OD) were measured digitally in each post-extraction site. A histological scoring system, considering both phlogosis and regenerative parameters, was used. Data were statistically analysed between and within groups with Student's t-test or Mann-Whitney rank sum test and with Paired t-test or Wilcoxon sign rank test respectively. Clinical examination at T1 revealed a good gingival and periodontal healing process in both groups, while a stronger mucogingival adherence was found in PRF-treated sites. Group PRF showed a higher increase of radiographic OD in comparison to Group C, although the difference was not significant ($P=0.312$). Within Group PRF a significant difference in OD measured at T0 and T1 was highlighted ($P=0.011$). No significant differences emerged within Group C ($P=0.169$). Histological evaluation showed less inflammation and greater regeneration in Group PRF compared to group C ($P<0.05$). Within Group PRF a significant difference in histological score emerged during the study ($P=0.024$), while did not within Group C ($P>0.05$). The study highlighted a potential capacity of the PRF to stimulate the natural healing process of extraction sites in dogs.

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STRAIN ELASTOGRAPHY IN HORSES: A COMPARISON BETWEEN NORMAL AND OSTEOARTHRITIC FORE FETLOCKS

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Sonoelastography is an ultrasound technique for evaluating soft tissue elasticity [1] validated in musculoskeletal disorders [2,3]. Early diagnosis is a critical component in treatment and prevention of osteoarthritis (OA) [4]. Synovitis and capsulitis produce pain and discomfort and increase the production of mediators contributing to the pathogenesis of OA [5]. Aim of this study was to compare the changes in elastic properties of the distal attachment of the fetlock joint capsule (JC) in pathologic versus normal joints, with strain elastography (SE). Horses with and without radiographic or ultrasonographic signs of fore fetlock OA were selected and assigned to the H (healthy) and P (Pathologic) group. SE was performed by 2 operators on transverse and longitudinal scans of JC. Qualitative and quantitative measures of the elastograms were assessed by 2 independent examiners on selected Regions Of Interest (ROI) [7] on the same most representative image. JC elasticity index (EI) was calculated. The strain ratio (SR) was obtained between the long digital extensor tendon (LDET) and JC. Intra and inter reader repeatability were assessed. Data were evaluated for normality before statistical analysis was performed. Confidence interval was set at 95%. Qualitative assessment of the images showed an excellent intra-and inter-rater agreement in group H and fair to excellent in P group, with mean score 4.95 and 4.79 in longitudinal and trasverse scan in H group and 3.91 and 3.95 in P group. Mean±standard deviation (SD) for EI and SR were 0.57±0.19 and 0.32±0.19 in transverse, 0.51±0.14 and 0.28±0.2 in longitudinal plane in H group; 0.98±0.58 and 0.46±0.48 in transverse, 0.93±0.51 and 0.59±0.46 in longitudinal plane in P group. Among the groups SE showed a statistically significant difference in both planes (Mann-Whitney U test). SE is a feasible and reproducible method to detect stiffness of the JC in horses. H group showed lower EI of the JC and SR compared to the P group, indicating lower stiffness and higher strain, also confirmed by the higher qualitative scoring of the elastograms in the H group.

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CARDIOPULMONARY CHANGES AND DESFLURANE REQUIREMENTS IN THE ANESTHESIA OF DOGS UNDERGOING TIBIAL PLATEAU LEVELING OSTEOTOMY, WITH CONSTANT RATE INFUSION OF FENTANYL, LIDOCAINE OR KETAMINE

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Inhalation anesthetics allow rapid control of the anesthetic depth by adjustments of the vapor deliver and of fresh gas flow (FGF), with relatively rapid induction and recovery [1]. Desflurane has marked low solubility and rapid induction and recovery responses to changes in inspired concentration if compared with other inhalant agents [2]. Combination of injectable anesthetic agents can reduce inhalation agent requirements resulting in less cardiovascular depression [3,4]. Aim of the work was to evaluate cardiopulmonary changes and desflurane requirements of intravenous (IV) constant rate infusion (CRI) of fentanyl, lidocaine, ketamine on separate occasions, in dogs undergoing tibial plateau leveling osteotomy. Eighteen dogs, 4-9 years, 17-35 kg, have been assigned to 3 groups of 6 dogs, in which one of 3 different CRI of agents were IV administered. Fentanyl 10 μg kg^{-1} h^{-1} (group F) or lidocaine 100 μg kg^{-1} $minute^{-1}$ (group L) or ketamine 40 μg kg^{-1} $minute^{-1}$ (group K) were IV administered, after IV bolus of 5 μg kg^{-1}, 2 mg kg^{-1} and 1 mg kg^{-1} respectively. Acepromazine 20 μg kg^{-1}, medetomidine 5 μg kg^{-1} and fentanyl 5 μg kg^{-1} were IV administered for sedation. Propofol at effect was IV administered for induction. Anesthesia was maintained with desflurane in oxygen and air at FGF 0.5 L $minute^{-1}$ with inspired oxygen fraction 0.4 in spontaneous ventilation. Heart rate (HR), mean arterial pressure (MAP), end-tidal carbon dioxide ($PetCO_2$) and desflurane (EtDes) concentrations, respiratory rate (RR), pulmonary tidal volume related to body weight (VT/BW) and minute volume related to body weight (VM/BW), peripheral hemoglobin oxygen saturation (SpO_2), rectal temperature (T) and time to estubation (ET) were evaluated by an operator unaware of the protocol adopted. Depth of anesthesia was assessed by palpebral reflex, eye position, jaw tone and autonomic responses to surgical stimulation. EtDes was increased or decreased depending on palpebral reflex, eye position, jaw tone and when MAP or HR increased or decreased respectively by 20% from previously recorded values in response to surgical stimulation. Data were recorded after intubation (T0), at skin incision (T1), at the first bone drilling (T2), at the midpoint of tibial osteotomy (T3), at the midpoint of skin suture (T4) at estubation (T5) and were analyzed by ANOVA ($p<0.05$). Results in mean values: HR was higher at T2 and T3 in group K (105 and 110 bpm) and lower in group F (82 and 76 bpm). MAP was higher at T2 and T3 in group K (108 and 103 mmHg). VT/BW was higher at T3 in group L (9 ml kg^{-1}). $PetCO_2$ was higher at T3, T4, T5 in group F (47, 50, 48 mmHg). EtDes was higher at T2 and T3 in group K (6.5 and 7%). SpO_2 was always over 95%. At the doses utilized, ketamine and lidocaine maintained higher values of cardio-circulatory functions than fentanyl. Requirement of desflurane is lower with fentanyl than with lidocaine and ketamine.

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TIBIAL PLATEAU LEVELLING OSTEOTOMY IN NINE CATS

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Cranial cruciate ligament deficiency (CCLd) in cats is less common than dogs and extracapsular procedures have been usually performed to manage joint instability [1]. In 2016 was firstly published a paper regarding tibial plateau levelling osteotomy (TPLO) application in 11 cats with CCLd: outcomes were very positive and further evaluations including longer terms follow up have been encouraged [2]. For these reasons the aim of this study was to evaluate the clinical and radiographic outcomes of cats in which TPLO surgery was performed for cranial cruciate ligament deficiency up to one year postoperatively.

Each cat underwent orthopaedic assessment, preoperative radiographic evaluation, surgical procedure, postoperative management clinical follow up at 1 month, 2 months and 1 year after surgery. Age, body weights, tibial plateau angles, meniscal tears, implants and osteoarthrisis (OA) progression has been recorded. Postoperative OA score was compared with that obtained 1 year after surgery using a paired t -test with commercially available software. Complete bone healing was observed 8 to 12 weeks after surgery associated with a full weight bearing. Radiographic evaluation performed 1 year after surgery showed no OA progression (*p value* > 0.1). Minor complication was achieved in one case (#7) in which a mild to moderate seroma was observed ten days after surgery. No major complications were recorded.

In feline surgery, TPLO applications looks controversial. In 2018 Bilmont and colleagues showed that a tibial plateau angle of 5°, 0° and -5° achieved after TPLO was not able to stabilize both the cranial tibial displacement and the tibial rotation in an ex-vivo model [3]. Our clinical outcomes were coherent with those obtained with another clinical study published in 2016 in which eleven cats underwent TPLO for cranial cruciate deficiency [2]. TPLO was performed without major complications, bone ossification was obtained after 8-12 weeks associated with absence of lameness and no OA progression. We hypothesized that muscle activation may play a very important action in stabilization of the stifle joint in cats and maybe justify the discordance between ex vivo and in vivo studies. In our experience TPLO was a suitable option for surgical treatment of cranial cruciate ligament rupture in cats but further evaluations for better understanding of the stifle joint biomechanics are highly encouraged.

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RETROSPECTIVE EVALUATION OF OSTEOARTHROSIS OF THE STIFLE IN DOG AFTER POROUS TTA: ROLE OF THE TIBIAL TUBEROSITY FRACTURE ON THE MAQUET HOLE

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The aim of the work was to study the progression of stifle osteoarthritis grade in dogs subjected to TTA (Tibial Tuberosity Advancement) for rupture of the Cranial Cruciate Ligament; the possible role of the tibial tuberosity fracture on the Maquet hole in the variation of the degree of osteoarthritis was also evaluated. Radiographic examinations were evaluated, immediately after surgery and at 3 months from porous TTA [1] of 58 dogs. The cases are divided into two groups; the first group (Fx) includes cases with fracture of the tibial tuberosity or with incomplete fracture of the Maquet hole and the second group (No Fx) includes cases without complications [2]. The degree of osteoarthritis was determined with the Wesseley method [3] and by a modified method of this. The results of the study showed that both groups had significant progression of osteoarthritis compared to T0 at three months after surgery. No statistical difference was found between the degree of osteoarthritis between the control group and the group with tibial tuberosity fracture. The two methods used in the evaluation of OA levels showed similar results.
Regardless of the degree of tibial tuberosity fracture Both groups showed an increase in the degree of osteoarthritis in T1 compared to T0. The absence of a statistically significant difference between the Fx group and the NO Fx group shows that OA progression in TTA patients is not significantly conditioned by fracture/incomplete fracture of the tibial tuberosity, which therefore represents a minor complication. the radiographic interpretation of OA scores with both methods by different observers with different experience showed no significant difference, therefore these two methods of evaluation of osteoarthrosis after TTA surgery are objective and comparable.

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CORNEAL LESIONS DURING GENERAL ANESTHESIA, A COMPARISON OF THREE DIFFERENT EYE DROP FORMULATIONS IN DOGS: PRELIMINARY DATA

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Corneal ulceration and erosion are known sequels of general anesthesia (GA) in dogs [1]. Park et al. reported several risk factors like duration of GA, number of topical medications or kind of surgical procedure [2]. However, no literature data are available about comparing the effects of different kinds of lubricant eye drops (LED) during GA in dogs. Aim of this prospective randomized clinical study was to compare Schirmer tear test-1 (STT) values and incidence of corneal epithelial defects using three different LED. This study was approved by OBPA of the University of Naples "Federico II" (2018/0052229). Dogs scheduled to undergo orthopaedic or spinal surgeries were enrolled. Brachycephalic dogs or dogs with pre-existing ophthalmic disease were excluded. After ophthalmic examination (OE) including slit-lamp biomicroscopy, STT, fluorescein and lissamine green staining, dogs were randomly allocated to receive as prophylactic LED during GA, either carmellose sodium (Celluvisc, 1% Dublin, IE) (GC), or 1% hyaluronic acid (HyCare, Chesterfield, UK) (GH), or 0.25% hyaluronic acid (Lacrivet, Milan, IT) (GL). STT were performed immediately after endotracheal intubation and every hour during GA until end of surgery. After each STT one LED was instilled topically according to randomization. The same ophthalmologist, blind to the treatment, performed OE after extubation and 24h after surgery. In case of corneal damage, the same LED used during GA was applied every 4hrs for the following 24hrs. Forty-four eyes were enrolled in the study. Differences over time in STT values between groups were analysed using two-way ANOVA. *Post-hoc* Sidak's test was used. Significance was set at $p<0.05$. Significant differences were found between groups in STT values at 1hr and 24hrs ($p=0.02$ and $p<0.01$ respectively). Dogs in GL had significant higher STT (3.5±2.7 mm/min) compared to GC (0.7±2.7 mm/min) at 1hr ($p=0.02$) and significantly higher STT at 24hrs than dogs in GC and GH groups ($p<0.01$). Fluorescein stain uptake was observed in 11.9% of eyes after extubation (10% in GL, 20% in GC and 7.7% in GH) and in 7.1% after 24hrs (10% in GL and 10% in GC). Lissamine green stain uptake was evident in 4.8% of eyes after extubation (7.7% in GH and 5 % in GL). No difference in duration of GA were found between groups. No corneal ulcer was observed. Decreased tear production is a prominent risk factor for corneal ulcerative disease during GA [1]. In the present study the LED characterised by low hyaluronic acid concentration and lower viscosity seems to keep higher STT values during the first hour of GA. Nevertheless, a clinically significant reduction in STT was recorded in all the eyes studied during GA. The LED containing hyaluronic acid showed higher efficacy in preventing corneal epithelial defects. Further studies are required to confirm this hypothesis.

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COMPUTED TOMOGRAPHY IN POLYTRAUMATIZED PATIENTS: A RETROSPECTIVE STUDY OF 63 CASES (2014 - 2017)

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In veterinary and human medicine, the term "polytrauma", although lacking a precise universally accepted definition [1], defines trauma patients whose injuries involve multiple body regions, organ systems or cavities, with the possibility of impaired respiratory or circular function. This determines the clinical and diagnostic complexity of a polytrauma; one study reported that about 50% of dogs with thoracic lesions also had skeletal fractures while other associated lesions were related to the nervous system and abdomen [2]. Consequently, the use of a single first-level diagnostic method is not enough to diagnose a trauma in all regions. In human medicine, according to the new Advanced Trauma Life Support (ATLS) guidelines, in case of major trauma, multilayer computed tomography (CT) represents the diagnostic gold standard since it provides a quick and complete overview of the lesions: it allows the identification of effusions and lacerations of organs and the use of a contrast medium allows the identification of active hemorrhage allowing an immediate therapeutic intervention [3]. This study aimed to describe the major CT findings that occurred in polytraumatized dogs and cats, the distribution frequency of the lesions associated to the main body's regions involved and the differences arising between these species. Research was carried out in the database of "I Portoni Rossi" Veterinary Hospital in Bologna, Veterinary Hospital "Mario Modenato" of Pisa University and Veterinary Clinic "Pet Care" in Bologna, to identify traumatized dogs and cats undergoing CT from 2014 to 2017. The following data were collected for each patient: gender, weight, type of study carried out and injuries reported. The lesions were classified according to the region involved: head, spine, chest, abdomen, pelvis and appendicular skeleton. Thirty seven studies involving cats and 26 involving dogs were included. The cats mainly presented lesions which involved both the skull and the chest simultaneously. The dogs presented lesions which affected the chest, abdomen and vertebral column simultaneously. In cats, the skull was more involved than in dogs ($P<0.001$). Regarding the cranial bone structures, more lesions were reported to the mandible and the maxilla in cats (43%); dogs were more affected by thoracic trauma ($P<0.0011$), by lesions of the vertebral column ($P<0.008$) and by abdominal trauma ($P<0.012$). The thoracic findings included pulmonary contusions (dogs 54%, cats 24%) and pneumothorax (dogs 38%, cats 11%). In veterinary medicine, as in human medicine, the application of a protocol which foresees CT in the management of major trauma cases could be an optimal method for obtaining a general picture of the lesions in polytraumatized animals in a short time, thus reducing the time between diagnosis and therapy.

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SONOGRAPHIC EVALUATION OF MEDIAL ILIAC LYMPH NODES-TO-AORTA INDICES IN THE DOG

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In dogs Medial Iliac Lymph Nodes (MILN), located laterally to the aorta near its trifurcation, are the largest lymph nodes of the iliosacral lymphocenter. They drain the skin of the caudodorsal abdominal wall, the skin, muscles and bones of the pelvis and pelvic limb, and the inguinal area. Ultrasonography allows non invasive evaluation of both peripheral and deep lymph nodes even if not clinically identifiable. The MILN are routinely evaluated during abdominal ultrasound examinations [1]. Benign and malignant lymphadenopathy might be difficult to differentiate because the features currently studied (size, shape, echogenicity and echotexture) may be similar, especially in the early stages of the disease [2]. The great variability of dog breeds has not allowed clinical radiologists to set a unique reference range of the MILN size. The aim of this paper was to evaluate the size of normal and pathologic medial iliac lymph nodes using a ratio between the aortic diameter and the lymph node diameters. We included in the study 37 dogs where one or both MILN were affected by lymphoma, metastatic neoplasia or inflammatory lymphadenopathy (diagnosis confirmed by means of cytopathology) and 63 healthy dogs used as a control group. Two experienced radiologists (SC and TM) acquired images and videos of the MILN and aorta, using a Toshiba Aplio 400 ultrasound equipment with either a 12 MHz Linear or a 7.5 MHz microconvex transducer. The recordings were subsequently analyzed by a different expert radiologist (DDS), unaware of the patient's medical history, who determined the length, height and thickness of the medial iliac lymph nodes and the ratio of these with the diameter of the aorta in transverse and longitudinal section. These data underwent statistical analysis with GraphPad Prism 7 and Reference Value Advisor. A positive correlation was found between the length, height and thickness of the medial iliac lymph nodes and the aortic diameter. There was a significant difference between ratio of right and left MILN of healthy dogs. There is a significant difference as regards length and thickness, but there is no significant difference as regards height. We have calculated the reference range of the ratio of height of MILN with the diameter of the aorta in longitudinal scanning in normal dogs and there was 0.2-1.2 for right lymph node and 0.3-1.2 for left lymph node. Sick dogs tend to have ratio's values greater than healthy dogs (0.3-2.6 for right lymph node and 0.3-2.9 for left lymph node). This reference range could be used in clinical practice to differentiate healthy and sick dogs.

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A CT BASED METHOD FOR THE ASSESSMENT OF RETROBULBAR CONE VOLUME IN DOGS

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Different regional anesthesia techniques are used alone or combined with general anesthesia as part of the anesthetic regimen and pain management in ophthalmic surgery. Among these, retrobulbar anesthesia (RBA) is the gold standard, producing oculo-extrinsic muscles' akinesia, peri-operative analgesia, preventing the oculo-cardiac reflex. Goal of RBA is to inject an adequate volume of local anesthetic (LA) into the retrobulbar cone (RC). In dogs, due to the large variability of skull shapes and sizes, there is no agreement among authors on how to calculate the volume of LA to inject. Aim of this retrospective study is to correlate different body variables to the RC volume measured via Computed Tomography (CT) in order to choose the volume of LA to inject into the RC. The RC volumes were measured in all the canine skulls' CT performed from 2009 to 2017 at the Interdepartmental Radiology Veterinary Center. Exclusion criteria were presence of lesion of the eyeball and/or the retrobulbar space. As useful variables to establish RC volume we pondered animals' size, weight, skull morphology (SM) and sex. All the CT studies were performed using slice thickness of 1 to 3 mm and were post-processed, using 'detail' and 'bone' convolution filters. Multiplanar reformatted images in axial, dorsal and sagittal planes were obtained too. On multiplanar images, the dorsal plan along the sagittal plane of the cone was obtained and the base and the height of the cone were measured. The base was traced between the insertion of the lateral and medial recti muscles on eyeball (RC diameter) while the height was measured between the eyeball base and the optic foramen. The RC volume was calculated according to the formula: base area multiplied by the height and divided by three. Univariate and multivariate analysis were used to evaluate the correlation factors between the calculated volume and the four variables considered. A sample of 354 dogs was collected. Sample size consisted of 140 large, 116 small and 98 medium size dogs; 4 dolichocephalic (D), 30 brachycephalic (B), and 320 mesocephalic (M); 178 males and 176 female; sample's mean weight was 19.1±14 kg (range 1 to 60 kg). From the univariate analysis a RC volume larger in males compared to females was found. Significant differences were found also regarding SM: B dogs had a larger volume than M ones. A large positive correlation between volume and weight was found. In the multivariate analysis, when all the variables were considered, the weight persisted as the strongest predictor with a still significant difference between SM. Our results demonstrate that body weight could be considered as the reference value to calculate the RC volume and, consequently, the volume of LA to inject. Further studies with the use of a contrast medium are needed to confirm our results.

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CONTINUOUS RATE INFUSION OF DEXMEDETOMIDINE VS SUBCUTANEOUS ADMINISTRATION IN ANAESTHETIZED HORSES UNDERGOING MRI EXAMINATION

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Up to 2005, dexmedetomidine use had not been reported in equine. Since then, several experimental and clinical studies have been published. The main reason for this increase relies on its beneficial pharmacological profile, including short half-life and rapid redistribution (1). The aim of the study is to compare the clinical effects and recovery quality after continuous rate infusion (CRI) or subcutaneous administration of dexmedetomidine in horses undergoing general anaesthesia. Fourteen horses scheduled for MRI examination were included. All horses were sedated with acepromazine 0.03 mg kg^{-1} intravenously (IV) and detomidine 10 µg kg^{-1} (IV). Anaesthesia was induced with ketamine 3 mg kg^{-1} (IV) and diazepam 0.04 mg kg^{-1} (IV) and maintained with isofluorane in 60% oxygen; end-tidal isoflurane concentration was maintained between 1.3-1.4 %. Horses were randomly divided in two groups. Group "Dex CRI" received dexmedetomidine intravenously at 1 µg kg^{-1} $hour^{-1}$, group "Dex SC" received 2 µg kg^{-1} of dexmedetomidine subcutaneously every 60 minutes. If nystagmus or incessant fighting against ventilator occurred, ketamine rescue at 0.1 mg kg^{-1} was given. In case of sudden movements, thiopental 0.5-1.0 mg kg^{-1} IV was given. Ringer's lactate was given at 3 mL kg^{-1} $hour^{-1}$, dobutamine was administered IV and the rate adjusted to maintain MAP>70 mmHg. Controlled mechanical ventilation using intermittent positive pressure ventilation was adjusted to maintain arterial carbon dioxide partial pressure between 38-45 mmHg. Heart rate, invasive arterial blood pressure, arterial blood gases, total dose of dobutamine administered, ketamine rescue needed, urine production were recorded. Time required until extubation and time to attain sternal and standing position were noted. The main anaesthesiologist assessed recovery quality graded on a standard scoring 5-point scale with a score of 1 representing the best recovery (2). Mann-Whitney U test was applied for non-parametric data and T-test for parametric data ($p \leq 0.05$). There was no statistically differences in physiological intra-anaesthetic parameters, in body weight (kg) (CRI 521±53; SC 506±76), age (years) (CRI 10.7±2.1; SC 10.8±4.1), anaesthesia duration (min) (CRI 139±9.,7; SC 144±16.2), number of ketamine rescue needed (CRI 1±1.15; SC 0.5±1.13), recovery score (CRI 1.8±1,2; SC 1.5±0,5). Also time until extubation (min) (CRI 11.5±5.0; SC 9.7±2.6), time to attain sternal (min) (CRI 41.5±12.2; SC 49.7±6.0) and standing position (min) (CRI 50.7±14.6; SC 57.2±6.,0) were not statistically different. There was statistical significance in urine production (L) (CRI 8.0±3.5; SC 11.1±4.4) and total dobutamine mcg/kg/min (CRI 0.89±0.35; SC 0.56±0.18). Subcutaneous administration of dexmedetomidine has product similar clinical effects to those achieved with CRI. It has permitted a significative reduction in dobutamine administration and a more stable depth of anaesthesia confirmed by the lower number of rescue ketamine boluses required even if not statistically different. Further studies are required to evaluate different dosages both in CRI and subcutaneous administration.

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ALFAXALONE VERSUS DESFLURANE FOR CATS UNDERGOING OVARIECTOMY

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To compare the quality of anesthesia, duration and quality of recovery of dexmedetomidine, methadone, midazolam and acepromazine combined with either alfaxalone or desflurane. Study design: Randomized, prospective clinical study. Animals: A group of 40 healthy client-owned cats undergoing ovariectomy. Methods after 30' from intramuscular (IM) premedication with a combination of acepromazine 0.05 mg•kg^{-1}, methadone 0.5 mg •kg^{-1}, dexmedetomidine 15 µg mg•kg^{-1} and midazolam 0.2 mg•kg^{-1} diluted with saline in order to obtain a standard volume of 0.6 ml, cats were randomly assigned to one of the two treatment groups. Group A (n=20), which was administered intravenous (EV) alfaxalone (1.5 mg•kg^{-1}). Group D (n=20), which was administered desflurane via facemask (18% in O^2 100% at 0.5 L for 1 minute or until intubation was possible). After induction, the vaporizer was set to maintain end tidal desflurane in a range of 5.5/6%. Heart rate (Hr), respiratory rate (Rr), systolic (SAP), diastolic (DAP) and mean arterial pressure (MAP) were measured and recorded in awake animal (T0) and 15' after premedication (T1). During anesthesia the same parameters plus end tidal CO_2, SpO_2 were measured and recorded before the skin incision (T2), during the right and left ovariectomy (T3, T4), at the end of the fascia closure(T5), at the end of skin closure (T6) and before the antagonization (T7). In cases of increased autonomic responses to surgical stimulation, an additional 1 mg kg^{-1} of alfaxalone (A group) was administered or was increase the percentage of inhaled desflurane (D group). After one hour from IM premedication, atipamezole (3.75 µg•kg^{-1}) was administered EV, and the times to extubation, sternal recumbency and standing position with active interaction were recorded. Quality of recovery was evaluated with a simple descriptive scale. Descriptive statistics were utilized to assess the normal distribution of data. ANOVA followed by a Bonferroni multiple comparison test were used to compare the intraoperative physiological variables between the two treatment groups. The Mann-Whitney test was applied for SDS assessment of recovery quality. Results: the additional anesthesia was required in four cats in A group. SAP, MAP and DAP were significantly lower in the D group although in the physiologic range. No animal has shown apnea. The time of extubation was lower in the A group ($p<0.02$) but the others recovery times were lower in the D group with $p<0.001$ and $p<002$ respectively. The quality of recovery was similar between groups with SDS scores: 0 (0-1) and 0 (0-1), respectively. Conclusions: Both protocols provided comparable good quality of anesthesia in term of ventilation and hemodynamic status. Although desflurane allowed faster recovery, alfaxalone showed less hemodynamic influence. In conclusion, under this study condition both drugs have proved valuable for cats under ovariectomy.

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PHARMACOKINETIC OF HIGH DOSE MEDETOMIDINE (0.13 MG KG-1) ADMINISTERED INTRAMUSCULARLY IN MALE CATS FOR SEMEN COLLECTION

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Medetomidine is α2-adrenoreceptive agonists used alone or in combination with other drugs for premedication in small animals. In clinical practice, the dose used in cats varies from 0.02–0.08 mg/kg [1]. Medetomidine can be used for semen collection in cats, where it induces a myorelaxant effect on the deferent duct, leading to sperm ejection and the release of spermatozoa within the urethra [2]. The dose of medetomidine needed to produce a good semen sample is much higher than the dose reported for anaesthetic and pain management procedures, and varies in cats between 0.13 and 0.14 mg/kg. However, in clinical practice, the effect on semen collection, in terms of quantity and quality, is variable among the patients. The authors hypothesized a variation of pharmacokinetic parameters in the cats in terms of distribution and elimination. The aim of the study was to characterize the pharmacokinetic of medetomidine (0.13 mg kg-1) administered intramuscularly (IM) in cats for semen collection using the urethral catheterization after pharmacological induction technique [2]. The study was approved by the local and national ethical committees. Eighteen client owned cats undergoing semen collection were included and were administered medetomidine (0.13 mg kg-1) IM. Venous blood samples were collected at 20, 30, 40, 50, 60, 75 and 90 minutes after medetomidine administration. Sperm collection was attempted at T20. After the last blood sample, atipamezole was administered IM and all cats were recovered. Plasma medetomidine concentrations were determined by LC-MS/MS analysis. The medetomidine concentration versus time curves were analysed for each individual by XY plot using WinNonlin 6.3 (Pharsight Corporation, Mountain View, CA, USA). All the cats appeared well sedated after 5 minutes. Semen collection was feasible in 15/18 cats. The medetomidine plasma concentration following the IM administration of a bolus was best described using a noncompartment model. Time of maximum concentration was observed at 40 minutes (range 20-90) and maximum concentration was 32.8 ng ml-1 (range 26.8-51.2). The median apparent clearance was 0.011284961 ml min-1 (range 0.000647-0.043548 ml min-1). In conclusion, medetomidine administered IM at 0.13 mg kg-1 reached its peak plasm concentration slowly. It also had a low total body clearance probably due to the cardiovascular alterations as previously described [3]. Further studies are needed to evaluate if the quality and quantity of semen collected up to 90 minutes after medetomidine administration correlate with the maximum medetomidine plasma concentration.

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RAPID INJECTION OF ALFAXALONE AND DEXMEDETOMIDINE FOR INDUCTION OF GENERAL ANAESTHESIA IN DOGS: QUALITY OF INDUCTION AND CARDIO-RESPIRATORY EFFECTS

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Following a multimodal approach, the use of sedative drugs in combination with general anaesthetics for co-induction of general anaesthesia is always more spreading for many procedures [1].The aim of the study was to compare the anaesthetic efficacy and the cardiorespiratory effects of two different dosages of dexmedetomidine (DEX) in combination with alfaxalone (AFX) administered through an intravenous rapid bolus (<5sec) for induction of general anaesthesia in dogs. Fourteen mixed-breed, client-owned, healthy dogs (ASA I or II), undergoing elective procedures, were randomly allocated in two groups. For each dogs the temperament was evaluated before premedication [2] as well as heart rate (HR), respiratory rate (fR), blood pressure (BP) and body temperature (BT). Induction of general anaesthesia was achieved through a rapid (<5 seconds) single intravenous injection of AFX (1.2 mg kg-1) combined with 2 mcg kg-1 (group A) or 3 mcg kg-1 (group B) of DEX. Quality of induction and intubation [3], time between drugs administration and orotracheal intubation and any side effects were recorded. Requirement of additional AFX was also recorded. Heart rate, fR, BP and BT were recorded 2 (T2), 5 (T5), 10 (T10) and 20 (T20) minutes after induction of general anaesthesia. Presence of electrocardiographic (ECG) alteration were recorded. Data were analysed by repeated measure ANOVA and Mann-Whitney U test. P was set at 0.05. Weight, age, gender, temperament and baseline physiological variables were not different between groups. Quality of induction and intubation were similar between the groups. Time required to obtain orotracheal intubation was significantly higher in group B (196 ± 69 sec) than in group A (135 ± 55 sec). No apnoea (>20 seconds) or emesis were observed. No patients required additional AFX for intubation. In both group A and B HR decreased after drug administration (T0: A 110±36, B 116±22; T2: A 58±15, B56±13) as fR (T0: A 72±29, B 86±31; T2: A 22±13, B 13±7) . Both variables were not statistically different between groups but statistically lower than baseline in each group at each time point. Mean arterial blood pressure (MAP) was higher in group A than in group B (MAP A 110±20; B 89±12). Body temperature did not present any differences between group A and B. No arrhythmia was observed. Both protocols were effective in producing induction of general anaesthesia in healthy dogs, allowing intubation without significant side effects.

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BRAIN RELAXOMETRY IN VETERINARY MEDICINE: STATE OF THE ART AND FUTURE DIRECTIONS

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The term Relaxometry is referred to quantitative estimation of relaxation times T1 and T2 in the brain to relate them to disease or other biological processes [1]. In fact, T1/T2 map may reveal abnormalities non-detected by clinical scans in many disorders. In veterinary medicine literature, only reports with high field scanners are available [2,3]. The aim of this work is to present preliminary results to assess the feasibility of this technique in veterinary medicine with a low field scanner.
A test was performed on phantoms filled with a solution of different contrast agent concentrations: 5 µmol of Magnegita- Gadopentetate dimeglumine 500 µmol/ml (Agfa HealthCare Imaging Agents GmbH, Köln, Germany) in the first phantom and 5.5 µmol of the same contrast agent in the second one. The aim was to assess the feasibility of this technique to identify difference in signal intensities non detected with clinical scans. These experiments were performed with an ESAOTE VETSCAN GRANDE scanner operating at 250 mT (ESAOTE S.PA, Genova, Italy). Relaxometry data were acquired with a protocol consisting of repeated acquisitions of SE T1w images corresponding to a variable TR. In a second experiment, we acquired a set of real data by means of standard-clinical and relaxometry scans on the brain of a 9 years-old male German Shepherd with left head tilt and bilateral nystagmus euthanized because of the rapid worsening of clinical conditions. Clinical scans were performed with SE T1w, FSE REL T2w and FAST FLAIR on different planes and relaxometry data were acquired with SE T1w images corresponding to a variable TR. In the first experiment, while no statistically significant differences could be detected between the clinical scans of the phantoms, the T1 map evidenced a significant 12% difference of the signal between them. This shows that the Relaxometry approach can achieve a higher contrast as compared to the standard protocol. In the second case we found hyperintense areas at the level of pons and medulla oblongata, suspected to be inflammatory or vascular in nature. The T1 map evidenced one more hyperintense area in the vermis cerebelli that, notably, appeared homogeneous from the clinical data. This finding was confirmed by histopathological examinations which showed the presence of vasculitis in the pons, medulla oblongata and vermis cerebelli. These results based on phantom simulations and a real case study validated by histopathological exams support the development of this approach as an advanced diagnostic tool since we achieved a higher contrast compared to standard protocols, even in a clinical setting where low field MRI scanners are employed.

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ASSESSMENT OF RADIOLOGY EXAMINATION IN THE DIAGNOSTIC PROCEDURES FOR RESCUED WILDLIFE UNGULATES

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Tuscany (Italy) has a high number of wildlife animals. The demographic growth and the increament of the road network and urban areas are the reasons why the conflicts between wildlife animals and human activities increased [1]. The animals-vehicle collisions [2,3] are the main causes of wildlife rescues [4] and admission to specialized Veterinary Hospitals (VH) for the first aid procedures [3]. The aim of this study was to analize ecological data in roe deer (RD) and fallow deer (FD) admitted at 4 VH over a 3-years period (2015-2018) and to assess the benefit of using radiological study as diagnostic tool in injured wildlife animals. A total of 1282 RD and 78 FD were included. Rescue reasons and outcome were recorded. All the subjects underwent to a physical exam in order to clinically identify the presence of a traumatic injury. In 216/1360 animals (199/216 RD; 17/216 FD) a complete radiological study was performed under sedation or general anesthesia. Prevalences were calculated for all the numerical data. The main reasons for admission were car collision (582/1282, 45.4% RD; 29/78, 37.2% FD) unknown reasons (494/1282, 30.5% RD; 34/78, 43.6% FD), accidentally entrapment in nets and fences (56/1282, 4.5% RD; 8/78, 10.2% FD) and rescue of healthy fawns by people (120/1282, 9.4% RD; 5/78, 6.4% FD). The clinically recorded lesions were: multiple lesions (fractures and internal trauma) (43/199, 21.6% RD; 5/17 29.4% FD); head trauma (29/199, 14.6% RD; 3/17, 17.4% FD); front limb (8/199, 4% RD; 1/17, 5.9% FD) and hind limb (22/199, 11% RD; 3/17, 17.6%, FD) fractures; vertebral fractures (44/199 22.1% RD; 1/17, 5.9% FD), pelvic fractures (26/199, 13.1% RD; 2/17, 11.8% FD). In 27/199 (13.6%) roe deer and 2/17 fallow deer (11.8%) no clinical traumatic lesions were detected. The outcome was: 145/1282 (11.3%) RD; and 13/78 (16.7%) FD died or were humanely euthanized, 7/1282 (0.5%) RD and 3/78 (3.8%) FD were stabilized and send to wildlife rescue associations, 47/1282 (3.7%) RD and 1/78 (1.3%) FD were released in protected areas. The 15.9% of the population enrolled was submitted to a complete radiological examination and the following lesions were detected: multiple lesions (fractures and internal trauma) (43/199, 21.6% RD; 5/17 29.4% FD); head trauma (29/199, 14.6% RD; 3/17, 17.4% FD); front limb (8/199, 4% RD; 1/17, 5.9% FD) and hind limb (22/199, 11% RD; 3/17, 17.6%, FD) fractures; vertebral fractures (44/199 22.1% RD; 1/17, 5.9% FD), pelvic fractures (26/199, 13.1% RD; 2/17, 11.8% FD). In 27/199 (13.6%) RD and 2/17 FD (11.8%) no radiological lesions were detected. The complete radiological exam seems to be useful as a diagnostic tool to better assess the compound pelvic and limb fractures in which the clinical exam may underestimate or underdiagnosed bone injuries, and head trauma associated to bone fracture or not. The proper evaluation of traumas might support the decision of the veterinarian regarding the outcome of the wildlife animals in relation to their ethological behaviour.

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SONOELASTOGRAPHY EVALUATION OF NORMAL CANINE CALCANEAL TENDON: PRELIMINARY RESULTS

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Sonoelastography is a noninvasive ultrasound (US) technique that aims to assess tissue elasticity [1].
In human medicine, this technique is applied to evaluate elasticity of calcaneal (Achilles) tendon [2].
The aim of this work is to assess the feasibility of shear wave elastography to study normal calcaneal tendons in awake dogs evaluating inter operator reproducibility of this techniques.
Healthy dogs without history of muscoloskeletal disorders were selected after a complete clinical examination. Left and right calcaneal tendons were first evaluated with B-mode US with Logiq S8 sonographic system (GE Healthcare) and a linear probe of 9L, 8,5-10 MHz in the longitudinal section with a slight flexed tarsocrural joint and a gel-pad; each tendon was divided in 3 anatomical regions, enthesis, intermediate portion and superficial digital flexor myotendinous junction. Shear wave elastography were performed in each region by two operators and quantitative evaluation (m/s and kPa) were performed by both operators with 3 measurements on the most representative images. A ROI 0.15 cm was settled, and statistical parameters were calculated with MATLAB software.
Ten adult dogs were enrolled (5 mix-breed, 2 Labrador Retriever, 2 Border Collie and 1 English Setter, mean age 4.25 years). Inter operator shear wave ICC values for m/s measurements were 0.43, 0.62 and 0.62 for the enthesis, intermediate portion and in the myo-tendinous junction respectively, for kPa measurements ICC values were respectively 0.3, 0.7 and 0.8.
Our results evidenced that shear wave is a reproducible technique to assess elasticity properties of middle third and myotendinous junction; the low reproducibility of enthesis measures might be justified because it could be difficult to standardize the flexion state of the tarsocrural joint in awake patients, similarly to what has been observed in human medicine where an ankle fixator used to standardize the position of the feet improved the measurements reproducibility [3]. Further studies are needed to confirm these results.

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RADIOGRAPHICAL AGE DETERMINATION IN PUPPIES USING THE DIMENSION OF THE OSSIFICATION CENTERS: PRELIMINARY RESULTS WITH THE DISTAL RADIUS

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In the last years, the increased illegal animal trafficking has made necessary to define a more precise method for the determination of age in puppies, since their movement is allowed only after they reach 3 months and 21 days (Reg.UE N.576/2013). To date, assessment of deciduous and permanent dental eruption and appearance of bone secondary ossification centers (OCs) are the most used methods. However, both methods have too large temporal windows. The evaluation of OCs involves their appearance, mineralization and complete fusion (1). The reported studies take into consideration temporal windows related to some breeds which are extended to the entire canine population (2). This affects the final assessment and make inaccurate the age estimation since its ranges can exceed more than 15 days. Aim of the study was to evaluate the relevance of the distal radius OC (DROC) in the estimation of puppies age. The study was approved by the Ethics Committee (PG/2018/0050378) and was carried out on three litters: Cavalier King, Golden Retriever and Bull Mastiff for a total of 25 puppies (16 females and 9 males), of known age and bred in the same conditions. Each radiographic study was performed on awake subjects, starting from the 7th day up to 4 months with a mean 11-days lapse. The right forelimb was radiographed in DPa. For this preliminary reports, only the DROC was take into account: the age of appearance and the linear dimensions (width and height) on the DPa view were recorded. The area of the DROC was calculated considering the nucleus as a rectangle. Mean, median, minimum, maximum, SD and 95% Confidence Interval were calculated for each linear dimensions and for the area. The Mann-Whitney test and Spearman's rank correlation were used to assess differences between sexes and the correlation with the age respectively. P was set at <0.05. The DROC was visible in all the subjects of the sample at 27th day of life and trended to grow constantly. Statistical analysis showed, as expected, a strong linear correlation between the DROC area and the age ($P<0.0001$ =; $R^2=0,99$), while there were no significant differences between the sexes ($P=0.49$). The formula to calculate the age, derived from the linear correlation, was: $x=y/1.28+17.45$; in which x=age (in days) and y=DROC area. These results, although preliminary, demonstrated that the DROC dimensions, togheter with its age of appearance, can help in evaluating the puppies' age. The estimated age could be obtained from the abovementioned formula once the size and area of DROC have been calculated. The accuracy of the results obtained will improve increasing the number of studied subjects.

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ORAL COMMUNICATIONS

SIFTVET

ASSESSMENT OF OCHRATOXIN A EXPOSURE IN ORNAMENTAL AND SELF-CONSUMPTION BACKYARD CHICKENS: PRELIMINARY DATA

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Ochratoxin A (OTA) is a mycotoxin produced by different moulds belonging to various species of *Aspergillus* and *Penicillium*. This mycotoxin is considered to be a possibly carcinogen to humans (Group 2B as classified by International Agency for Research on Cancer) [1]. The presence of OTA has been reported in a wide range of foodstuffs, including animal-derived products. Indeed, OTA may affect the poultry industry, representing a potential health hazard for humans consuming contaminated poultry meat. Despite efforts to reduce the amount of this mycotoxin in food, a certain degree of contamination seems unavoidable [2]. The purpose of this study was to evaluate the presence of OTA in purebred chickens raised for self-consumption and/or for beauty competition in backyard farms, an important (and often overlooked) area. For this study, 52 chickens of 6-7 months of age reared in six different backyard farms were sampled in four different regions of Italy. In particular, 18 chickens were from Friuli-Venezia Giulia, 6 from Lombardy, 15 from Tuscany, and 13 from Lazio. The animals, raised using free-range method, were fed with dry and wet home-made diets consisting of cereal mix, by-products, and also common edible kitchen waste. From each animal, samples of bile and kidney were collected and stored at -20°C until analysis. OTA was detected using an HPLC-FLD method. At the start of the study, bile samples from all the animals were analysed, and 6 of them (11.5%) were positive for the presence of OTA. In particular, 2 were from Friuli-Venezia Giulia (3.85 µg/kg and 7.29 µg/kg), 1 from Lombardy (13.09 µg/kg), 1 from Lazio (22.39 µg/kg) and 2 from Tuscany (17.83 µg/kg and 170.42 µg/kg). In a second step, the kidneys of the animals whose bile was found positive for OTA were analysed, and the mycotoxin was not detected in any sample. The limits of detection (LODs) were 2.1 µg/L for bile and 0.1 µg/kg for kidneys, while the limits of quantification (LOQs) were 4.0 µg/L for bile and 0.2 µg/kg for kidneys. This study confirms the excretion and concentration of OTA in poultry bile, according to Armorini et al. (2015), and the suitability of using bile as a matrix for screening measurements of OTA in chickens. The detection of the mycotoxin in bile but not in kidneys suggests that these animals were exposed to OTA but that their meat could be considered safe, since this mycotoxin concentrates at much higher levels in kidneys compared to other tissues. In conclusion, this study that considered a relatively low number of samples shows that OTA can be present in backyard poultry farms and it would be useful to continue the research extending it to farms in other areas.

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THE EFFECT OF A RED ORANGE AND LEMON EXTRACT ON OCHRATOXIN-A INDUCED NEPHROTOXICITY IN RATS

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Ochratoxin A (OTA) is the most widespread and dangerous mycotoxin which contaminates various food commodities including grains, dried fruits, nuts, coffee, meat products, wine and beer (1). The high thermal stability makes the eradication of OTA from the food chain very difficult (2). Its sub-chronic and chronic toxicity in humans and in several animal species includes nephrotoxicity, neurotoxicity, teratogenicity, immunotoxicity and hepatotoxicity (3); however, several studies demonstrated that the kidney is the target organ (3). Since the mechanism underlying such effects remain unclear, the focus of this work was to investigate the antioxidant effects of a by-products natural Red orange and Lemon Extract (RLE), rich in anthocyanins and phenols, on OTA-induced nephrotoxicity. We have analyzed, on 24 adult Sprague Dawley rats, oxidative stress by the measurement of malondialdehyde production and by SOD and GPx parameters by ELISA Kit; renal function by clearance of inulin and histological examination by haematoxylin-eosin and Masson's trichrome staining. Statistical analyses were performed using the GraphPad Software. The rats were treated with OTA (0.5 mg/Kg b.w.) and/or RLE (90 mg/ kg b.w.) by gavage for 14 days. We demonstrated that several oxidative stress indicators were altered in the kidneys (1.5±0.04 vs 2.0 ±0.06 umol/l in GPx and 62.9% vs 100% on inhibition in SOD) coupled to a strong reduction of Glomerular Filtration Rate (GFR) (0.51±0.8 vs 0.86±0.08 ml/min), to a body weight decrease (315±22 vs 364±19 gr) and an increase of serum creatinine (1.25±0.08 vs 0.92±0.09 mg/dL) and urea levels in serum (22.8±5.25 vs 18.12±4.45 mg/dL). Histopathological examinations revealed tubular and glomerular necrosis in OTA-treated groups. Moreover, OTA treatment induced a more severe interstitial fibrosis compared to control group. Co-treatment of RLE and OTA was associated with a partially restore of fibrosis and tubular and glomerular necrosis compared to the OTA group. Treatment with RLE restored the body weight, normalized the reactive oxygen species and prevented the glomerular hyperfiltration. In conclusion, this study demonstrated that there is a strong relation between oxidative stress and OTA-induced renal injury and that RLE prevents this renal injury.

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OCCURRENCE OF OCHRATOXIN A IN TYPICAL SALAMI PRODUCED IN CAMPANIA (ITALY): PRELIMINARY DATA

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Salami is a typical Italian food product and each region produces specific types of salami, often linked with local traditions. Ochratoxin A (OTA) is a mycotoxin produced by several fungal species of the genera *Penicillium* and *Aspergillus*, and it is classified as possibly carcinogenic to humans [1]. Contamination of food commodities has been reported from all over the world [2]. The aim of this study was to carry out a monitoring action to assess the presence of OTA in artisan salamis collected in Campania (Italy). A total of 31 different salamis were randomly purchased from farms and small salami factories. For each salami, the casing was careful removed, and the outer and inner edible portions were collected. The aliquots were minced and stored at –20°C until analysis. The casing and the edible parts were then analysed separately. The extraction of OTA was performed using an acetonitrile-water mixture (80:20), while sample cleanup was carried out using Ochraprep® immunoaffinity columns. The samples were analysed by LC–MS/MS. The limits of detection (LOD) and quantification (LOQ) were 0.125 and 0.25 µg/kg, respectively. The results show that 7 salamis were positive for the presence of OTA on the casing, but only one exceeded the guidance value of 1 µg/kg established for OTA by the Italian Ministry of Health [3]. In this sample the concentration detected on the casing was 9.2 µg/kg, while in the outer and inner edible part the OTA level was 3.87 and 0.30 µg/kg, respectively. The presence of OTA on the casing in a concentration higher than in the edible portions seems to indicate that this contamination is of environmental origin and that OTA can cross the casing. Several studies have shown that OTA contamination of dry-cured meats and dry-fermented products would be largely dependent on the characteristics and the environmental conditions of the manufacturing plants, particularly with reference to temperature, relative humidity, and environmental mycoflora composition [4,5]. Some authors reported that indirect transmission of OTA from animals exposed to contaminated feed to pork products occurs rarely [5]. Hence the importance of ensuring first of all the control of the environmental conditions of the manufacturing plants, without, however, overlooking the entire chain of meat production.

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NATURAL ANTIOXIDANTS ATTENUATE AFLATOXIN B1 TRANSCRIPTIONAL EFFECTS IN A CATTLE FETAL LIVER CELL LINE (BFH12)

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Aflatoxin B1 (AFB1) is probably the most studied mycotoxin worldwide. Classified as a Group I carcinogen for humans, AFB1 recognizes the liver as the major target organ, which in turn plays a crucial role in mycotoxin bioactivation and detoxification. AFB1-contaminated feed and food commodities pose serious health problems and cause substantial economic losses; in dairy cows, AFB1 and its derivative AFM1 are mycotoxins of greater incidence, as this latter can be excreted through, and then contaminate, cow's milk and dairy products [1]. Today, a growing interest in the prevention of AFB1 using antioxidant phytochemicals (AOPs), e.g., polyphenols, and flavonoids, has been recorded [2,3]. Therefore, *in vitro* studies were performed to assess the modulatory effect of curcumin (CUR), curcuminoids (CUM), quercetin (QUE), and resveratrol (RES) on AFB1 target genes. Following cytotoxicity assays identifying the IC_{50} value for AFB1 and each of aforementioned AOP, the bovine fetal hepatocyte cell line (BFH12) was at first pre-incubated for 24 h with a cytochrome P450 (CYP) inducer (i.e., PCB126) and, then, for 16 h with 2.5, 5, and 10 µM CUR and CUM as well as to 10, 20, 30 µM QUE and RES. Lastly, cells were incubated for further 48 h with 5 µM AFB1 alone or in combination with aforementioned AOP concentrations. The possible variation (a reversal) of AFB1 transcriptional effects was assessed by using qPCR assays. Statistical data analysis was performed using one way ANOVA followed by Tukey's multiple comparison test. Overall, the tested AOPs showed a protective effect on liver cells, albeit to a variable degree. Additionally, QUE and RES were the most and the least effective AOP, respectively. Specifically, QUE, CUR, and CUM triggered a significant (P<0.01, P<0.001) reversal of AFB1 inhibitory effect on *NAD(P)H dehydrogenase [quinone] 1* (*NQO1*), and *Cu-Zn superoxide dismutase* (*Cu-Zn SOD*) mRNA levels. Comparable protective effects were also noticed for *CYP1B1* (QUE, P<0.05), and *glutathione transferase A1* (*GSTA1*; CUR and RES, P<0.05, P<0.01). Interestingly, CUR, CUM, and QUE significantly counterbalanced AFB1-dependent up-regulation of *CYP3A28* (P<0.05, P<0.01), *Mn SOD* (P<0.05, P<0.01, P<0.001) and *glutathione peroxidase 1* (*GPX1*; CUR and CUM; P<0.05, P<0.01). Present results suggest that AOPs could be used as dietary supplements to counteract AFB1 effects. Further studies (e.g., RNAseq) are envisaged to better characterize AOPs protective role and unveil other transcriptional pathways modulated by AFB1.

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POLYPHENOL PARTIAL REVERSAL OF CADMIUM INDUCED OXIDATIVE DAMAGE IN THE HEPATOPANCREAS OF CRAYFISH *PROCAMBARUS CLARKI*: PRELIMINARY RESULTS

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Cadmium is a non-essential heavy metal known to induce a broad spectrum of toxicological effects and biochemical disruptions, posing serious hazards to human and animal health. Cadmium is reported to induce oxidative stress by producing free radicals and depleting glutathione (GSH) levels, as well as reducing antioxidant enzyme activities. Among the endogenous defense mechanisms of the cell to protect itself from the damage induced by free radicals, the antioxidant enzymes such as: glutathione peroxidase (GPx), glutathione reductase (GR) and Glutathione S- transferase (GST), appear intervene in restoring the optimal quantity of glutathione [1]. Too low levels of antioxidants or inhibition of antioxidant enzymes cause oxidative stress and can damage or kill cells. Many potential drugs of plant origin have been studied to reverse the toxic effects of heavy metals, as they are rich in antioxidants capable of terminating the free radical production and thus preventing the oxidative stress. Dietary phenolic compounds are natural antioxidants regarded as potent free-radical scavengers in biological systems, capable of improving the cellular antioxidant defense system [2]. Here we present preliminary data on *in vitro* effects caused by cadmium exposure with or without the addition of polyphenols on GPx, GR and GST activities, in the hepatopancreas of the freshwater crayfish *Procambarus clarkii*. The hepatopancreas of adult crayfish was carefully removed, cut into slices and exposed to 10^{-5} M and 10^{-3} M of $CdCl_2$ in the presence (experimental) or absence (control) of two different concentrations of polyphenols extracted from barley malt and hops (10 and 100 µg/ml). After incubation of 24 h, proteins were extracted from the hepatopancreas by homogenizing the tissue in ten volumes (w/v) of cold RIPA buffer containing tissue protease inhibitor cocktail (Sigma-Aldrich, 1:500, v/v) [2]. Then, the activities of glutathione-S-transferases (GST), glutathione reductase (GR) and glutathione peroxidase (GPx) were analyzed. Hepatopancreas slice viability was determined by measuring adenosine triphosphate (ATP) levels and evaluating the genomic DNA integrity by DNA fragmentation assay. Both GST and GR activities increased from 7.1 and 0.053 U/g of protein in the control samples to 35.2 and 0.289 U/g of protein in the samples treated with 10^{-3} M of $CdCl_2$, respectively. Samples treated with 10^{-3} M of $CdCl_2$ and 100 µg/ml of polyphenols showed an increase of GST and GR activities with respect to the control (63.0 and 0.357 U/g of protein vs 7.1 and 0.053 U/g of protein, respectively). GPx activity decreased from 0.15 U/g of protein in the control samples to 0.001 U/g of protein in samples treated with 10^{-3} M of $CdCl_2$. Samples treated with 10^{-3} M of $CdCl_2$ and 100 µg/ml of polyphenols showed an increase of GPx activity like to the control (0.15 U/g vs. 0.121 U/g of protein). Our results indicate that cadmium exposure caused oxidative stress and enzyme activity impairment at the concentration of 10^{-3} M and that the damage was partially reversed by the addition of polyphenols. Although preliminary, we can hypothesize that polyphenols as feed integrators could represent a valid strategy to minimize the oxidative damage induced by cadmium, at least in crustaceans.

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EFFECTS OF NATURAL ANTIOXIDANTS ON AFB1-MEDIATED TOXICITY IN DIFFERENT CELL MODELS

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The risk of feed contamination by AFB1 has recently increased due to climate change, which is influencing the geographical pattern of both fungal growth and toxinogenesis [1]. Thus, there is an increasing interest toward new approaches to both counteract AFB1 toxicity in farm animals and reduce the contamination risk of derived products (i.e. AFM1 in milk). Some natural compounds have already proved to afford protection against some of the negative effects of AFB1 in laboratory species and broilers through the modulation of AFB1 metabolism [2]. AFB1 is mostly biotransformed in the liver; however extra-hepatic organs (e.g. the mammary gland) may play an additional role in the generation of toxic metabolites. Our aim was to evaluate the AFB1-mediated cytotoxicity, as well as its modulation by selected natural antioxidants (i.e. Curcumin – C –, Curcuminoids – CD –, Quercetin – Q – and Resveratrol – R) in different cell models: a bovine mammary epithelial cell line (BME-UV1), primary cultures of bovine mammary gland (PC), and a mouse liver cell line (AML12). PC were derived from mammary gland samples collected at the slaughterhouse from lactating dairy cows, and cultured as described [3]. All cell models were incubated with increasing concentrations of AFB1 (12 nM - 60 µM) or each antioxidant alone (0.12-50 µM) for 24 and 48hrs, in order to determine the concentrations to be used in the co-incubation experiments. To evaluate the protective effects of the antioxidants against AFB1 cytotoxicity, cells were pre-incubated with or without each antioxidant (5 µM) for 16hrs, and subsequently exposed to AFB1 (20-40 µM) in the presence or absence of each antioxidant for 24 and 48hrs. Cell viability was evaluated by the WST-1 or Neutral Red Uptake assays. Data were analyzed by ANOVA followed by Dunnett's or Bonferroni's post hoc test ($P<0.05$). As expected, results indicate that AFB1 cytotoxicity occurs in a time- and dose-dependent manner in all cell models, albeit with a different degree of sensitivity: LC50 at 24hrs is equal to 0.7 nM, 3.7 µM and 34 µM in BME-UV1, PC and AML12, respectively. The tested antioxidants afforded a significant protection against AFB1 in BME-UV1 and AML12, though no positive effects were observed in PC. Q was the most effective in both cell lines ($P<0.0001$), increasing viability of AFB1-treated cells up to 62% and 80% in BME-UV1 and AML12, respectively. R and CD were significantly ($P<0.05$) effective though to a lesser extent (enhanced viability up to 40% in both cell lines), while C significantly ($p<0.01$) protected cells (of approximately 30%) only in AML12. We can conclude that both the sensitivity to AFB1 and the capability of natural antioxidants to positively modulate its toxicity may be influenced by the target tissue, and likely relies on the expression profile of the biotransformation enzymes involved in the mycotoxin metabolism.

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PROTECTIVE EFFECT OF QUERCETIN ON METHIMAZOLE INDUCED OXIDATIVE STRESS IN A FELINE KIDNEY EPITHELIAL CELL LINE (CRFK)

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Methimazole (MTZ) is an antithyroid drug widely used in the therapy of feline hyperthyroidism to decrease the level of thyroid hormones (mainly 3,3',5-triiodothyronine, T_3). Chronic administration of MTZ is associated with several adverse effects [1]. Although the exact mechanisms underlying toxicity have not been elucidated, oxidative stress is thought to play a significant role in triggering tissue damage [2]. The oxidative stress is the result of an imbalance between the production of reactive oxygen species (ROS) and the capacity of free radical scavenging. For this reason, antioxidant substances found in plants, vegetables, and fruits may have a protective role. Among them, quercetin (Q) is a powerful antioxidant, which is able to interact with free radicals and to terminate the production of unstable molecules [3]. The aim of this study was to evaluate the protective effect of Q in a feline kidney epithelial cell line (CRFK) upon MTZ exposure. CRFK were seeded in 96-well plates (6,000/well) and after 24 hours were incubated with MTZ (4 µM) or menadione (M) (6 µM) as a positive control, in the presence or absence of Q (6 µM) for 24, 48, and 72 hrs. In order to prevent Q inactivation, the medium was replaced every day. MTZ concentration was selected according to the maximum plasma levels achieved in orally treated cats, while Q and M concentrations were chosen based on cytotoxicity curves. ROS production and cell viability were assessed by DCFH-DA and Neutral Red Uptake assays, respectively. All the experiments were performed independently three times with six replicates for each experimental condition. The results are expressed as the percentage of ROS production with respect to untreated cells (mean±SEM) and normalized on cell viability. The statistical analysis was performed by one-way ANOVA followed by Tukey's Multiple Comparisons Test. As expected, M and MTZ induced a significant ($p<0.05$ or less) time-related increase in ROS production with respect to controls at all experimental time-points (M: 294±47%, 427±68%, 615±80%; MTZ: 119±7%, 126±12%, 186±16%, at 24, 48 and 72 hrs, respectively). Conversely, the rate of ROS production in cells incubated with MTZ in the presence of Q was 89±5%, 80±4% and, 119±14% at the same time-points. The results show that the co-incubation with Q afforded a protective effect against the MTZ-induced oxidative stress (MTZ vs MTZ+Q $p<0.001$). Our findings contribute to extend the knowledge about MTZ-related side effects in a feline in vitro model and suggest that the dietary supplementation of Q could be a promising tool to limit the extent of adverse reactions during the MTZ treatment of feline hyperthyroidism.

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ANTIMICROBIALS IN FARM ANIMALS: HPLC-MS/MS DETECTION OF FOURTEEN ANTIMICROBIALS IN SAMPLES OF BOVINE AND SWINE MANURE AND AGRICULTURAL SOIL BEFORE AND AFTER FERTILIZATION

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The overuse of the antibiotics in intensive animal farming, due to prophylactic and therapeutic treatments has a relevant responsibility for the widespread of antibiotic resistance. The environmental consequences resulting from agricultural soil fertilization with drug-contaminated manure represent a critical point, since the real scenario of environmental exposure to antimicrobials is still quite incomplete [1,2]. The aim of the present study is to determine the concentrations of 14 active compounds belonging to four antimicrobial classes (fluoroquinolones, β-lactams, macrolides and polymyxin) in bovine and swine manure and in agricultural soil before and after manure application. Eleven dairy cow farms (5 based in Lombardy and 6 in Veneto) and ten swine farms (5 based in Lombardy and 5 in Veneto) were enrolled. From each farm, one sample of mass manure and two soil samples (one before and one after fertilization) were collected. All samples were analysed, according to a validated HPLC-MS/MS method for the detection of flumequine, ciprofloxacin, danofloxacin, enrofloxacin, marbofloxacin, ampicillin, amoxicillin, ceftiofur, cefquinome, erythromycin, spiramycin, tilmicosin, tylosin and colistin. In cow farms, only 1 manure sample showed a positive result with the detection of ampicillin. In swine farms, 8 out of 10 farms (80%) were detected as positive. Particularly, in 21 out of 30 samples (70%) at least one antimicrobial was detected, homogeneously distributed within manures, unfertilized and fertilized soils (7 out of 10 samples for each matrix). Within the investigated antimicrobials, flumequine was the most detected in swine farms (19 out of 21 samples, 90.5%), followed by marbofloxacin and enrofloxacin (2 out of 21 samples, 9.5% for both antimicrobials). Specifically, 19 out of 21 samples resulted with only one antimicrobial concentration above the detection limit (17 flumequine, 1 marbofloxacin and 1 enrofloxacin), whereas 2 out of 21 samples resulted with two antimicrobial concentrations above the limit (1 flumequine + marbofloxacin and 1 flumequine + enrofloxacin). Considering the preliminary results of this study, dairy cows farms may have a limited impact on the presence of antimicrobials in the environmental, while intensive swine farming is more involved in environmental contamination due to the antimicrobials use in their production. Further investigations for evaluating the presence of antimicrobial resistance gene in soil and manure may be crucial to determine the role of intensive animal farming as potential source of diffusion of resistance in the environment. Furthermore, to determine also the potential toxic effects of environmental antimicrobial contamination on non-target organisms further studies are recommended.

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SIMULTANEOUS DETERMINATION OF FOUR ANTIMICROBIAL CLASSES IN SOIL AND MANURE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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In intensive animal farming, antimicrobials are used for individual and mass treatment. Since manure is commonly used for the fertilization of agricultural soils, an environmental load of drug residues may result. The definition of this load and the following consequences on the environment are topics of increasing interest, especially the occurrence and the spread of antimicrobial resistance in the environment as consequences resulting from the soil fertilization with drug-contaminated manure [1]. To quantify the presence of antimicrobials in soil and manure from intensive farms, a reliable, sensitive and simple analytical method was developed for the simultaneous determination of different veterinary antimicrobials (fluoroquinolones, β-lactams, macrolides and polymyxin) by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). The antimicrobials detected were flumequine, ciprofloxacin, danofloxacin, enrofloxacin, marbofloxacin, ampicillin, amoxicillin, ceftiofur, cefquinome, erythromycin, spiramycin, tilmicosin, tylosin and colistin. On fortified soils and manures samples (from bovine, swine and poultry farms), a solid phase extraction was performed with Oasis® HLB cartridges, then they were analyzed by HPLC-MS/MS on a Synergi™ Hydro-RP C18 column (150mm x 2mm, 4 μm) with a binary gradient of methanol and 0.1% formic acid. The method was validated, in accordance with Decision 2002/657/EC [2] by evaluating the required parameters: decision limit (CCα), detection capability (CCβ), recovery, trueness, linearity, specificity, repeatability and reproducibility. In soil, CCα values fall into the range 5.02-10.37 ng/g for most compounds except amoxicillin and colistin (100.73 and 20.53, respectively) and the CCβ values were within a range of 5.42-10.98 ng/g for most compounds and with a value of 21.05 and 101.87 for amoxicillin and colistin. For manure, the CCα values fall into a range from 0.50 to 1.13 ng/g for most compounds and with a value of 20.04 and 100.07 for amoxicillin and colistin. The CCβ values were in a range from 0.70 to 1.34 for most compounds except again amoxicillin and colistin (20.78 and 100.97, respectively). Recoveries of the 14 analytes were between 84 and 99% in fortified soil, and between 87 and 98% in fortified manure. For all antimicrobials, precision in terms of relative standard deviation was ≤18% and ≤17% in soil and manure, respectively. All the parameters considered for the validation of the method were within the limits of acceptability, therefore it is possible to state that the proposed analytical method is appropriate for the simultaneous determination of quinolones, fluoroquinolones, β-lactams, macrolides and polymyxins in soil and manure.

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TESTING THE MUTANT SELECTION WINDOW HYPOTESIS WITH PASTEURELLA MULTOCIDA IN RABBIT EXPOSED TO ENROFLOXACIN

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Mutant prevention concentration (MPC)-based antimicrobial dosing is an attracting way to minimize the emergence of antimicrobial resistance [1]. Investigations concerning pathogens in food producing animals like rabbit are very few. This study was performed to evaluate the sensitivity of pathogenic *Pasteurella multocida* isolated from nasal swab collected from rabbits (n=7) affected by respiratory disease before and after 5 days of 24 h continuous water medication with enrofloxacin (ENRO - 10 mg/kg). Two blood samples by day and lung of 3 animals dead 1 (n=1) or 3 (n=2) days after treatment were collected. Potency of ENRO measures included Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration and MPC [2-4]. Efficacy measures included Relative Susceptibility (RS; MIC/break point of sensitivity), Bactericidal Index (BI; MBC/MIC) and Mutant Selection Window (MSW; MPC/MIC). A liquid chromatography coupled to mass spectrometry (LC-MS/MS) method was applied to identify and quantify ENRO and ciprofloxacin (CIPRO) in plasma and lungs [5]. Plasma purification was performed by liquid-liquid extraction with acetonitrile whereas the Quick Easy Cheap Effective Rugged Safe (QuEChERS) methodology was adopted for lungs. Based on the susceptibility break points (S≤0.25 □g/ml) [6] all isolates were high sensitive to ENRO (RS=0.15±0.06 s.e.m.) and a good bactericidal activity was recorded (BI=2.08±0.75 s.e.m.). ENRO MPCs revealed a significant decrease (P<0.001; Mann Whitney test) in bacterial susceptibility (RS=0.94±0.17, s.e.m.) and the MSW values ranged between 4.17 and 16.67. ENRO+CIPRO plasma concentration (from 0.109 and 0.223 □g/ml) detected in each animal exceeded the MIC of corresponding isolates but not the MPC values in six out of seven animals. ENRO+CIPRO concentration in lung tissue (1.913, 0.447 and 0.196 □g/g) was about 8 (n=1)-2.5 (n=2) fold higher than plasma concentration and was above the MICs and above (n=5) or borderline (n=2) the MPCs recorded against the tested strains. The results suggest that the conventional medication regimen of ENRO in rabbit can prevent the mutant selection in lung but not in other *P. multocida* colonized organs or tissue in which an effective drug concentration cannot be achieved.
The study was approved by CESA-DiMeV “Animal Welfare Body” (approval n° 2/16).

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ANTIMICROBIAL ACTIVITY OF SINGLE AND BLENDED ESSENTIAL OILS AGAINST PROBIOTICS AND *SALMONELLA* ISOLATES COLLECTED FROM FARMED POULTRY AND SWINE

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Antimicrobial resistance (AMR) represents a serious threat to human health, as well as to food-producing animals and the environment. AMR is a growing phenomenon, while antibiotic efficacy against human and zoonotic infections is decreasing [1]. Salmonellosis is the 2nd most common zoonosis in Europe, and recent studies highlight multidrug resistance (MDR) of numerous *Salmonella* strains to the most commonly used antimicrobial agents [2]. EOs could meet some of the strategic objectives outlined in the WHO's Global Action Plan on AMR. *Salmonella* is the most frequently isolated bacterial agent in food-borne infections. Food-producing animals are the main reservoirs of infection, with animal-derived foods (meat, eggs and milk) sources of transmission.
The aim of the study was to evaluate antibacterial efficacy of single EOs (*Lavandula intermedia* and *Origanum vulgare)* and of GR-OLI (25% commercial solution of an unknown mixture of *Eucalyptus globulus, Satureja montana, Citrus aurantium* var. *dulcis, Thymus vulgaris, Melaleuca alternifolia, Citrus limon, Lavanda hybrida, Melaleuca cajeputi, Thymus capitatus*) against *Salmonella* (29 strains) isolated from swine and poultry farms and against beneficial microorganisms used as probiotics, including *Saccharomyces cerevisiae, S. boulardii, Enterococcus faecium* and *Bifidobacterium thermoacidophilum* (6 strains). EO efficacy was evaluated through MIC analysis according to EUCAST guidelines [3]. The action of scalar concentrations between 2% v/v and 0.06% v/v for OEs and between 8% v/v and 0.25% v/v for GR-OLI was evaluated. Moreover, the EOs with sub-MIC values capable of reducing bacterial growth were tested in CaCo-2 cell adhesion and biofilm experiments using *Salmonella* (12 strains).
GR-OLI and *O. vulgare* proved more effective than *L. intermedia* against multidrug-resistant *Salmonella* strains. The EOs evaluated had higher MIC values against probiotics than against *Salmonella*. GR-OLI also decreased biofilm formation and reduced adhesion of *Salmonella* strains to CaCo-2 cells. GR-OLI showed higher efficacy against multidrug-resistant *Salmonella* strains than against drug-sensitive *Salmonella* strains. Our data on the evaluated EOs require further investigation and confirmation, also exploring the possibility of resistance development. However, sub-MIC doses of GR-OLI could prove to be an interesting strategy for reducing Salmonella presence in intensive swine and poultry farms.

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POSSIBLE APPLICATIONS OF OXIDATIVE STRESS EVALUATION IN BLOOD OF AVIAN SPECIES: A PRELIMINARY STUDY

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In physiological conditions, an equilibrium between pro- and anti-oxidant factors exists; when the former exceed the capacity of their removal/inactivation, Oxidative Stress (OS) occurs [1]. The OS level in blood of selected bird species was assayed, by measuring the Plasmatic Antioxidant Activity (PAT – mmol/L ascorbic acid), and the oxidative plasmatic potential (D-ROMs – mmol/L H_2O_2); the OS Index (OSI) was then obtained (D-ROMs/PATx1000). In this preliminary study, blood samples were collected from 18 healthy chickens (*Gallus gallus domesticus*), sampled during usual health monitoring (9 from industrial and 9 from rural organic farming), and from 18 clinically healthy wild birds (*Pica pica*), captured as part of an ornithological study (authorized with ISPRA Prot. 8093/T-A31 of 02/21/19 and Region ER Det. 3751 of 01/03/19). For OSI test, blood was analysed using FRAS-5 analytical system (H&D); furthermore, a haematological evaluation was carried out with a haemocytometer (Natt & Herricks solution staining), and on blood smear (Diff-Quick staining) for differential count. Statistic evaluation was performed by Mann-Whitney test, and values were expressed as means±SD. In *G. gallus*, OSI was significantly higher (P=0.011) in subjects from intensive farming (14.7±7.10) than in those bred in rural conditions (5.64±10.32). In *P. pica* group mean OSI value was 8.14±4.61; in this species, a possible correlation between WBC count and OS was found, since OSI values were significantly higher (P=0.0073) in subjects with WBC>20x10^3/dl with respect to those with WBC<20x10^3/dl. Obtained results suggest the occurrence of higher OS levels in chickens bred in intensive conditions, compared to rural farming, which could be related to management methods and productivity levels. In addition, in *P. pica* the higher OSI values measured in subjects with WBC>20x10^3/dl may indicate a correlation between OS level and immune response, as previously observed [1]. Collectively, present data open up good prospects for the application of OSI measurement both in avian medicine and in animal welfare monitoring; moreover, a possible utility of this technique in the field of ecopathology could be suggested, since previous evidence exists about indirect monitoring of pollution levels through the evaluation of OS in bioindicator species [2,3,4].

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USE OF MUSSELS TO MONITOR CONTAMINATION OF THE NORWEGIAN FLEKKEFJORD FJORD: PRELIMINARY DATA

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Mussels are filter-feeder organisms that accumulate contaminants with few metabolic transformations [1] [2]. Thus, they are considered suitable sentinels species to monitor the presence in the environment of various persistent bioaccumulative and toxic (PBT) substances [3] [4]. In Norwegian fjords, salmon fishing and farming are a major economical resource hampered by the contamination of the marine sediment, favoured by the the seabed low depth. Flekkefjord fjord suffered the input of several contaminants, including polychloribyphenils (PCBs) and polycyclic hydrocarbons (PAHs), which lead to its inclusion in the list of the 18 Norwegian sites of National interest for recovery. In the present study, mussels were used to monitor contamination by PAHs, ICES-6 non dioxin like PCBs, polybrominated diphenyl ethers (PBDEs), organochlorine and organophosporous pesticides in Flekkefjord fjord. Five sites were selected at different distances from the bottom of the fjord and suspended ropes were placed at two different depths (5 and 15 m). A pool of 50 mussels of *Mytilus edulis* was collected in each site just after placing the rope (control) and after 1 month (t1) to monitor a possible bioaccumulation of contaminants. Samples were extracted by a QuEChERS method and analysed by GC-MS/MS. PBDEs were not detectable in all samples. Hexachlorobenzene and DDE were found in 7 control and 5 t1 samples below limit of quantification (LOQ; 1 ng g^{-1}). Traces of transchlordane and phorate were detected in t1 and control samples, respectively. PCBs were detected in all samples with lower levels in control [<LOQ (1 ng g^{-1}) to 6.99 ng g^{-1}] than t1 samples (<LOQ to 25.9 ng g^{-1}) with a different footprint between the two groups. Benzopyrene and benzofluoranthene concentrations were both similar to each other and at the two sampling times (3.09-3.58 ng g^{-1}). These results are preliminary to further data that will be collected following the planned sediment remediation works. In fact, such works will possibly worsen water pollution, due to reshuffling, thus negatively affecting the food chain of the fjord with a particular impact on the salmon production.

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POLYCHLORINATED BIPHENYLS AND ORGANOCHLORINE PESTICIDES IN DONKEY MILK FROM SOUTHERN ITALY

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Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are lipophilic organochlorine compounds (OCs) widely employed in the past that can still be detected in abiotic and mainly biotic matrices of the ecosystems, due to the high bioaccumulation and biomagnification along the food chains [1]. For about 90% of cases, the daily human exposure to these compounds is due to the consumption of fish, meat, and dairy products [2]. Often used as substitute for maternal milk as well as for the nutrition of elderly and children with pathologies, such as allergies and food intolerances [3], donkey milk is among the foodstuffs potentially contaminated by OCs. In the present study, we analysed the presence of several OCs in the milk of donkeys reared in farms of the Southern Italy (Caserta, Salerno, Cosenza and Potenza provinces). Concentration levels of 4 OCPs (*p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD, overall denoted with ΣDDT, and HCB), 18 NDL-PCBs, where NDL stands for Not Dioxin Like, among which the six indicator congeners, 3 non-ortho DL-PCBs (77, 126, 169), and 5 mono-ortho DL-PCBs (105, 118, 156, 157 and 167) for 56 samples, from Caserta, Salerno, Cosenza and Potenza provinces, were determined. The 8 DL-PCBs analysed are a subset of the 12 DL-PCBs for which, together with 16 dioxins, the EU legislation set maximum levels relative to a number of foodstuffs. Sample units underwent a liquid/liquid manual extraction. The cleaned extracts were analysed using a HRGC/LRMS equipment. The mass spectrometer operated in EI mode and a SIM program was constructed for acquisition and quantification. The results showed the prevalence of the NDL-PCBs; overall the lowest and highest values of their mean concentration, related to Caserta and Cosenza samples, respectively, were 93.13 and 353.15 ng g^{-1} on lipid weight (lw) basis. These numbers were instead 75.84 and 271.85 for the the six indicator PCBs, 3.4 and 42.45 for the DL-PCBs. For any province, the concentration level of OCPs was quite low, with the highest value of ΣDDT for the Potenza sample (54.95 ng g^{-1}). The p,p'-DDE was detected in all provinces but Salerno. Regarding the risk evaluation, six sample units from Salerno were characterized by concentrations of the DL-PCBs exceeding the MRL for the EU (expressed as WHO-PCDD/F-PCB-TEQ using the WHO-TEFs). Such MRL is 0.11 pg g^{-1} fixed on a wet weight basis for milk containing less than 2% fat, as in our samples. The results obtained show a potential risk for human health.

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DELAYED ACUTE TOXICITY OF TWO VETERINARY FLUOROQUINOLONES IN *DAPHNIA MAGNA*

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Previous toxicity tests on *Daphnia magna* embryos showed high mortality rate and reproduction inhibition in apparently healthy daphnids returned to pure medium after embryonic exposure to the veterinary fluoroquinolones (FQs) Enrofloxacin (ENR) and Flumequine (FLU) [1,2]. In this study, after conducting the standard 48-h acute immobilisation test on *D. magna* neonates [3] with the two compounds, the survived daphnids were returned to pure medium and followed-up for two weeks in order to check their ability to survive, grow and reproduce. The experiment was run in order to evaluate if the standard immobilisation test, which does not take into account delayed acute toxicity, may overestimate the EC_{50s} of FQs. For each compound, eight concentrations were assayed in the range 40-0.7 mg/L. Four groups of 5 young daphnids were exposed to each concentration or used as controls. After 48-h, immobilised individuals were counted and discarded, while the healthy ones were returned to pure medium, to be regularly fed and followed-up in order to check their survival and reproduction ability. After first clutch delivery (day 12) they were collected, fixed in ethanol (70%), and their length measured, to evaluate daily growth. Both FLU and ENR were able to elicit delayed toxicity after 48-h exposure of *D. magna* neonates. At 48-h, immobilisation rates were in the range 0-75% (ENR) and 0-85% (FLU) and the calculated EC_{50s} were 16.72 mg/L (ENR) and 25.35 mg/L (FLU). However, during the following days all the individuals exposed to the highest concentrations (22.2 and 40 mg/L) died, while those exposed to 12.3 mg/L reached a mortality rate ≥80%, and showed a zero reproductive capacity of the few survived individuals. At lower concentrations, these delayed effects were still present but much attenuated. Severe effects on body length were also detected, randomly, in some specimens, which were usually unable to produce any eggs. Overall, results have shown that the standard immobilisation test, by neglecting delayed toxicity, leads to a not negligible overestimation of the acute EC_{50} of FQs. For example, Robinson et al. [4] reported no acute effects at 10 mg/L exposure for FLU and ENR while at the end of our study an EC_{50} <10 mg/L was calculated for both compounds. As EC_{50} is important for regulative purposes, we suggest that a follow-up should be added to the acute immobilisation test guideline, in order to allow the detection of delayed effects.

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CONFIRMATORY STUDIES ON THE TRANSCRIPTIONAL REGULATION OF BOVINE *CYP3A28* GENE

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Several transcription factors, including the pregnane X-receptor (*PXR*) and the constitutive androstane receptor (*CAR*), contribute to the complex regulation of human cytochrome P450 3A (CYP3A), representing the main CYP isoform in human liver [1]. Three genes (*CYP3A28*, *CYP3A38* and *CYP3A48*) have been identified in bovine *CYP3A* locus, but molecular mechanisms involved in bCYP3A regulation are still unclear [2]. We have identified the *CYP3A28* proximal promoter and a distal fragment (-6899/-4937 bp, F3) as regions responsive to bPXR and bCAR; moreover, two motifs (an ER6 and a DR5 motif) seem to be involved in bPXR and bCAR transactivation, respectively. In the present study, chromatin immunoprecipitation (ChIP) assays were performed in a bovine fetal hepatocyte cell line (BFH12) to confirm the relevance of ER6/DR5 motifs in *CYP3A28* regulation. Preliminary time- and dose-dependent *CYP3A28* induction studies were made using prototypical human or mouse *PXR/CAR* ligands, i.e. dexamethasone, PCN, mifepristone (RU486), SR12813, CITCO, and FL81. ChIP experiments were then executed on dimethylsulfoxide (DMSO), RU486- and FL81-treated BFH12 cells. First, the cross-reactivity of anti-hCAR, anti-hPXR and anti-human retinoid X receptor alpha (*RXRα,* the PXR/CAR heterodimer partner) antibodies was assessed by immunoblotting. Then, nuclear chromatin was sonicated and immunoprecipitated using the aforementioned antibodies. Finally, precipitated DNA fragments were amplified in qPCR with specific primers flanking ER6 and DR5 elements. Each antibody recognized appropriately sized and localized proteins in untreated bovine liver cytosolic and nuclear extracts, as confirmed by stable hCAR- and hPXR-expressing hepatocytes (positive controls). ChIP assays in DMSO- and RU486-treated cells showed that RU486 weakly increased the recruitment of RXRα, for the binding to ER6. It was not possible to reliably detect binding of PXR, probably due to its low expression in BFH12 cells. As regards DR5, ChIP experiments suggest this motif could recruit both bCAR and bPXR; DR5 is the main DNA binding site recognized by CAR, and the immunoprecipitation of both PXR and RXRα was qualitatively and slightly increased by RU486. Unfortunately, FL81 (the chosen CAR ligand) did not affect CAR-mediated immunoprecipitation of DR5. Our data would confirm the presence of two regions potentially responsive to bPXR in *CYP3A28* gene promoter (the proximal ER6 motif and the F3 distal element containing DR5). Moreover, ChIP results would suggest that bCAR is recruited to the DR5 element; nevertheless, further molecular studies are needed to confirm ChIP results here obtained.

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VALIDATION OF COMMERCIAL IMMUNOASSAY KIT FOR THE DETERMINATION OF DRUGS IN EQUINE PLASMA

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The use of local anesthetics and corticosteroids in racehorses can configure the practice of doping when they are intentionally used to conceal pathologic conditions [1]. Anti-doping test for drugs in racehorses includes screening test followed by a chromathographic confirmatory method. The most commonly used screening method is the enzyme linked immunoassorbent assay (ELISA), because of its sensitivity, rapid time of execution and relative low cost. ELISA kit are available on the market to detect several drugs in equine urine and plasma.

The aim of this study was the intra-laboratory validation of commercial ELISA kits for the determination of the local anesthetics lidocaine and mepivacaine and the corticosteroids triamcinolone and dexamethasone in equine plasma samples. ELISA kit were purchased from Neogen (Lexington, KY, USA). Validation parameters were: sensitivity (IC_{50}), limit of detection (LOD), intra and inter assay coefficient of variation (CV), cross-reactivity (CR) with interfering molecules and background concentrations (calculated on 40 drug-free samples).
Lidocaine ELISA kit validation: IC_{50} = 0.78 ng/ml; LOD = 0.12 ng/ml, intra-day and inter-day CV = 3.0% and 6.7%, respectively. CR for mepivacaine, bupivacaine and ropivacaine were 2.29%, 0.55% and 0.47%, respectively. The highest background concentration (0.10 ng/mL) was below the IC_{50} of the standard curve. **Mepivacaine** ELISA kit validation: IC_{50} = 12.01 ng/ml; LOD = 0.80 ng/ml; intra-day and inter-day CV = 3.5% and 5.1%, respectively. CR for lidocaine, bupivacaine and ropivacaine were 100%, 100% and 71%, respectively. Mepivacaine ELISA kit did not discriminate between local anesthetics. The highest background concentration (1 ng/mL) was below the IC50 of the standard curve. **Triamcinolone** ELISA kit validation: IC_{50} = 4.60 ng/ml; LOD = 0.37 ng/ml, intra-day and inter-day CV=12.4% and 21.0%, respectively. CR for dexamethasone, and methylprednisolne were 4.0% and 1.0%, respectively. The highest background concentration (0.10 ng/mL) was below the IC50 of the standard curve. **Dexamethasone** ELISA kit validation: IC_{50} = 6.23 ng/ml; LOD = 1.24 ng/ml; intra-day and inter-day CV=11.1% and 22.2%, respectively. CR for triamcinolone and methylprednisolne were 1.0% and 0.4%, respectively. The highest background concentration (0.05 ng/mL) was below the IC_{50} of the standard curve.
In conclusion, validation data for lidocaine and dexamethasone commercial ELISA kit obtained in this study agree with those reported by manufacturer [2], while for mepivaciane and triamcinolone commercial ELISA kit the comparison is not possibile because validation data reported by manufacturer refers to buffer only.

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EVALUATION OF FOUR COMMERCIAL ELISA KITS FOR THE DETECTION OF CANINE AND EQUINE PROCALCITONIN

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Procalcitonin (PCT), the precursor of the calcitonin hormone, is a small protein (13 kDa) that is synthesized by the C-cells of the thyroid glands. PCT concentrations in plasma of healthy subjects are very low, but they increase rapidly in response to a pro-inflammatory stimulus. PCT is markedly elevated in severe forms of systemic inflammation or in bacterial infections, and this condition persists until recovery. For these reasons PCT is considered an efficient diagnostic biomarker for sepsis in humans and other animal species, such as horses and dogs. Equine PCT has a homology within the amino acid sequence to the human and canine PCT of 83% and 73%, respectively [1]. Dog PCT has a homology to the human PCT of 67% [2]. Several commercial ELISA Kit for the determination of PCT are available: Recombinant Canine Procalcitonin ELISA Kit (rcPCT, Biovendor), Canine Procalcitonin ELISA Kit (cPCT, TSZ ELISA), Equine Procalcitonin ELISA Kit (ePCT, Mybiosource), human Procalcitonin ELISA Kit (hPCT, Sigma Aldrich). The aim of the present study was the evaluation of four commercial ELISA Kit for the detection of PCT in canine and equine plasma samples.

Plasma from 10 dogs (5 healthy and 5 septic) and from 10 horses (5 healthy and 5 septic) was collected and stored at −80°C. The ELISA assays were performed according to the manufacturer's instructions. The optical density (OD) of the samples was determined with a microplate reader. Statistical differences were evaluated with T-test. Correlation analysis was performed with Spearman test. A p value ≤ 0.05 was considered significant.

Canine PCT: PCT concentration measured by using rcPCT ELISA Kit in plasma samples of healthy and septic dogs was in the range of <LOD-40 pg/ml and 96-902 pg/ml, respectively ($p \leq 0.05$; LOD=3.6 pg/ml). On the contrary cPCT, ePCT and hPCT ELISA Kit were not able to detect canine PCT. **Equine PCT:** PCT concentration measured by using ePCT ELISA Kit in plasma samples of healthy and septic horses was in the range of 28-50 pg/ml and 148-203 pg/ml, respectively ($p<0.05$, LOD=10 pg/ml). PCT concentration measured by using hPCT ELISA Kit in plasma samples of healthy and septic horses was in the range of <LOD-92 pg/ml and 112-420 pg/ml, respectively ($p<0.05$; LOD=10 pg/ml). Furthermore, Spearman test revealed a significant positive linear correlation between PCT concentration measured with ePCT and hPCT. On the contrary, both cPCT and rcPCT ELISA Kits were not able to detect equine PCT.

In conclusion, the results of the present study do not support the use of the cPCT ELISA Kit for the detection of PCT in dogs, whereas rcPCT ELISA Kit seems to be a more suitable assay. Moreover, both ePCT and hPCT ELISA Kits are able to determine PCT concentrations in equine plasma samples.

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ORAL COMMUNICATIONS

SIRA

TIMED ARTIFICIAL INSEMINATION IN MILK-PRODUCING JENNIES

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Estrus synchronization and timed artificial insemination (TAI) are commonly used in order to improve genetic selection and to manage big herds of livestock without estrus detection [1]. Despite a raising interest for donkey species [2], at the best of our knowledge only one paper reports results after TAI in jennies [3]. The aim of this study was to describe the outcome of two protocols for TAI in milk-producing jennies based on the use of PGF and hCG. Ninety lactating or not lactating Amiata jennies, aged 3-21 years, were submitted to AI using a dose of $1X10^9$ spermatozoa diluted in INRA 96 and stored at 15°C for a maximum of 6 hours from collection. AI was performed at the moment of ovulation induction with hCG. In protocol 1 (SHORT), 54 jennies were treated with PGF on Day 0 and submitted to the reproductive tract ultrasounds (US) on Day 7: those evaluated to be in heat (follicle ≥ 28 mm and absence of CL) were submitted to AI and to ovulation induction, while the others were treated with PGF on Day 14 and re-submitted to the US on Day 21, when they were inseminated and submitted to induction of ovulation, if in heat. In protocol 2 (LONG), 36 jennies were treated with PGF on Day 0 and submitted to the US on Day 7. Those in heat were submitted to AI and ovulation induction, while the others were treated with PGF. This protocol was repeated weekly for 10 weeks. In the SHORT protocol, the percentage of jennies inseminated/treated, pregnant/inseminated and pregnant/treated were 76%, 56% and 43%, respectively, while in the LONG protocol, the same percentages were 94%, 47% and 44%. The lactation or not lactation status as well as jennies' age class had no effects on the previous parameters ($P>0.05$). In this study the pregnancy rates of the two protocols were fairly similar, so it seems to be unpractical to adopt the LONG protocol that is more time consuming and requires more animal handling in order to obtain the same results. In conclusion, these results indicate that it is possible to achieve reasonable pregnancy rates in jennies using TAI with a short-time stored fresh semen and reducing at the minimum the animal handling.

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POSTPARTUM UTERINE INVOLUTION IN MARTINA FRANCA JENNIES

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Because of the scarce knowledge about postpartum (PP) uterine involution in donkeys, the present study was aimed to investigate the PP uterine involution in Martina Franca jennies evaluated by ultrasonography and histology. The study was performed on 9 multiparous jennies with a normal, singleton foaling, at term. Transrectal ultrasonography was performed the day after foaling, at 3, 7, 14, 21 and 28 days postpartum (PP). The cross-sectional diameter was measured in the post pregnant (PPH) and non-post pregnant uterine horns (NPPH) at the level of the tip (T), middle (M) and corpora-cornual junction (CCJ) regions. The ovaries were also scanned to assess the first PP ovulation. At the same times, also endometrial biopsies were performed for the computerized evaluation of epithelial thickness (ET), for microcaruncolar (MC) and glandular (GL) area and perimeter, and for GL number assessment. The interval between parturition and foal heat was also recorded. Possible differences of ultrasonographic or histologic data among all sampling times were statistically evaluated by ANOVA for repeated measures. According to ultrasonographic records, the mean T diameters did not differ significantly between PPH and NPPH at any time, whereas the mean M diameter of the PPH (day 1, 76.0±0.80 mm; day 3, 71.6±0.70 mm; day 7, 62.6±0.70 mm) was larger than the NPPH (day 1, 59.1±0.80 mm; day 3, 56.7±1.10 mm; day 7, 54.0±1.40 mm) only until day 7 ($P<0.05$). The diameter of the CCJ in the PPH was not measurable until day 3 PP, then the mean diameter of the CCJ resulted larger in the PPH (96.3±1.45 mm) in comparison to NPPH (77.8±1.40 mm) only at day 7 ($P<0.05$). The foal heat appeared on average 6.5±0.9 days PP, and ovulation occurred 11.9±1.3 days PP. The histology showed that the mean ET significantly increased from day 1 (1043±270 µm) to 3 and 7 days (1257±430 and 1278±411 µm, respectively) ($p<0.001$), and again to day 14 (1380±413 µm) ($p<0.001$), concurrently with the foal heat, with a subsequent decrease at 21 and 28 PP day, returning to thickness similar to 3 and 1 PP day, respectively, during the diestrus. Mean MC perimeter and area at day 1 were 40731±13651 µm and 130787±76562 µm2, respectively, showing clear signs of cariorexis and cytoplasmatic vacuolization, as indicators of involution, on day 3. Endometrial mean GL area and perimeter showed a significant ($p<0.001$) constant trend of decrease from 1 to 28 days, failing to provide suitable data about uterine involution. On the other hand, in comparison to day 1 (GL mean number=989), an increase was seen from day 7 (n=1840), with the highest value at day 21 (n=4227). On the base of the overall ultrasonographic (disappearance of significant differences among uterine sections measurements) and histologic findings (significant last increase of ET), the present study results suggest that, in the observed group of 9 Martina Franca jennies, the PP uterine involution can be considered complete at 14 days PP, earlier than the 22.5±1.7 days reported for the French donkey [1], and, differently to what reported by [1], without a significant influence of the time of foal heat appearance.

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POST-THAWING PARAMETERS OF DROMEDARY CAMEL (*CAMELUS DROMEDARIUS*) EPIDIDYMAL SPERMATOZOA SUPPLEMENTED WITH SEMINAL PLASMA

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Seminal plasma (SP) may improve parameters of frozen-thawed spermatozoa. Aim of the study was to evaluate the effects of the addition of 15% SP on dromedary camel (DC) frozen-thawed epididymal spermatozoa. Two ejaculates were obtained from two fertile males, and the SP recovered (centrifugation at 10,000 x g for 30 min), mixed in equal amount, and stored at -80 °C. Spermatozoa were collected from epididymis through retrograde flushing from 14 testes, collected by orchiectomy and kept 24 hrs at 4°C [1]. Samples were divided in two aliquots and diluted with extenders (Tris 268.28 mM/L, Citric Acid 79.7 mM/L, Lactose 152.64 mM/L, Glucose 27.75 mM/L) with (15%SP V:V) and without seminal plasma (C). Following dilution (conc 50x106 sptz/mL, 4% Glycerol) and equilibration (180 min), total and progressive motility (TM, PM) were evaluated and a cut-off value of 40% PM was used to select samples for freezing procedures (2 x 13). Samples were placed in 0.5 mL paillettes and exposed to liquid nitrogen vapours (15 min at 4 cm) followed by immersion. After thawing (46°C x 20 secs), TM and PM, membrane integrity/viability (eosin/nigrosin), and membrane functional integrity (HOS-test) were evaluated. Data were analysed with Wilcoxon test and P value was set at 0.05. Pre-freezing TM was higher in samples with 15% SP addition: 69.39±2.42% (C) vs 73.90±1.80% (15% SP) (P=0.045) whereas PM did not show statistical differences 52.00±1.58% (C) vs 54.03±1.68% (15% SP) (P=0.248). The freezing procedure induced a drop of TM and PM but the two treatments did not statistically differ. TM: 22.09±1.32% (C) vs 24.22±1.37% (15% SP) (P=0.279); PM: 9.99±0.61% (C) vs 10.20±0.85% (15% SP) (P=0.701). The SP addition reduced post-thawing sperm membrane viability but improved membrane functional integrity; viability: 68.30±2.02% (C) vs 54.10±2.20 (15% SP) (P=0.001); membrane functional integrity 44.19±2.45% (C) vs 52.72±1.67 (15% SP) (P=0.013). As observed by other authors, the SP may have induced the pre-freezing sperm activation [2] whereas the post-thawing effects on viability and membrane functional integrity could have been caused by the dilution of extender components with protective SP proteins that, in low amount, may have protected the sperm membrane functionality [3,4]. Analysis of seminal plasma and the evaluation of different concentration added to extenders will clarify the benefit of using seminal plasma in dromedary camel semen freezing procedures.

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EFFECTS OF TEMPERATURE (15°C VS 25°C) DURING 48-H OOCYTE HOLDING ON BLASTOCYST DEVELOPMENT AFTER ICSI IN THE HORSE

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In vitro production of blastocysts by intracytoplasmic sperm injection (ICSI) is an important tool in both clinical and research activities. In clinical equine ICSI programs, holding oocytes at room temperature (25°C) overnight allows shipment of immature equine cumulus-oocyte complexes (COCs) from the mare's location to the ICSI laboratory [1]. Furthermore, oocyte holding aids the scheduling of oocyte in vitro maturation (IVM) procedures. This overnight holding (generally for 18 hrs) before placement into maturation culture has no detrimental effect on meiotic and developmental competence [2]. However, in some instances, longer shipping or holding periods are required. The aim of the present study was to determine whether prolonged oocyte holding time before IVM was possible, and whether reduced temperature might be beneficial during this time. Cumulus-oocyte complexes (COCs) recovered from the ovaries of slaughtered mares were held at 15°C or 25°C for 48 hrs in Earle's/Hank's' M199-based medium (EH) and cultured for IVM as previously described (2). Sperm preparation via swim-up, piezo- ICSI, and 2-h post-ICSI oocyte culture were conducted in CZB media (2). Embryos were cultured in DMEM/F-12 with 10% FCS for up to 10 days in an atmosphere of 5% O2, 5% CO2 and 90% N2 (2). Rates of maturation to metaphase II (MII) and blastocyst formation were recorded. Blastocysts were confirmed after staining with Hoechst 33258 as containing more than 64 nuclei with organization of an outer presumptive trophoblast layer. Data was analyzed by Chi-square test, with Fisher's exact test used when a value less than 5 was expected in any cell. The rates of in vitro maturation did not differ significantly between 15°C and 25°C groups (28/59, 47% vs 26/51, 51%, respectively). Interestingly, 4 blastocysts out of 28 injected oocytes (14%) were obtained in the 15°C group, whereas only 1 blastocyst out of 26 injected oocytes (3.8%) was obtained in the 25°C group, although this difference was not significant. Our study demonstrates that in vitro production of equine blastocysts can be achieved after 48 hrs of oocyte holding before maturation, at temperatures as low as 15°C. The possibility to use holding periods up to 48 hrs would ease shipping procedures around the world. Further studies are necessary to investigate additional quality parameters of embryos derived from oocytes held at 15°C *vs* 25°C.

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STUDY OF DOPPLER SONOGRAPHY OF CORPUS LUTEUM DURING THE BOVINE OESTRUS CYCLE

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Doppler ultrasound has recently emerged as one of the main innovations in cattle practice and has mainly found application in the evaluation of luteal blood perfusion (LBF). It has been evaluated during different phases of the oestrous cycle, but the technique is poorly standardized [1-3]. Aim of the study was to evaluate visual and quantitative changes of size of corpus luteum (CL) and LBF in dairy cattle during an oestrous cycle at 2 follicular waves using colour flow mode (CFM) and power flow mode (PFM) doppler ultrasound. The study was preliminary approved by the Ethics Committee of the Department of Veterinary Sciences of the University of Messina (reference number 010/2016). Ten Friesian cows were selected and synchronized. The CL was evaluated at 3/4-day intervals until the next oestrus onset. The emergence of the 2 follicular waves was also monitored. After identifying the spiral luteal artery entry at the base of the CL, multiple scans of the vertical plane at the maximum diameter of the CL from the apex to the base were recorded in B-mode, CFM and PFM for each cow and session. An Esaote mylab 30 gold was employed with the following setting: pulse repetition frequency 2.1 (CFM) or 2.8 (PFM), gain 70%. The real area of CL (RACL) was calculated subtracting the area of the eventual inner cavity. The LBF was quantified off-line by means of a visual score and of an image analysis system (Digimizer 4.1) and expressed as percentage of coloured area on RACL. Student and ANOVA tests were used for statistical analysis. Data were presented as means and standard deviations. There were significant individual variations for the RACL and LBF, calculated in CFM. PFM was the most accurate method for evaluating LBF, although, at day 15-16, it was also affected by individual factors ($p<0.01$). The study of RACL and LBF during a 2-wave oestrous cycle distinguished 3 phases: a phase of CL formation (3-4 days) with LBF of 0.3±0.3 cm2 on a RACL of 3.1±0.9 cm^2, a central phase (7-16 days) with LBF of 0.7±0.4 cm2 on a RACL of 4.8±0.8 cm^2 and a regression phase (19-20 days) with a minimum LBF of 0.1±0.1 cm^2 in a CL of 3.3±0.6 cm2. Visual score allowed easily differentiating these phases being powerful to recognize non-functional CLs (no blood flow) with relevant clinical applications. According to this study, there is no significant difference of LBF from 7 to 16 days of the oestrus cycle; although the RACL significantly changes in this period, the dimensional variations are too thin to be clinically appreciated. However, this study contributed to standardize the technique and to define the values of RACL and LBF during a 2-wave oestrous cycle of dairy cattle.

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IMPROVEMENT OF EMBRYO RECOVERY IN HOLSTEIN COWS TREATED BY INTRA-OVARIAN PLATELET RICH PLASMA BEFORE SUPEROVULATION

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Reproductive strategies aimed to stimulate the early follicular growth or inhibit follicular atresia would be beneficial for priming the ovarian follicles reserve in donor cows enrolled in superovulatory programmes. In this context, hormonal or physical treatment have been largely employed with the main goal of reducing the inhibiting action of the dominant versus subordinate follicles but none of these treatments have been able to influence the recruitment process of the small ovarian follicle turnover [1]. An alternative method to prepare bovine donors before superovulation could be the administration of regenerative elements directly into the ovary, with the aim to stimulate small follicles recruitment, which in turn will allow for the presence of follicles of the appropriate size responsive to the exogenous gonadotrophin administration. Platelets rich plasma (PRP), indeed, contain many factors known to inhibit apoptosis and fibrosis, enhance angiogenesis and stimulate mitosis at level of granulosa cells that are necessary during follicular development. The aim of the present study was to investigate if intra-ovarian administration of PRP before superovulation could increase the number of follicles responsive to the FSH/LH treatment in order to improve embryo recovery from donor cows. Eight Holstein-Friesian cows, 3 to 4 years old, with a history of normal fertility, were enrolled in this study. To produce PRP, whole blood from the mammary vein of each cow was collected into blood collection bags containing CPDA-1. The whole blood was centrifuged at 100xg for 30 min to allow supernatants plasma collection that underwent a further centrifugation at 1500xg for 10 min. The resulting platelet pellet was diluted with the plasma of each cows to obtain a concentration of 1x109 platelet/ml. Two days before a luteolytic PGF2alfa dose, in each animal the right ovary was considered as control while the left one was injected by ultrasound guidance with 5 ml of autologous PRP. Nine days after induced oestrus, all cows were superovulated with 50 mg Folltropin administered i.m. in ten decreasing doses and inseminated twice by the same cryopreserved semen. Seven days after AI, right and left uterine horns were separately flushed. All data were evaluated by Student's T-test. By ultrasound evaluation, before PRP treatment, the average number of follicles, on the right ovaries was 9.18±1.35 and on left ones 7.32±1.67 ($P>0.05$). Two days post-treatment with PRP, the average number of follicles on control ovaries was 7.67±2.52 and in treated ovaries 8.00±2.00 ($P>0.05$). At the last Folltropin injection, i.e. onset of proestrus, in the control ovaries the average of follicles was 11.33±2.89 and in treated ovaries 20.00±9.17 ($P<0.05$). By flushing, 6.67±2.31 grade 1-2 blastocysts were collected from the uterine horn omolateral to control ovaries and 14.67±9.29 from the treated ones ($P<0.05$). Our data suggest that PRP could stimulate latent follicles and *in vivo* embryo production.

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FLUORIMETRIC EVALUATION OF THE MITOCHONDRIAL MEMBRANE POTENTIAL OF BOAR SPERM CELLS

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The ability of the sperm cell to fertilize the oocyte is related to chromatin and mitochondrial integrity, both are required for fertilization. Mitochondrial integrity is absolutely necessary for sperm movements. Astenozoospermia is the frequent reason of male infertility and it is the result of structural and bioenergetic modifications, hormonal and genetic defects. Aim of this study is to evaluate the mitochondrial membrane potential (MMP) of boar spermatozoa by a fluorometric technique based on the safranine spectral variations. The functional metabolic condition has been evaluated by lactate to state mitochondrial integrity. Semen was recovered from twenty Large White boars by an artificial vagina. Semen samples were filtered and evaluated for several parameters: volume, colour, pH, concentration, total and progressive cell motility, viability and morphology. Only ejaculates showing sperm cell concentration >200x106/ml, motility >60% and morphology >60% were employed. Sperm cells were lysed in an hypotonic buffer that allows to recover functional mitochondria according to Ferramosca et al. [1]. Mitochondrial metabolism was studied by a spectrofluorometer at 25°C (Perkin-Elmer LS50B). Aliquots containing 0.11mg of demembranated cells (Hypotonic Treated Cell, HTC) were resuspended in a PBS containing safranine O (25nmol/mg prot.) and 5mM succinate that induced a decrease of safranine absorbance (>9U.F.) and the generation of a MMP. When the discoupling agent FCCP (1μm) was added a prompt MMP decrease and a safranine fluorescence increase (>4U.F.) was observed. To evaluate lactate metabolism into mitochondria, L-lattato (5mM) was added to HTC cells; again a prompt increase of MMP and a safranine decrease (<9U.F.) was observed together with the typical response to FCCP.
In conclusion MMP of functional mitochondria can be studied by modification of the safranine fluorescent spectrum. MMP is a good indicator of the respiratory chain activity so that it can be employed for the evaluation of mitochondrial integrity. The access of cationic lipophilic dyes into mitochondria depends on □□ while the fluorescence of accumulated fluorochromes corresponds to MMP [2]. Further studies will be necessary to evaluate the relationship between MMP and the fertility potential of spermatozoa from astenozoospermic subjects.

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GNRH INCORPORATION IN INSEMINATIVE DOSES IN RABBIT: EFFECT ON SEMEN QUALITY AND REPRODUCTIVE PERFORMANCES

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The most frequently used method for ovulation induction in commercial rabbit does is the intramuscular administration of gonadotropin-releasing hormone (GnRH) or its synthetic analogues. Unfortunately, these hormonal treatments, particularly when repeatedly used, are generally followed by a decreased fertility due to the appearance of plasmatic anti-GnRH antibodies [1]. Several authors recently demonstrated ovulation induction by including GnRH analogues directly into the seminal dose, and administering them through vaginal absorption [2]. The present study was aimed at determining if lecirelin acetate, a GnRH analogue, could be used for does insemination by its inclusion in the seminal dose. Twenty does of 9 months age (Grigio del Monferrato, autochthonous Italian breed) were individually housed and divided into two groups, on the basis of GnRH administration method: control group which received 0.2 ml of intramuscular (C) lecirelin (Dalmarelin, Fatro®) and intravaginal group (IV) inseminated by adding 0.3 ml of the same GnRH in the seminal dose (10±1 million spermatozoa in 0.5 mL of diluent). The experiment was performed for six consecutive reproductive cycles at 42 day- intervals. Sperm motility and morphological evaluations were performed on each of the heterospermic pooled semen before each insemination. Does inseminated by intravaginal addition of Dalmarelin showed a higher or equal sexual receptivity compared to c group ($P<0.001$), which resulted in a higher fertility rate as the cycle increased. Regarding the number of live-born kits, only one cycle showed a significant effect ($P<0.01$), with differences recorded within cycles 2, 4 and 6 without significant effects on the group. The volume of the seminal dose was very low and this could explain the best results of the IV group, which had a similar GnRH amount of C. The addition of GnRH to rabbit sperm did not significantly impair motility parameters. Progressive motility was significantly ($P<0.001$) positively correlated with motility characteristics, including VAP, VSL, ALH, BCF, STR, and LIN. In conclusion, our present findings supported that the incorporation of GnRH in the seminal dose could be used for ovulation induction in rabbit does. Despite this, further studies are needed to identify the optimal dose for intravaginal administration.

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HAEMATOLOGICAL PARAMETERS, ACID-BASE BALANCE, ELECTROLYTES AND BLOOD METABOLITES IN ALPINE KIDS FROM BIRTH TO 72 HOURS OF LIFE

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Goats are receiving a growing attention in veterinary medicine, but physiological data in newborn and adult goats are still scarce [1, 2]. The first weeks of life represent a critical stage; neonatal diseases and mortality are still the main causes of economic loss in livestock production. The availability of specific physiological ranges would support the veterinarian in making a correct diagnosis and prognosis and allow him to better protect animal health and welfare. The aim of this study was to fill the knowledge gap on the physiological parameters in newborn goat kids by acquiring data on the haematological parameters, acid-base balance, electrolytes and blood metabolites of Alpine goat kids in the first three days of life. Twenty Alpine goat kids, farmed at the Department of Veterinary Medical Sciences of the University of Bologna, were included. Inclusion criteria were: supervised and physiological parturition, adequate suckling of colostrum, normal daily physical examination for the first six days of life, absence of clinically evident disease for at least one week after the last sample was collected. Venous blood was drawn from the jugular vein at birth before the assumption of colostrum (T0), at 24 hours (T24) and 72 hours (T72) of life. The collected samples were immediately analysed for haematology and blood gas analysis. Preliminary results, analysed with the repeated measures analysis of variance, show that the sampling time (age) had a significant ($p<0.05$) effect on most of the measured parameters: a marked decrease in haemoglobin ($p<0.0001$), haematocrit ($p<0.0001$) and platelet count ($p=0.01$) was recorded from birth to 72 hours of life. On the contrary, leucocytes were lower at birth than at T24 and T72 ($p=0.055$). The increase in the WBC count was due to the increase in neutrophils ($p=0.016$), basophils ($p<0.0001$) and monocytes ($p<0.0001$), while lymphocytes remained unchanged. No significant changes were found in the number of RBCs and reticulocytes, which were present at a high percentage. As regard the acid-base balance, no difference was found in pH, pCO2, tC02, HCO3. Among the electrolytes, K+ and Na+ did not show differences, while Ca++ was lower at birth than in subsequent times ($p<0.001$) and Cl- was higher at birth than after. Glucose at birth was much lower ($p<0.0001$) than at 24 and 72 h of life, while lactate did not show any difference between sampling times. The increase in WBC, neutrophils and monocytes was probably due to the maturation of the immune system during the first days of life. Differently from adult goats in newborn kids' neutrophils dominate the profile. These data are not indicative of an acidotic status in the newborn kids, but the very low levels of glucose emphasize the necessity for the kids to suckle an adequate quantity of colostrum within the first hours of life.

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EMBRYONIC DEVELOPMENT IN THE *BOA CONSTRICTOR*: PRELIMINARY ULTRASOUND OBSERVATIONS

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The *Boa constrictor* is one of the most common reptiles bred in captivity. A thorough knowledge of the reproductive activity of females and a precise monitoring of embryonic development are important for a successful breeding [Stahl et al., 2001; Bertocchi et al., 2018]. Although studies on ultrasound monitoring of the reproductive activity of different reptile species are reported, it is important to study the characteristics of each species due to the existence of significant interspecific variability [Andrews, 2004]. With regards to *Boa constrictor*, scientific contributions in this area are still rather scarce. The aim of the present study is to evaluate the ovarian activity and the embryonic development of Boa constrictor by ultrasound, an absolutely non-invasive technique (OPBA ethical approval PROT. N. 07/CE/2019). We retrospectively analyzed the ultrasound scans done routinely on the farm to monitor the activity of the reproducing females. In particular, a total of 30 adult captive born female Boa constrictors have been examined during the reproductive period. The snakes were housed in single racks, each with a temperature gradient up to 28°C, and under a natural light-dark cycle. Upon detection of follicles with a diameter between 15-30 mm, the female was housed with the male for a week. After the 7-day exposure to the male, ultrasound scans were performed weekly until the gestation term. For each female, was performed a series of brief lateral scans of the lower half of the body, after applying a layer of conductive gel. Each scan was performed on non-sedated animals kept in sternal recumbency, using a portable ultrasound system and a 7.5-MHz linear array transducer (Esaote MyLab TM ClassicC®). Ultrasound features including dimensions and echogenicity of the ovarian follicles were determined, and the development of the embryonic structures was described. Ovarian follicles were highlighted on both sides within the caudal half of the females' body. In the early stages of the reproductive cycle these follicles appear as roundish anechoic structures with a chain arrangement, which become clustered at ovulation and after a few days returns to a linear arrangement. Immediately after ovulation, a slight decrease in size (a few mm) can be detected, the shape is oval, and the inside of the structure can present in one of two ways: a uniform widespread echogenicity, or a decidedly more uneven appearance with hyperechoic and anechoic areas with a concentric arrangement. As gestation progresses the size of the structures increases and if we take as a reference point for gestational age the post-ovulatory shed, after about 30 days the embryonic development leads to the appearance of a "snail" structure which will then grow to occupy most of the available space, taking on a "spiral" appearance, after the 60th day. Approximately 25 days after the post-ovulatory shed, we were able to detect the cardiac activity of embryos. In some cases, reabsorption was observed at different stages of development. As with other snake species, it seems also for Boa constrictor, the best approach for follicle visualization is the ventro-lateral one [Schilliger, 2010]. In different species of reptiles, ultrasonography has also been recognized as a valid technique for assessing embryonic vitality and development [Bertocchi et al., 2018]. This seems to be confirmed for Boa constrictor too. Indeed, the present study suggests that ultrasound is an excellent non-invasive technique to evaluate the reproductive activity of *Boa constrictor*, allowing to https://doi.org/10.1371/journal.pone.0199377.

COMPARATIVE STUDY OF TWO INCISIONAL INFILTRATION TECHNIQUES IN DOGS UNDERGOING OVARIECTOMY: PRELIMINARY RESULTS

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Incisional infiltration with local anaesthetic is an important part of multimodal analgesia. Usually, this technique is performed by injection of local anaesthetic directly into the surgical field using a syringe [1]. Comfort-in™ is a needle-free injection system that works by forcing the liquid medication at an elevated speed without piercing the skin; high pressure allows the more rapid administration of drugs by penetrating to the skin in devoid of an injection [2]. The aim of the study is to compare the analgesic effects of midline infiltration of 2% lidocaine using the traditional technique and the Comfort-in™ in dogs undergoing ovariectomy. The study was approved by the Ethical Committee of the Department of Veterinary Sciences of Messina (No. 021/2018). Twenty-two ASA 1 female mixed-breed dogs aged between 2 and 7 years from a private shelter were randomly divided into two groups: 1) lidocaine (2 mg/kg) infiltration with the syringe (group S; n=11); 2) lidocaine (2 mg/kg) infiltration with the Comfort- in™ Technology (group C; n=11). The dogs were premedicated with im medetomidine (5 □g/kg) and tramadol (3 mg/kg). General anaesthesia was induced and maintained with iv tiletamine and zolazepam (5 mg/kg). The infiltration of the midline incision was performed 10 minutes before surgery. During anaesthesia, heart and respiratory rates, non-invasive arterial pressure, oxygen haemoglobin saturation and body temperature were measured every 5 minutes and before skin incision, at skin incision, and at skin suture. The data recorded were analysed using a repeated measures analysis of variance and a p- value<0.05 was considered significant. There were no significant differences in the monitored parameters when compared to the baseline values and between groups. The surgery was completed in all the animals, and there were no anaesthetic or surgical complications. The results of this study confirm that the infiltration using the Comfort-in™ Technology provided effective analgesia in dogs undergoing ovariectomy, in the same way as the traditional infiltration. On the other hand, advantages of the use of incisional lidocaine has been showed by different studies [1,3] and consisted in less use of intraoperative and postoperative analgesics and in a lower postoperative pain score. Comfort-in™ lowers the risk of needle injury that is extremely important when anaesthetic drugs are handed. Further studies are needed in order to demonstrate whether this technique can be a reliable alternative method to perform incisional infiltration, especially in sedated or awake animals.

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HAIR CORTISOL CONCENTRATIONS IN BEEF CALVES FROM BIRTH UP TO 8 MONTHS OF AGE

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Although steroidogenesis in maternal, placental and fetal compartments is interdependent, the maternal and fetal hypothalamic-pituitary-adrenal (HPA) axes represent separate biological systems, and glucocorticoids, whether of fetal or maternal origin, are likely to influence development and to have long-term effects on HPA [1]. Hair analysis represents a promising approach for the non-invasive measurement of steroids, allowing for a retrospective analysis of the total exposure to steroids over time, and avoiding the influence of acute events or circadian fluctuations [2]. Cortisol (C) is the main steroid produced by HPA activation, and its measurement in hair coat has been already reported in cows and in calves older than 3 months [3,4], but no information is available on newborn calves. The object of this study was to evaluate in RIA [2] the hair cortisol concentrations (HCC) in beef calves from birth to 8 months of age. Hair samples of 12 beef calves born by spontaneus delivery were collected by shaving at calving (T0) and monthly up to 8 months of age (T1-T8) only on the re-growth area. Calves sex, weight and Apgar score were registered immediately after birth. Differences of HCC among sampling times and correlation between HCC and sex and birth weight were statistically analyzed through non-parametric tests. All Apgar scores were ≥8, thus considered as normal. Statistical analysis revealed that HCC in calves were influenced by sampling time ($P<0.0001$), with higher levels at T0 compared to T1 ($P<0.01$) and to all the subsequent samples ($P<0.001$). HCC at T1 were higher compared to T2-T8 ($P<0.001$); HCC at T2 were higher compared to T3 ($P<0.05$) and also compared to all subsequent sampling time ($P<0.01$). HCC at T3 were higher than in all the following samplings ($P<0.05$), while no further changes were detected from T4 forward. No correlation was found between HCC and newborn sex (7 males, 5 females) and between HCC and birth weight. These data demonstrate that C is quantifiable in the hair coat of newborn calves, and that HCC are influenced by the age of calves. The higher HCC detected at birth (T0) probably reflects the high serum C concentrations present late in pregnancy that are stimulated by the fetal HPA, the route by which parturition is initiated in cows. The finding of higher HCC until 3 months of age, although with decreasing values, suggests that C secretion continues after birth and that it could be involved in the developing events occurring during the first months of age.

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NEWBORN VIABILITY IN CHIHUAHUA DOGS: DO THEY NEED A BREED-RELATED APGAR CLASSIFICATION?

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Perinatal mortality in dogs is extremely high, reaching values of 30-40%, mainly due to the long process of whelping and the high risk for dystocia in some breeds. The prompt neonatal recognition of newborns that need assistance, and the provision of adeguate support was reported to improve the chance for survival. A dog-adapted Apgar score was used for the easy and quick assessment of newborn dogs viability, independentely by the breed. However, a modified Apgar score (AS) was proposed for two brachicephalic dog breeds at high risk for dystocia, for a better evaluation of the actual newborn viability in these breeds [1]. Because also toy- and small-sized breeds ar recognized as at high risk for dystocia [2], this study was aimed to assess if Chihuahua (CHH) newborns need a breed-related Apgar viability classification for a better evaluation of newborns assessment in this breed. The study was performed on 38 CHH bitches submitted to elective Caesarean Section at term of pregnancy. At birth, each puppy was evaluated by AS and grouped in one of the three viability classes [3], and puppies with AS<7 were submitted to a different degree of neonatal assistance. Neonatal birthweigth and gender were also recorded. Puppies with malformations were excluded by the study. Survival was assessed at 24 hours and 7 days after birth. The ROC analysis and the Youden test were used to assess the cut-off AS for surviving/not surviving puppies. The 38 CHH bitches gave birth to 118 alive puppies (litter-size 3.9±1.43, birthweigth 142±38.12 g), 57 males and 61 females, but 2 had severe cleft palate and were euthanised. Puppies survival was 63/66 (96%) in 7-10 class AS, 34/36 (94%) in 4-6 class AS and 6/14 (43%) in 0-3 class AS. The statistical analysis showed that the area under the curve for the ROC analysis was 0.82 ($p<0.0001$) and the cut-off for surviving/not surviving puppies was AS 4, with a sensibility of 0.65, specificity of 0.93, and negative predictive value of 0.96. Therefore, the results showed that the cut-off for viability classification of CHH newborns according to the chance of not surviving is similar to the ones previously reported. However the high number of puppies in AS 4-6 viability class was higher (31%) in comparison to the 10% previously reported [3]. Therefore, the results from the present study seem to suggest that CHH newborn puppies do not need a different AS classification for the quick detection of puppies that will not survive. On the other hand, the higher number of puppies classified in AS 4-6 highligths the need for the detection of puppies that could benefit of a proper neonatal assistance.

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RETROSPECTIVE ANALYSIS OF TWO ANALGESIC STRATEGIES DURING RADICAL MASTECTOMY IN CATS

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Mastectomy is a surgical procedure that often involves elderly patients with an increased perianestetic mortality. The use of loco-regional anesthesia reduces the endocrine, inflammatory and metabolic response to surgical stress and determines an improvement in the surgery outcome (1). The aim of our study was to evaluate in a retrospective way the analgesic efficacy of a loco-regional anesthetic strategy (subarachnoid anesthesia combined with blockage of intercostal nerves using Levobupivacaine) in comparison with the use of Sufentanil Citrate administered in constant rate infusion used in several subjects. A total of 10 cats (11.2 to 16.8 years old, 2.8 to 4 kg of body weight) were analyzed. All subjects received the same anestetics: Alfaxalone 3-5 mg/kg and Methadone 0.25 mg/kg both IM as premedication; Alfaxalone (1-2 mg/kg EV) for the induction and Sevofluorane (end expiratory values ranging between 2.3 and 2.7%) for the maintenance. Afterwards, 5 cats (group LR) received loco- regional Levobupivacaine, 5 cats (Group CRI) constant rate infusion of Sufentanil. At the end of the surgery all cats were administrated with Meloxicam SC (0.20 mg/kg). Procedures with animals were performed following good veterinary practices for animal welfare (D.Lgs 116/92) with the informed consent of the owners. During the surgery evaluation criteria were the hemodynamic stability and the need for more pain-relieving medications. In the postoperative period the level of comfort/discomfort and pain were used to get a score according to the multimodal EUNESP-Botucatu scale. Post operative observations started when subjects reached the sternal decubitus (0 hour) and followed at 1, 2, 4, 8 hours. Subjects that had a score higher than 7 received supplementation of analgestic drugs. The scale rates were used to highlight the differences between the two groups. Intraoperative analgesia was satisfactory in both groups, with good hemodynamic stability; in one cat of group LR and two cats of group CRI it was necessary to use an additional bolus of Sufentanil (0.5 mcg/kg). Two cats of group LR required one more dose of Methadone at 1 hour post sternal decubitus, while all cats from group CRI required many more doses (4 cats at 0h, 3 at 1h, 1 at 2h, 3 at 4h, 1 at 8 h). Analgesia reached in cats of group LR was much more satisfying than in CRI group. What emerged from this study, despite the small range of subjects, demonstrates that the use of loco-regional anesthesia techniques, using long-acting local anesthetics, ensures long-lasting and high quality analgesic coverage, capable of minimize the surgical stress that inevitably emerges during invasive surgical procedures such as radical mastectomy.

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TELOMERE LENGTH AND CANINE MAMMARY CARCINOMAS PROGNOSIS: PRELIMINARY STUDY

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Mammary tumors are the second most common neoplasia in dogs; therefore, they represent a significant clinical problem, furthermore, recent studies indicate that spontaneous canine mammary carcinomas (CMCs) resemble human breast cancer by clinic and pathology as well as its behavior and prognostic indicators [1, 2] Biological aging pathways may be a mechanism for cognitive impairment in cancer survivors. The goal of the current study was to examine whether indicators of biological aging, namely elevated levels of DNA damage, reduced telomerase enzymatic activity, and shorter peripheral blood telomere length (TL) would be related to cognitive function in a cohort of survivors of breast cancer. [3, 4]. The association of TL with breast cancer prognosis in human had been examined through a systematic review [3]. Our study had the aim to relate peripheral blood TL and canine mammary carcinomas (CMCs) in the bitch. To this end, six female bitches aged 6-8 years old, of middle size, that were submitted to mastectomy for CMC during the last 24 months, were subjected to blood sampling to evaluate TL. As control, 5 clinically healthy bitches free of cancer diseases, and of the same range of age and size were subjected to the same blood withdrawal. From each animal, 8 ml of blood was collected and taken in Qiagen tubes and sent via airmail to the lab of Dana Farber/Harvard Cancer Center Genotyping and Genetics for Population Sciences. DNA extraction was performed on blood white cells, and TL was measured by a modified quantitative polymerase chain reaction qPCR method, as previously described [4]. Our results were submitted to ANOVA test and show that breast cancer patients had significantly shorter TLs than control subjects ($p<0.05$). The results of the current study suggest a significant association between measures of biological aging and breast cancer. Future prospective studies are needed to confirm a causal role of biological aging as a driver of declines in cognitive function after cancer treatment.

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EFFECTS OF OOCYTE EXPOSURE TO NANOMOLAR LEVELS OF OCHRATOXIN-A ON EMBRYO DEVELOPMENT IN THE JUVENILE SHEEP

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Ochratoxin A (OTA) is a mycotoxin produced by various fungal species of the genera *Aspergillus* and *Penicillium* with widespread occurrence in stored foods and feedstuffs [1]. Contamination with OTA can induce reprotoxic, embryotoxic and teratogenic effects in laboratory and farm animals [2]. Ruminants are relatively resistant to toxic effects of OTA, due to its degradation to the less toxic metabolite ochratoxin alpha by rumen microbiota. Nevertheless, nanomolar levels of OTA have been found in the blood of sheep fed with contaminated feed [3]. The aim of the present study was to evaluate the effects of oocyte exposure to OTA nanomolar levels on embryo development in the juvenile sheep. Cumulus-oocyte complexes (COCs), recovered from the ovaries of slaughtered juvenile sheep, were exposed to 1, 10, 100 and 1000 nM OTA during *in vitro* maturation (IVM) [4]. IVM medium with vehicle (1% methanol) was used as control. In three replicates, 50-100 COCs/condition were analyzed. After IVM, oocytes underwent in vitro fertilization and embryo culture up to day 7. Embryo development was monitored by phase contrast and epifluorescence microscopy after staining nuclear chromatin with Hoechst 33258 [4]. Data were analyzed by Chi-square test (statistical significance at $P<0.05$). At any tested concentration, OTA did not affect oocyte maturation rates (72.2%, 71.5%, 72.9%, 74.5% versus 71.5%) and total cleavage rates/matured oocytes (88.5%, 80.0%, 71.4%, 86.3.5% versus 87.3%) for 1, 10, 100 and 1000 nM OTA respectively vs control. OTA also did not affect blastocyst formation rates/cleaved (6.2%, 8.0%, 5.2% versus 3.6%; for 10, 100 and 1000 nM OTA respectively vs control). Interestingly, no blastocyst formation was found after oocyte exposure to 1 nM OTA. In conclusion, oocyte exposure to nanomolar OTA levels had no apparent effects on embryo development. Further studies are in progress to evaluate additional oocyte and embryo quality parameters**.**

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THE MYCOTOXIN BEAUVERICIN IMPAIRS EMBRYO DEVELOPMENT AND BLASTOCYST QUALITY IN THE JUVENILE SHEEP

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Beauvericin (BEA) is a mycotoxin that disturbs nuclear and cytoplasmic in vitro maturation (IVM) of prepubertal sheep oocytes [1]. The aim of this study was to examine long-term carry-over effects of BEA exposure during IVM on embryo cleavage and blastocyst quality. Cumulus-oocyte complexes recovered from the ovaries of slaughtered prepubertal sheep (<6 months) were cultured for IVM in presence of 0 (Control), 0.5, 1 and 3 □M BEA [1,2]. After IVM, oocytes were subjected to in vitro fertilization and in vitro embryo culture [2]. Embryo developmental stage was assessed at day 8. Blastocysts were classified according to expansion and hatching status and their diameter was evaluated. After that, embryos were stained with Hoechst 33258 to evaluate number of nuclei and chromatin integrity, and with MitoTracker Orange CMTM Ros and 2′,7′-dichlorodihydrofluorescein diacetate to asses mitochondrial activity and ROS levels (expressed as the percentage of the signal of the control sample). Data were analyzed by the Chi-square test except for mitochondrial activity and ROS levels data compared by one-way ANOVA (significance at $P<0.05$). Data are presented for 0.5, 1, 3 µM BEA vs Control, respectively. A total of 523 oocytes were used (n.5 replicates). The result of percentages of embryos at the 16-31 cell after oocyte exposure to BEA were 16/134, 12%, 6/134, 4.5%, 6/128, 4.7% vs 15/127, 12% ($P<0.05$), and for morula- stage 4/134, 3%, 2/134, 1.5%, 2/128, 1.6% vs 9/127, 7% ($P<0.05$). From these results it is possible to underline that the exposure to 1 and 3 µM BEA decreased these rates. After exposure to BEA, the 2–3 cell stages were 8/134, 6%, 15/134, 11.2%, 15/128, 12% vs 3/127, 2.4% ($P<0.01$) but in this case 1 and 3 µM BEA increased the rate. No significant effects were found on blastocyst formation rates (3/134, 2%, 6/134, 4.5%, 4/128, 3% vs 4/127, 3%). Blastocysts derived from oocytes exposed to 1 and 3 µM BEA had not hatched after 8 days, in contrast, a few embryos obtained after exposure to 0 and 0.5 µM BEA did hatch (1/3, 0/6, 0/4, vs 1/4). Blastocyst diameter (131±34, 125±16, 125±13 vs 133±24 µm) and numbers of nuclei (79±15, 87±16, 87±9 vs 82±9) did not vary. Interestingly, increased percentages of embryos with more than 20% affected blastomeres with lobulated nuclei and/or micronuclei were observed after BEA exposure (1/3, 4/6, 3/4 vs 0/4). No effects on mitochondrial pattern and activity were detected (91±30, 103±36, 95±30 vs 100±21) whereas oocyte exposure to any BEA concentration reduced ROS generation ability (64±16, 87±23, 78±20 vs 100±23; $P<0.01$), possibly indicating viability loss. In conclusion: exposure to BEA during maturation of sheep oocytes compromised embryo development and blastocyst quality, possibly reducing fertility.

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BENEFICIAL EFFECT OF RESVERATROL ON *IN VITRO* FERTILIZATION OF OVINE OOCYTES UNDER CADMIUM EXPOSURE

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Cadmium (Cd) is a highly toxic heavy metal with adverse effect on female reproduction. Exposure to Cd during in vitro maturation (IVM) negatively affected oocyte maturation, fertilization and embryo development [1,2]. A recent study demonstrated that Cd exposure impaired oocyte fertilization through oxidative damage [1]. This study was aimed to evaluate the effect of supplementation of resveratrol (Resv), a natural phenol with antioxidant properties [3], to the IVM medium on in vitro fertilization (IVF) of ovine oocytes under Cd exposure. Cumulus-oocyte complex (COCs) recovered from ovaries of slaughtered juvenile sheep (30-45 days old) were matured in presence of 2µM Cd with 0µM (n=147, Cd- group) or 1µM (n=141, Cd-Resv group) Resv. COCs matured in absence of Cd were used as control (n=149, CTR group). After IVM oocytes were fertilized in vitro in synthetic oviductal fluid medium with fresh semen [4]. Reactive oxygen species (ROS) levels of metaphase II oocytes were evaluated by confocal laser scanning microscopy after staining with 10 µM 2',7'-dichlorodihydrofluorescein diacetate. Fertilization assessment was performed at 16 hours post IVF by fixation and staining of fertilized oocytes. The presence of two pronuclei and two polar bodies indicated a normal fertilization, the presence of more than two pronuclei and two polar bodies was designated as polyspermic fertilization. STATA\IC 11.0 software was used to data analysis, Kruskal-Wallis test was employed for ROS levels and Chi-square test to analyze fertilization rates. with Results showed that the intracellular ROS levels were lower ($P<0.05$) in CTR and Cd-Resv groups compared to Cd-group. Oocytes of CTR and Cd-Resv groups had higher normal fertilization (CTR: 61.7%; Cd-Resv: 58.2%) and lower polyspermic fertilization (CTR: 24.2%; Cd-Resv: 26.2%) rates compared to Cd-group (36.0% and 44.9%, respectively) ($P<0.05$). In conclusion, Resv treatment during IVM alleviated oocyte oxidative stress induced by Cd-exposure and enhanced the IVF outcome.

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AMNIOTIC MICROVESICLES SUPPLEMENTATION DURING *IN VITRO* BOVINE EMBRYO CULTURE SHIFT MICRORNAS PROFILING TOWARD IN VIVO PRODUCED BLASTOCYSTS

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Microvesicles (MVs), carrying microRNAs (miRNAs), are involved in communication mechanisms in biological processes such as gametogenesis, fertilization, implantation and embryo development. *In vitro* produced bovine embryos exposed to amniotic MVs have higher quality compared to control (CTR)[1] and, after cryopreservation, provide higher in vitro embryo hacting and recipient pregnancy rate [2]. From these results, MV supplementation during embryo culture is expected to alter miRNAs expression and potentially influence embryo implantation. The aim of this study was to evaluate miRNA profiling of *in vitro* produced blastocysts with or without MV supplementation, using in vivo produced blastocysts as CTR. *In vitro* embryos were produced based on our protocol [1]. Presumptive zygotes were randomly transferred in SOFaa without MV supplementation or cultured by adding 50x106 MVs/ml in the SOFaa on day 5 post fertilization [1]. Embryo developmental rate was evaluated at day 7 (blastocyst stage, B7) and B7 were immediately cryopreserved in liquid nitrogen for genomic study. To obtain in vivo embryos, three cows with a history of normal fertility were superovulated by Folltropin and inseminated by the same cryopreserved semen. After flushing, only B7 were immediately cryopreserved for genomic study. Samples for RNA isolation were obtained from pools of embryos (n=10) for each condition (*vivo*, *vitro* and *vitro*+MVs). Total RNA was isolated by NucleoSpin1 miRNA kit. The libraries were prepared using TruSeq Small RNA Library Preparation kits (Illumina). Illumina sequences were input to miRDeep2 for miRNA detection and discovery. Our results show that the number of miRNAs found to be differentially expressed among three comparisons (*vivo* vs *vitro*, *vivo* vs vitro+MVs and *vitro* vs *vitro*+MVs) were 20, 15, and 2 respectively. The analysis of these miRNAs showed that in vivo produced embryos are clearly separated from in vitro embryos, but the in vitro+MVs group resulted closer to in vivo samples, and this agree with the quality of embryo and the pregnancy rate after MV supplementation [1,2]. In vitro and in vitro+MVs embryo differ significantly for two miRNAs (miR 130a, miR-181b) that are found to be higher in our in vitro embryos cultured without MVs as well as in degenerate bovine embryos compared to good blastocyst [3]. In conclusion, this is the first study reporting the complete miRNAs profiling of in vitro blastocysts compared to those obtained in vivo. Microvesicle addition during *in vitro* production seems to counteract the adverse effect of in vitro culture and regulate the expression of specific miRNAs involved in the success of embryo implantation.

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RELATION BETWEEN CANINE SEMEN CHARACTERISTICS AND CARBONYLATION IN SEMINAL FLUIDS PROTEINS

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Oxidative stress is one of the major factors affecting semen quality in mammals. Reactive oxygen species damage biomolecules, yielding reactive carbonyl groups (e.g., aldehydes, ketones, and lactams) in lipids, nucleic acids and proteins [1]. The aim of this study was to evaluate the total proteins concentration (TPC) and the protein carbonyl content (PCC) in canine seminal plasma (1st and 2nd fraction) and prostatic fluid (3rd fraction) and to evaluate their relationship with semen characteristics. A fractionated semen sample was collected from 18 dogs and semen volume, sperm concentration and total number, motility and morphology were evaluated. Ejaculates showing >70% subjective motility, >60% normal spermatozoa and >200x106 spermatozoa (>100x106 if dogs weighted <10 kg) were considered normal. Aliquots of seminal plasma and prostatic fluid were centrifuged at 700 g for 6 minutes and the supernatants were stored at -80°C either as such (centrifuged seminal plasma, CSP and centrifuged prostatic fluid, CPF) or after being filtered through a 1.2µm filter (filtered seminal plasma, FSP and filtered prostatic fluid, FPF). TPC was determined by the Lowry technique [2], PCC by the Levine et al. [3] method. The correlation between TPC or PCC and single semen characteristics was evaluated by Spearman Rho test. Differences in TPC and PCC between semen and prostatic fluid, or normal and abnormal ejaculates were evaluated by Paired t-test and Mann-Withney test, respectively. Prostatic fluid had a higher PCC content than seminal plasma in both centrifuged and filtered samples ($P<0.01$). A significant negative correlation was identified between semen volume and PCC in CSP, and between the volume of collected prostatic fluid and PCC in CPF ($P<0.05$). In abnormal ejaculates (n=7) PCC in FSP was higher than in normal (n=11) ones (0.903±0.402 and 0.527±0.223 nmol/mL/mg protein, respectively; $P<0.05$). On the contrary, TPC in CSP was higher in normal than in abnormal samples (45.79±13.57 and 33.01±11.62 mg/ml, respectively; $P<0.05$). In conclusion, prostatic fluid and seminal plasma differed in PCC, and seminal PCC was higher in abnormal ejaculates. These results are in line with the only study on PCC in dog semen [4], which suggested how poor semen quality and infertility in dogs could be associated with increased protein peroxidation [4].

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CANINE SEMEN QUALITY AND PROSTATIC FLUID COMPOSITION DURING OSATERONE ACETATE (YPOZANE) TREATMENT FOR BENIGN PROSTATIC HYPERPLASIA

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Benign prostatic hyperplasia (BPH) is a common prostatic condition of adult intact dogs in which androgenic hormones play a role [1]. Steroidal antiandrogens such as osaterone acetate (OA) compete with androgen receptors at prostatic level with a specific inhibitory action on prostatic volume causing a decrease in prostatic size [2]. Little is known about the effects of OA on semen quality and seminal plasma composition [3]. Eight adult male dogs (>5 years) of different breeds with BPH diagnosed through history, clinical exam and prostatic ultrasound were selected for the study. On the day of the diagnosis (D0) a) a fractionated semen sample was obtained by using an artificial vagina; b) semen quality was assessed using the second fraction; c) the third fraction was centrifuged and the supernatant stored at -18°C for further examination; d) the dog was treated orally (0.25-0.5mg/Kg/7days) with OA (Ypozane). This methodology was repeated on D60, D120 and D180 with exception of "d". Electrolytes (Na, K, Zn, Cu, Cl, Mg), glucose, cholesterol and triglycerides were evaluated on all third fractions of the ejaculate using chemiluminescence (Immulite 1000, Siemens, Milano, Italy). ANOVA test and a Krustal-Wallis tests was performed with statistical package SAS 9.4 (SAS institute, Inc. Cary, NC, USA). Prostatic volume of every dog decreased during the treatment with maximum values at 60 days post treatment (mean reduction of 45.7%, $P<0.05$). Sperm concentration decreased during the first 2 months of treatment by 62.4% sperm/mL ($P>0.05$) and increased over the next 4 months with maximum values at D180. The proportion of sperm tail abnormalities at D0 (10.6□2.4) increased significantly at D60 (25.7□2.4, $P<0.05$) while other sperm defects did not change significantly. In prostatic fluid, Zinc concentration (□g/mL) increased during treatment (4897.2□765.1) with higher values at D180 (9155.52□1081.98, $P<0.05$) while remaining electrolytes, glucose, cholesterol or triglycerides did not show any difference. Based on this data, the effect of OA treatment caused an increase in sperm tail defect during the first 2 months which disappear from the 4th month of treatment. Prostatic fluid Zn concentration increased during OA treatment with higher levels at the end of the treatment. This data correlates positively with the increase of sperm concentration at the end of the treatment suggesting that Zn may have a positive effect on canine sperm concentration similarly to what has been reported for humans [4].

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ORAL COMMUNICATIONS

SOFIVET

EXTRACELLULAR VESICLE (EV) DERIVED FROM EQUINE MESENCHYMAL STEM CELLS: *IN VITRO* ISOLATION AND CHARACTERIZATION

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Extracellular vesicles (EVs) are released by a variety of cells. EVs carry RNA. They carry RNA and also participate in cell-to-cell communication, interacting with the extracellular (Malda et al., 2016). The interest in EVs relies on their proposed capacity to improve tissue repair and regeneration. It has been observed that EVs participate in cell therapy by inhibition of cell apoptosis, stimulation of regeneration and the activation of cell programs during an injury (Tetta et al., 2011). Mesenchymal cells (MSCs) have been largely proposed as a treatment choice in equines' joints tissue regeneration. Release and presence of EVs have already been identified in vitro in MSCs (Baglio et al., 2012). However, little is known about EVs derived from MSCs in equines and further studies to characterize them and to understand their role in tissue repair are required. Thus, our objective was to isolate EVs from equine MSCs and evaluate their characteristics. It is important to mention that in fetal bovine serum (FBS), usually supplemented in *in vitro* cell cultures, contains its own EVs. This might result in misinterpretations when studying MSCs derived EVs (Wei et al., 2016). Therefore EV deprived FBS was prepared by ultracentrifugation and equine bone marrow cells were evaluated and compared by the use of different types of FBS: normal FBS, EV deprived FBS, pellets derived from the ultra-centrifugation and a commercial EV free FBS. We could observe by the population doubling growth assay no significant differences.

EVs from different equine MSCs (bone marrow, synovial fluid, and adipose cells) were evaluated using the nanoparticle tracking analysis (NanoSight) and no differences were observed in respect to EVs average size, size distribution and concentration in all MSC derived EVs. The EVs isolated from the different type of MSCs cells were analyzed by the use of transmission electron microscope (TEM). We could confirm the positive isolation of EVs and observe their morphology and shape. The present study provides knowledge about the EVs derived from equine MSCs and might give the paths about their role in tissue regeneration and repair *in vitro*.

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DIFFERENTIAL AQUAPORINS DISTRIBUTION IN THE CANINE EPIDIDYMIS: HYPOTHESIS FOR THEIR ROLE

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Aquaporins (AQPs), a family of integral membrane proteins, seem to be particularly involved in spermatogenesis and sperm maturation, facilitating the water passage along male reproductive tract and particularly by assisting the sperm passage throughout the epididymis [1, 2]. Among mammalian species, there are few are studies on domestic animals [3]. The dog has recently received particular scientific attention due to possible applicative aspects for clinical studies and assisted procreation technologies. The present study aimed to analyze the tissue distribution of AQPs (AQP7, 8, 9) in epididymis of adult dogs by using immunohistochemistry (IHC), western blotting (WB) and real-time RT-PCR analyses. The epididymis samples obtained after surgery by dogs (n=5) were properly processed as previously reported [4]. Results evidenced that AQPs are differentially distributed along the regions of epididymis showing AQP7 immunoreactivity (IR) was mainly detected in the apical portion of principal cells and in basal cells of all epididymis segments. AQP8 IR was observed in the principal, basal and narrow cells. In addition, AQP9 IR was detected quite exclusively along the principal cells of all segments of epididymis. Western blotting analysis evidenced for all AQPs the presence of multiple immune reactive bands in all tracts analyzed. Real time RT-PCR experiments revealed the expression levels of AQPs mRNA in all epididymis segments. In particular, AQP7 mRNA level expression was higher in the cauda than corpus and caput; AQP8 mRNA level was higher in the corpus than caput and cauda; AQP9 mRNA level was higher in the caput than corpus and cauda. Our results evidenced showed the presence of AQP7, -8 and -9 along the dog epididymis segments, giving also the first indication of AQP8 presence in epithelial cells of this tissue. These data were confirmed by WB and real-time RT-PCR, thus supporting the idea that the localization and expression of tested AQPs are species-specific. Considering the recent potential use of AQPs for cellular cryopreservation, further studies are needed to evaluate their role in the sperm membrane permeability during cryopreservation.

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ANIMAL ASSISTED INTERVENTION (AAI) IN PENITENTIARY: THE EFFECTS OF INTERACTION WITH DOGS ON CONVICT'S EMOTIONAL INTELLIGENCE

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The interspecific relation between humans and dogs during AAI may significantly enhance the life conditions of those at risk of social marginalization. **Aim of the project**: With the current project we have assessed whether the social bond formed between shelter dogs and convicts may have improved emotional intelligence reducing impulsive behavior in the latter group.
This study was conducted at Eboli's Penitentiary (SA) and 20 convicts were divided in two equal groups. The first group was engaged in weekly basic education activities that involved a group of five shelter dogs. Each dog was properly selected according to its ethological characteristics (docility, reactivity, relational competences). The second group (control group) was engaged in recreational activities that did not involve the presence of dogs. Emotionality and impulsivity where assessed in convicts belonging to both groups (with and without the presence of dogs) at the beginning and at the end of the project by the "Emotional Quotient Inventory (EQ-i)" and the "Barratt Impulsiveness Scale (BIS-11)".
Results showed a significant increase on the Mood scale in the experimental group subjects who had interactions with dogs; on the other hand, no increase was observed within the control group ($t(2) = -7.559$; $P=0.017$, t-test for paired samples). Cortisol levels from saliva samples have been measured in dogs before, during and after each activity (the dogs worked 2 hours per day with a 20-minute break every hour) (1; 2). Results indicate that the dogs' welfare has been optimally preserved since no significant differences of the cortisol levels measured before and after the AAI sessions have been found ($P>0.05$, mixed generalized linear model). Moreover, results demonstrate that the dogs' cortisol basal level significantly decreased at the end of the experimental period, suggesting a general decrease of their stress levels.
This study provides evidence on the validity of Assisted Animal Education programs in improving the psychological profile of social classes at risk of marginalization and shelter dog welfare.

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SYNTHETIC TORPOR IN PIGS: A PRELIMINARY STUDY ON THE ACTIVATION AND INHIBITION OF THE RAPHE PALLIDUS NEURONS

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The induction of a deep reversible hypothermia, also referred to as synthetic torpor, has been addressed as a potentially useful procedure for different pathologies [1] and as method for improving humans prolonged space travel [2]. The activation of the neurons of the Raphe pallidus (RPa), a region of the brainstem involved in the autonomic outflow to brown adipose tissue (BAT) and other organs [3], was proven to determine several metabolic effects. In rodents, its pharmacological activation increases BAT activity alongside with heart rate and expired CO_2 [4], while its inhibition induced hypothermia up to synthetic torpor [5]. Since the use of a non-clinical large animal model would help understanding the mechanisms underlying synthetic torpor and their translatability to humans, the aim of this work was to test the effects of pharmacological inhibition and activation of RPa neurons in the pig. Four adult female commercial pigs were enrolled in the study. On the day of the experiment, pigs were anesthetized and intubated in order to maintain anesthesia using isoflurane; fentanyl was administered for analgesia. Surgical approach consisted in a complete C1 laminectomy followed by occipital craniectomy and cerebellar tonsils dissection in order to visualize the foramen of Magendie and the fourth ventricle floor. GABAzine and muscimol were administered using a microinjection syringe in the RPa region. Monitored parameters included heart (HR) and respiratory (RR) rates, invasive blood pressure (IBP), expired CO_2 ($ETCO_2$), and rectal temperature. Shivering was visually assessed and a thermocamera allowed to evaluate the vascular responses. At the end of the procedure, animals were euthanized, and the brainstem sampled for histology. The injection of GABAzine determined a significant increase ($p<0.05$) in HR, IBP and $ETCO_2$, with evident shivering; the thermocamera showed a temperature decrease in the ear pinna. The injection of muscimol brought all the parameters back to baseline values, with $ETCO_2$ further decreasing. No differences in rectal temperature were noticed. This preliminary study in pigs confirms what already described in rodents upon RPa neurons activation. Overall, these results seem to strengthen the hypothesis that RPa is a key target for the induction of synthetic torpor, potentially translatable to humans.

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CIRCULATING CONCENTRATIONS OF L-ARGININE AND ITS METABOLITES ARE ALTERED IN A SHEEP MODEL OF NUTRITIONALLY-INDUCED INTRA-UTERINE GROWTH RESTRICTION

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Uterine vasodilatation, muscular relaxation and improved endothelial function are essential in pregnancy for the adequate blood supply, nutrition, and development of the fetuses. Nitric oxide (NO) is the main regulator of these phenomena [1]. The aim of this study was to investigate the blood concentrations of L-arginine, ADMA, SDMA, and L-homoarginine, modulating NO synthesis, in single, twin and triplet pregnancies in ewes undergoing either dietary energy restriction or normal nutrition. From d 24 to 100 of pregnancy, the ewes were fed ryegrass hay and two different iso-proteic concentrates fulfilling either 100% of ewes' energy requirements (control group; n=30, 14 singleton pregnancies 12 twin pregnancies and 4 triplet pregnancies) or only 50% (feed-restricted group; n=29; 11 singleton pregnancies, 15 twin pregnancies and 3 triplet pregnancies). Blood samples were collected monthly to measure by capillary electrophoresis the circulating concentrations of arginine, ADMA, homoarginine, SDMA and of other amino acids (alanine, cysteine, homocysteine, glycine, serine, taurine, and tryptophan) not involved in NO synthesis to rule out possible direct effects of diet restriction on their concentrations. No differences between groups were observed in the circulating concentrations of most of the amino acids dosed. L-homoarginine increased markedly in both groups during pregnancy ($p<0.001$). SDMA ($p<0.01$), L-arginine and ADMA concentrations were higher in feed-restricted ewes than in controls. The L-arginine/ADMA ratio, an indicator of NO production by NOS [2], decreased towards term without differences between the two groups. The ADMA/SDMA ratio, an index of the ADMA degrading enzyme activity [3], was higher in controls than feed-restricted ewes ($p<0.001$). Obtained results show that circulating concentrations of L-arginine, of its metabolites and the ratio between NO synthesis boosters and inhibitors are altered in energy-restricted ewes delivering low birth weight lambs, and that these alterations are more marked in ewes carrying multiple fetuses.

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ANGIOGENIC PATTERNS OF TESTICULAR INACTIVATION IN THE ROE DEER DURING THE PRE AND POST RUT PERIODS

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In the light of its reproductive peculiarity, the roe deer (*Capreolus capreolus*) represents a spontaneous model of testicular inactivation. Indeed, during winter, bucks show a complete suspension of spermatogenesis, that starts again in spring with a peak during the breeding season (July/August) [1]. The underlying mechanisms to the regulation of the cyclic annual testicular changes are still not fully clear, although they seem to be imputed to the spermatogenic cell line since the other testicular cell populations remain stable, and apoptosis is not present [2]; nonetheless, the angiogenic factor seems to be pivotal [3]. The aim of this study was to evaluate the angiogenetic patterns, during pre and post-rut periods, and their correlation with the testicular involution. Eighteen adult bucks were sampled during the hunting season between June 1st and July 15th (pre rut, n=9) and August 15th and September 30th 2018 (post rut, n=9) in the South-Western Bologna Apennines (IT). Upon killing, animals were transferred to the local biometrical centre, where the scrota were collected. Once isolated, testes weights (TW) were registered and parenchyma immediately sampled. Analyses included Matrix Metalloproteinase-2 (MMP2) activity by zymography, qRT-PCR for gene expression of metallopeptidase inhibitors 1 (TIMP1) and 2 (TIMP2), two isoforms of Vascular Endothelial Growth Factor (VEGF 120 and 166) and its receptor (VEGFR1 and VEGFR2), and Testosterone (TEST) quantification by RIA. Differences between groups were investigated, after distributive normality evaluation (Shapiro-Wilk test), using the non-parametric Mann Whitney *U*-test (C.I. 95%); correlations between parameters were assessed by Spearman rank correlation test. Statistical differences (pre VS post-rut) were only recorded for proMMP2 ($p=0.018$), TEST ($p=0.0092$) and TW ($p= 0.0041$). TEST was correlated with TIMP1 ($\rho=0.564$), TIMP2 ($\rho= 0.488$), VEGF120 ($\rho= -0.428$) and proMMP2 ($\rho= -0.680$), while proMMP2 was correlated, aside from TEST, also with TW ($\rho= -0.777$), TIMP1 ($\rho=-0.486$) and TIMP2 ($\rho=-0.494$). On the basis of these results, the increase in activity of proMMP2 during the post rut period seems to be directly related to the involution of the testes. This is coherent with its well-acknowledged role in tissue remodelling [4] that, in this case, follows functional inactivation. To fully understand the role of angiogenesis, more extensive sampling, possibly during the entire year, should be performed to guarantee statistical power since coupled sampling cannot be performed.

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PATHOGENIC ROLE OF DELTA 2 TUBULIN IN BORTEZOMIB INDUCED PERIPHERAL NEUROPATHY

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Tubulin and microtubules (MTs) play critical roles in neuronal function (1,2) and are well-established targets for certain anticancer drugs that can also induce chemotherapy induced peripheral neuropathy (CIPN) (vinca, alkaloids, taxanes) (3). The contribution of these MT changes to the onset of CIPN is not well understood, but strongly predicted to be a determining factor. We hypothesized that seemingly unrelated CIPN-inducing drugs may share an underlying mechanism of pathogenesis based on acute alteration of one or more tubulin post-translational modifications (PTMs). *Methods.* To examine this, we measured the relative levels of selected tubulin PTMs in the cell bodies of dorsal root ganglia (DRG) and in sciatic nerves (SNs) isolated from rats treated with acute doses of the proteasome inhibitor Bortezomib (Bort) prior to any manifestation of neuronal injury or neuropathic behavior. All the experiments are also performed in vitro on primary culture of DRG neurons dissected from adult mice.

Among the tubulin PTMs examined, delta-2 tubulin (D2), an irreversible tubulin PTM and marker of hyperstable MTs, was significantly increased in L4-L5 DRG and SNs of Bort treated rats prior to any manifestation of neuronal injury or neuropathic behavior. We examined the pathogenic potential of D2 accumulation in dissociated adult DRG neurons and found that induction of D2 alone did not result from perturbation of MT dynamics but was sufficient to cause axonopathy and damage mitochondria motility in adult DRG neurons. Conversely, reducing D2 accumulation alleviated both axonal degeneration and inhibition of mitochondria motility promoted by Bort. Our results suggest that the mechanisms of CIPN drugs may converge on the acute perturbation of tubulin PTMs, and that disruption of the tubulin tyrosination/detyrosination cycle plays a crucial role in the onset of axonal injury in BIPN through its control of mitochondria dynamics.

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SHORT-TERM ADMINISTRATION OF DIFFERENT GLUCOGENIC MIXTURES MODIFIES BLOOD PARAMETERS AND FOLLICULAR COUNT IN SARDA EWE

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Short-term glucogenic treatments improve oocyte quality in ewes submitted to ovum pick up [1]. This effect has been related to a positive energetic status [2], but was associated with an increase in plasma osmolarity [3]. This study aimed at determining the lowest dose of glucogenic treatment able to modify ewe's metabolic status and ovarian dynamics, without impacting on blood cells viability. Two glucogenic mixtures (M: 70% glycerol, 20% propylene glycol, 10% water; G: 90% glycerol and 10% water) were administered b.i.d. during 4 days to non-lactating Sarda ewes (n=5 per groups) at 100%, 75%, 50% and 25% amount of energy offered in previous experiments (M100: 400mL per day; G100: 484mL per day) [1, 2]. The treatments determined a dose-related increase in circulating concentrations of glycerol, glucose, insulin, and a decrease in urea and NEFA plasma concentration ($p<0.0001$), with the 75% and M50% groups having similar values to the 100% ones. The M50% group, despite having a shorter metabolic effect compared to the 75% and 100% ones, determined a lower increase in plasma osmolarity. A dose-related effect ($p<0.05$) on haematocrit, red cell distribution width, mean corpuscular volume and mean corpuscular haemoglobin concentration was found, with 50% groups showing intermediate values between 75% and 25% ones. In a second phase, we evaluated the effect of selected treatments (G75, M75, M50; n=5 per group) on ewe's ovarian follicular population during a synchronized oestrus cycle, compared with a control group stimulated with PMSG (CON; n=5). Glucogenic formulations were administered from D -2 to D 1 (D 0 = second PGF administration), and oestradiol concentration were determined on D 2. From D-2 to D 1, follicular population was counted and measured by 3D ultrasonography. No differences were found among groups in oestradiol concentration, ovarian volume, vascularity index, flow index and their relationship. The number of follicles >4 mm was higher in G75% and M75% compared to the control group ($p<0.05$). In conclusion, M50% treatment had an intermediate metabolic effect, and a follicular population similar to PMSG treated group, while 75% groups were identified as the lowest doses able to positively impact on ewe's metabolic status and ovarian follicular population.

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TELOMERE DYNAMICS IN DOGS: PRELIMINARY STUDY

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Telomeres are protective regions of repetitive DNA at the ends of chromosomes that maintain fidelity of genetic information during replication and are critical for genomic stability. Telomeres shorten each time a cell divides, this loss is owing to the incomplete replication of linear chromosomes by the DNA polymerases also called the 'end-replication problem'. Steady shortening imposes a finite lifespan of cells and establishes the "molecular clock" of biological aging. Oxidative stress, inflammation, and environmental pollution accelerate the telomere shortening process. Telomeres can be restored by the telomerase, a ribonucleic enzyme that maintains telomere length (TL) and is active during early gestation but is repressed during extra-uterine life in somatic cells [1]. In contrast to the telomeres of somatic cells, those in sperm do not shorten with age because of upregulated telomerase, ensuring the transmission of intact chromosomes over generations. A preliminary study was carried out on blood and semen of male dogs (collected as part of routine clinical checks to which animals were subjected) to assess the telomere dynamics and some semen parameters in this species. The samples were collected from six male dogs (1 Akita, 1 Boxer, 1 English bulldog, 3 Neapolitan Mastiffs). DNA extraction was performed on white blood (WB) cells and semen, and TL was measured in the laboratory of Dana Farber/Harvard Cancer Center Genotyping and Genetics for Population Sciences, a modified qPCR method, as previously described [3]. Semen quality and fertility test were assessed. Semen and WB TL ranges were 0.8147 to 1.2357 and 0.6820 to 1.1491 Exp ddCT respectively, considering that anything over 1.00 is long compared to under 1, that results short. Results of semen parameters ranged as follows: Seminal volume: 8 to 35.5 ml; pH: 6.4 to 6.8; semen concentration: 168 to 940 x 10^6 spz/ml; total motility: 30 to 90%; progressive motility: 20 to 80%; viability: 60 to 90%; morphological anomalies: 3 to 10%. Data showed a parallelism with human telomere biomass. Analogies have been found between humans and dogs, such as telomere lengths, telomere shortening, absence of telomerase activity of somatic cells, which make the canine species an interesting model to understand the dynamics affecting TL during the course of the life of individuals and of the animal itself in relation also with the lifespan of the dog breed. In addition to understanding the dynamics, the results can extend to reproduction, specifically to infertility, as the shortening of spermatozoa TL has been associated with infertility in men [3].

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HORSES PHYSIOLOGICAL REACTION TO AN EXPRESSIVE SOCIAL HUMANOID

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The studies on the interaction between horses and humans have increased considerably in the last years, revealing the complexity of such interaction. Starting from the famous history of Clever Hans [1] till recent findings in which horses seem to be able to recognize human odor of fear and happiness [2], a deep channel of emotional communication can be postulate in horse-human interaction. We hypothesize that horses perceive changes of the human facial expression of emotion by using the visual channel. To verify the hypothesis we use the FACE (Facial Automation for Conveying Emotions), a human-like android robot which shows emotional information through facial expressions (www.faceteam.it) [3]. Therefore, it is able to socially act like human beings. Twelve female Standardbred horses (hosted at the "M. Modenato" Veterinary Teaching Hospital, University of Pisa) coped with three validated facial expression (happiness, sadness, anger) expressed by FACE. By using smart textiles device, the ECG and breathing dynamic have been collected and integrated in the same algorithms, for the correct analysis of the Heart Rate Variability in each horse. The horse was equipped with smart textile device and lead to the testing box. Before exposing to the robot, the horse was left alone (10 minutes) for basal data collection; after basal acquisition the box door was closed and FACE positioned in front of the box. Once the robot was placed, the box door was re-opened and the horse could see FACE in front of her (two wooden poles prevent the horse to touch the robot). The test began with further basal (2 minutes) condition, with FACE neutral facial expression. Immediately after, the three facial expressions (2 minutes each) were administered to the horse. The sequence of the FACE's facial expression was randomized for each horse. The corrected series of the ECG traces (2 minutes each) were then analyzed for extraction of the following features in the time domain of the heart rate variability: mean inter beats interval (RR, ms); its standard deviation (SDRR, ms), and root mean square of successive squared RR differences (RMSSD, ms). The direct comparison (Wilcoxon rank test) with neutral face revealed significant difference with happiness (RR, $p=0.002$; SDRR, $p=0.003$; RMSSD, $p=0.027$), sadness (RR, $p=0.042$; SDRR, $p=0.034$) and angry (RR, $p=0.007$; SDRR, $p=0.007$; RMSSD, $p=0.021$). These preliminary results indicate that the human emotional facial expression alone can activate the autonomic nervous system response in horses, with the main component of the sympathetic branch. Furthermore, to our knowledge this is the first study approaching the field of animals-robot emotive interaction.

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ANGIOGENIC PROPERTIES OF PORCINE VASCULAR WALL MESENCHYMAL STEM CELL SECRETOME

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Vascular Wall-Mesenchymal Stem Cells (VW-MSCs) are a promising resource in regenerative medicine thanks to their excellent angiogenic features [1]. Recently, stem cell secretome is intensively investigated as an alternative to whole-cell-based therapies, since it is enriched with proangiogenic factors and its composition change widely following different stimulations [2]. Previously, we described a new method to isolate and culture VW-MSCs from the tunica media of pig thoracic aorta: porcine VW-MSCs (pVW-MSCs). These cells have excellent pro-angiogenic features either for the ability of differentiating in endothelial cells and the capacity to sustain a capillary network [3]. Taking into account the translational value of the pig model, it is fundamental to characterize the secretome of pVW-MSCs both under physiological and LPS-induced inflammatory conditions. pVW-MSCs were isolated, characterized and expanded [3] from 3-mo-old female pigs (Large White) euthanized for other experimental purposes, to generate three primary cell culture replicates. Conditioned media were prepared treating pVW-MSCs with or without LPS (0; 0.1 and 10 μg/ml) for 4 hours, collected after LPS treatment or after 24 hours of recovery time. Cells and conditioned media were collected and stored until subsequent analysis. In order to investigate the possible effect of LPS on gene expression, a RT² Profiler PCR Array (RT² Profiler™ PCR Array Porcine Cytokines & Chemokines, Qiagen) was performed. Cytokines and chemokines presence in pVW-MSCs conditioned media were studied by multiparametric ELISA. In addition, pVW-MSCs conditioned media were tested for *in vitro* endothelial cells angiogenesis by tube formation and scratch test analysis. Our results clearly demonstrated that after LPS treatment, pVW-MSCs showed an altered gene expression profile: with a significant increase of cytokines (TNF-α, IL-1α, IL1-β, IL-6 and IL-8) and chemokines (CXCL2, CXCL10, CCL1, CCL20). The analysis of conditioned media revealed that pVW-MSCs secreted high levels of IL-8, GM-CSF, IFN-γ and other immunomodulatory proteins, such as IL-6 IL-18 IL-4 IL-2 IL-10. LPS induced a significant IL-6 and IL-8 increase, conversely, the amount of GM-CSF, IFN-γ, IL-2, IL-4, IL-10 and IL-18 showed a significant transient decrease. Conditioned medium from unstimulated pVW-MSCs induced *in vitro* endothelial angiogenesis, which was more evident when the conditioned medium derived from LPS stimulated pVW-MSCs. In conclusion, our results clearly demonstrated the angiogenic properties of pVW-MSCs secretome and its possible future applications in translational regenerative medicine models.

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IN VITRO CULTURE CONDITION EFFECT ON MITOCHONDRIA FUNCTION IN MOUSE EMBRYOS

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Somatic cell nuclear transfer (SCNT) allows the asexual reproduction of individuals by transplanting a somatic cell nucleus into an enucleated oocyte [1,2]. SCNT has tremendous potential for the rescue of endangered species [3] and the highly efficient production of transgenic animals [4]. Yet, despite several technical improvements [5,6,7], cloning efficiency remains very low (2-5%) [8]. We have recently shown that the major cause of abnormalities observed in cloned fetuses are mitochondrial dysfunctions in placenta collected from cloned sheep [9]. Investigations on mitochondria in SCNT are limited to the mtDNA hetero/homoplasmy in cloned offspring, whereas no data are available for an eventual role of mitochondria dysfunction on the developmental failure of cloned embryos/animals. Here we wanted to know whether mitochondrial abnormalities are observed already in cloned embryos since mitochondrial replication does not occur after the hatched blastocysts stage.

SCNT, *in vitro* cultured (IVC) and natural mated (NM) mouse embryos were produced with high percentage (80% - IVC; 42% -SCNT). Then, 2 cell embryos and blastocyst were analysed for mitochondrial structure and functionality.

The results showed a statistically lower expression of major mitochondrial proteins as well as the corresponding mRNA in SCNT and IVC embryos compared to NM group. Then, embryos stained with Mitotracker green and recorded on time-laps showed minimal fusion process in SCNT and IVC blastocysts compared to NM. Additionally, a decreased density of mature mitochondria, very high degree of cytoplasmic vacuolisation, numerous cytoplasmic vesicle and autophagosomes were observed in SCNT blastocyst.

The data indicate that *in vitro* culture conditions affect mitochondrial functionality and lack of nuclear-mitochondrial interaction at blastocyst stage can explain the potentially high development rate of SCNT embryos

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COMPARISON OF SLOW AND RAPID FREEZING FOR LONG TERM STORAGE OF FREEZE-DRY RAM SPERMATOZOA

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Semen lyophilization is an interesting technique that might be a cheap alternative to long-term storage under liquid nitrogen. The first significant result of this method was achieved by Wakayama and Yanagimachi in the 1998 [1] demonstrating for the first time the birth of healthy offspring from epididymal freeze-dried (mouse) spermatozoa. From this work on, the most used approach for lyophilisation is that of deep-freezing, that is directly immersing the semen sample into liquid nitrogen before vacuum drying. Recently we have shown that it is possible to establish a "dry" bank of ejaculated and epidydimal freeze-dried ram spermatozoa [2,3]. In order to improve and make the technique more reliable, here we focused on the freezing phase, comparing two different protocols: i) Fast-freezing, where the semen is plunged directly into liquid nitrogen (LN group); ii) Slow-freezing, where the sample is progressively cooled to a final temperature of -50°C (SL group). Briefly, for the preparation of the LN group sample the protocol reported in [2] was followed, while for the SL group the semen was frozen with a freezing rate of 1°C/min until -50°C degrees, when the sample was placed inside the lyophilizer. Dry spermatozoa from both groups was used for Intracytoplasmic Sperm Injection (ICSI) and the embryo development was evaluated at 24h (2-Cells stage) and 7 days (expanded blastocyst) after fertilization. At 24h post fertilization the SL-group showed a higher number of cleaved embryos than LN-group (42/100 (42%) versus 19/75 (25.3%), P=0.0253, SL and LN respectively). At 7 days after fertilization the blastocyst rate in SL-group was higher [7/100 (7%)] than in LN-group [2/75 (2.7%)], although not statistically different. Our data shows that lyophilisation can be conveniently achieved in ram spermatozoa without previous freezing in liquid nitrogen, thus simplifying the procedure. This data supports the idea that lyophilisation might be a valuable and cheaper alternative to liquid nitrogen for long-term storage of ram semen.

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EXPRESSION OF PROTEASE-ACTIVATED RECEPTOR 2 IN INTESTINAL TRACT DURING COLON TORSION IN HORSE

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Proteinase-activated receptor 2 (PAR-2) is a G-protein-coupled receptor activated from different serine proteases highly expressed in intestinal wall with multiple functions. Experimental data obtained from animal models have suggested a role for PAR-2 in the pathogenesis of inflammation of the gastrointestinal tract [1,2]. Recently the expression of PAR-2 in the equine small intestine after epiploic foramen herniation was described [3]. In order to complete the description of PAR-2 in horses with large colon disease, the aim of the present study was to investigate the expression of PAR-2 receptor in pelvic flexure of horses affected by large colon torsion. In this study, nine horses were recruited after admission to the Veterinary University Hospital (DIMEVET) for severe abdominal pain and colon torsion were recruited. Small full thickness samples (3x2 cm) were taken after the surgical evacuative colotomy and, after several washings with PBS, the intestinal mucosa was scraped using two glass slides. After RNA and protein extraction, quantitative Real Time PCR and western blot analysis for PAR-2 receptor was performed. At the same time, tracts of large colon from healthy horse were taken from the local slaughterhouse and then analyzed. To evaluate the expression of key molecules involved in inflammatory response, a focused panel of equine cytokines and chemokines genes (RT² Profiler™ PCR Array Horse Cytokines & Chemokines, Qiagen) was used. Our data showed no significant statistical difference of PAR-2 mRNA expression in pelvic flexure tracts between healthy and pathological samples. On the contrary, PAR-2 protein (44kDa) amount was statistically lower in pathological samples ($p<0.05$, Student's t-test). Moreover, a significant increase of the smaller band (25kDa) was observed ($p<0.05$, Student's t-test), indicating a huge activation of the receptor during intestinal torsion, in agreement with previous data described in the equine small intestine [3]. Finally, the array assay and subsequent validation, showed that three genes (CXCL1; IL8, MIP-2BETA) were upregulated in pathological samples ($p<0.05$, Student's t-test), suggesting that, activation of PAR-2 has a pro-inflammatory effect through the overproduction of inflammatory cytokines, in agreement with those obtained in other model [4]. This data could potentially give indications on intra and postsurgical therapy in equine colonic displacement.

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ANNUAL PROFILE OF HEAT SHOCK PROTEINS EXPRESSION IN EQUINE SPERMATOZOA AND ITS RELATIONSHIP WITH SEMINAL PARAMETERS

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Heat Shock Proteins (HSP) are a family of chaperons that protect cells from high temperatures [1]. In most mammals, spermatogenesis is negatively affected by high testicular temperatures even only reaching as high as body temperature [2]. Volpe et al. [3] showed that equine spermatozoa express HSP60, HSP70 and HSP90. The aim of this study was to evaluate, during all year, the expression of the three chaperons in spermatozoa collected from six adult stallions of proven fertility, routinely employed for A.I. Moreover, we aimed to highlight a possible correlation among HSP expression and some seminal parameters: concentration, viability, total motility (MOT), progressive motility (PMOT) and mitochondrial membrane potential. Monthly, a semen sample was collected from each stallion by an artificial vagina. Seminal parameters were evaluated by CASA-System (IVOS 12, Hamilton-Thorne, USA). Viability was studied by SYBR Green 14 and Propidium iodide while mitochondrial status by JC-1 (Molecular Probes). The expression of the three chaperons was analyzed by western blot employing the following human monoclonal primary antibodies: anti-human HSP60, HSP70, HSP90 and GAPDH (Stressgen Biotechnologies Corp., Victoria, BC, Canada.), the last used for signal normalization. The intensity of each positive band was quantified by Quantity-One Software (BioRad, Milan, Italy) and results expressed in arbitrary units as Mean±SD. All data were analyzed for statistical significance by the SPSS software. Data were checked for normal distribution by Kolmogorov-Smirnov test and analyzed by one-way ANOVA for differences and by Pearson's test for correlations. Our results showed a significant difference ($P<0.001$) in the expression of the three chaperons during the year, with the highest level in April and August for HSP60, in July and August for HSP70 and in March and July for HSP90. Moreover, between MOT and PMOT, a positive correlation ($P=0.000$; $R=0.449$) was found. MOT was also correlated to HSP60 ($P=0.000$; $R=0.411$), to HSP70 ($P=0.000$; $R=0.433$) and to HSP90 ($P=0.000$; $R=0.464$). Surprisingly only HSP90 was correlated to sperm viability ($P=0.010$; $R=0.302$), while all the chaperons were positively correlated to sperm concentration (HSP60: $P=0.000$; $R=0.424$; HSP70: $P=0.006$; $R=0.318$; HSP90: $P=0.001$; $R=0.384$). Mitochondrial status was only correlated to viability ($P=0.000$; $R=0.623$) and to sperm concentration ($P=0.001$; $R=0.384$). Taken together, these results underline the possible role of the three HSPs in modulating the physiology of the equine spermatozoa being more expressed during the breeding season and in the period of the year in which environmental temperature is highest, thus minimizing the unfolding state of the sperm proteins especially those having a role in cell to cell interactions that are necessary for fertilization.

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EXPLORING THE USEFULNESS OF HAIR CORTISOL AS STRESS BIO-MARKER IN NEBRODI BLACK PIG FARMS

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Cortisol is a steroid hormone produced by the Hypotalamic-Pituitary-Adrenal (HPA) axis. It is considered to be the main biomarker of chronic stress altough its blood levels might vary depending on several factors (circadian rhythms, food intake). Moreover, blood sampling might induce acute stress-related cortisol rise. In the present study, the authors evaluated the possible measurement of cortisol from hair in order to assess stress levels in Nebrodi black pig (Sus scrofa), purposing a non-invasive alternative to the standard measurement on serum. Sampling was carried out in different farms located in the Nebrodi area. Hair and serum samples were collected from ninety-five pigs. Welfare was assessed by the "Twelve Criteria of animal welfare". Hair cortisol measurement was carried out as previously described by Davenport et al. [1]. A significant positive correlation was observed between hair cortisol and stress levels. From the serum analysis it can be seen how the values remain below 20 µg/ml for the serum and for 20 pg/mg for the hair extract. These results show correlation between hair cortisol and serum cortisol, but due to the low levels detected, the realiability as a stress biomarker and could not be demonstrated. Therefore, the measurement of the hormone from these samples could potentially provide insight into the welfare status of pigs during their lifetime. However, further larger scale studies are needed in order to allow a more effective and standardized evaluation of cortisol in relation to animal welfare in different farming conditions of the Nebrodi black pig and to its conservation in the Sicilian territory.

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ORAL COMMUNICATIONS

SOIPA

LEISHMANIA INFECTION AND BLOOD MEAL SOURCES IN PHLEBOTOMINE SAND FLIES FROM SICILY

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Leishmaniasis is a zoonotic protozoan vector-borne disease transmitted by phlebotomine sand flies [1]. Detection of natural *Leishmania* infection in vectors is a key factor to assess the risk of transmission and to address control strategies timely. Additionally, the study of blood meal sources in sand flies provides data on their feeding habits and allows the identification of potential hosts/reservoirs for *Leishmania*. In this study we investigated the presence of *Leishmania* DNA and identified blood meal sources in sand flies caught in five selected sites in Sicily along two transmission seasons.

The genomic DNA was extracted from 1,866 female sand flies (*S. minuta* n=1,264; *P. perniciosus* n=594*; P. sergenti* n=4*; P. perfiliewi* n=3*; P. neglectus* n=1), including 176 blood-fed specimens. Detection of *Leishmania* DNA was carried out by the amplification of the internal transcribed spacer 1 (ITS1), and cytochrome B gene (cyt-b) was subsequently targeted to confirm ITS1 positive amplification results. As some of the ITS1 positive sequences revealed high identity with *Trypanosoma* spp. using BLASTn, the sequences were also tested for *Trypanosoma* spp. by the amplification of small subunit ribosomal RNA (SSU rRNA). Identification of food sources in engorged females was performed by amplifying the host mitochondrial cyt-b gene.

Twenty-eight sand flies (1.5%) out of 1,866 scored positive to *Leishmania* spp. In particular, *Leishmania tarentolae* was isolated in 26 specimens of *Sergentomyia minuta*, while *Leishmania donovani/infantum* was detected in 2 sand flies, *S. minuta* (n=1) and *Phlebotomus perniciosus* (n=1). Interesting, seven *S. minuta* specimens (0.4%) tested positive to *Trypanosoma* sp. vertebrate host of 108 out of 176 blood-fed females was successfully identified. Wild rabbits (27/82) represented the most preferred mammal species for *P. perniciosus*, whereas *S. minuta* mainly fed on humans (16/25); several other vertebrate hosts (e.g. horse, goat, swine, dog, chicken, cow, cat, donkey, rat) have been recognized in both species.

The presence of *L. infantum* DNA in *P. perniciosus* confirms the role of this species in the maintenance and spread of leishmaniasis in Sicily. Though sand flies are commonly regarded as generalist feeders, the higher frequency of blood meals on wild rabbits suggests a kind of preference of *P. perniciosus* to this mammal species and puts forward the hypothesis on its involvement in the epidemiology of leishmaniasis as sylvatic reservoir. Finally, the detection of *L. infantum* and *Trypanosoma* sp. DNA in *S. minuta*, together with the anthropophilic feeding-behaviour herein observed, incite to clarify the ability of this species in the transmission of pathogens to humans and other warm-blooded animals.

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IDENTIFICATION OF PHLEBOTOMINE SAND FLIES THROUGH MALDI-TOF MASS SPECTROMETRY AND IN-HOUSE REFERENCE DATABASE

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Phlebotomine sand flies are vectors for many pathogens responsible for human and animal diseases worldwide. Their identification at species level is of importance in epidemiological studies and control programmes [1]. MALDI-TOF MS has been increasingly investigated as an alternative approach to the conventional identification of arthropods species [1-4]. To establish an in-house protein spectra database for a quick and reliable species identification of phlebotomine sand flies, 166 field-caught sand fly specimens, morphologically identified as *Phlebotomus perniciosus* (n=56; 26 males and 30 females), *Phlebotomus neglectus* (n=4 males), *Phlebotomus sergenti* (n=6; 4 males and 2 females) and *Sergentomyia minuta* (n=100; 45 males and 55 females), were subjected to MALDI-TOF MS analyses. Out of 166, 149 specimens (89.8%) produced consistent species-specific protein spectra.
SuperSpectra for *P. perniciosus* and *S. minuta* were generated, while no databases have yet constructed for *P. neglectus* and *P. sergenti* due to the low number of specimens examined. Through validation test, 80 sand flies (n=20 *P. perniciosus*; n=60 *S. minuta*) were analyzed and confirmed using the new generated SuperSpectra. Results herein reported support the use of MALDI-TOF MS for phlebotomine sand fly species identification advocating its usefulness in survey studies in order to improve basic knowledge on these important vectors of zoonotic pathogens. MALDI-TOF MS, indeed, presents remarkable advantages respect to conventional morphological approach and/or PCR-based methods allowing rapid, simple and reliable identification [4].

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EFFICACY OF IMIDACLOPRID 10% /MOXIDECTIN 2.5% SPOT ON (ADVOCATE®) AND DOXYCYCLINE IN THE TREATMENT OF DOGS NATURALLY INFECTED WITH *DIROFILARIA IMMITIS* IN ITALY

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Dirofilaria immitis, the filarial nematode responsible for heartworm disease in both cats and dogs, is endemic in different areas of the world and is spreading in previously unaffected areas because of climate changes and the diffusion of the mosquito Aedes albopticus, active and biting also during the day [1]. New pharmacological protocols more efficacious both against microfilariae and adult worms are constantly required. Here we evaluated the efficacy of Advocate® (10% imidacloprid + 2.5% moxidectin) and doxycycline treatments on dogs naturally infected with D. immitis. Fourteen dogs were treated with Advocate®+doxycycline (moxi/doxy) and six with the adulticidal drug melarsomine dihydrochloride (Immiticide®) as control treatment. Moxi/doxy protocol foresaw the administration of doxycycline (10 mg/kg BID) for the first 30 days + topical administration of Advocate® every four weeks for 9 months. The control group was treated with Immiticide® (2.5 mg/kg) at the moment of enrollment, followed one month later, by two injection of the same dose, 24 h apart. The presence of circulating antigens (indicating the presence of adult worms) and the number of microfilariae (mf) were evaluated at the moment of enrollment and then at various time points (1,2,3,4,5,6,7,8,12,18,24 months). Echocardiogram and radiographs were performed at month 0,6,12,18,24. All dogs, except one, treated with moxi/doxy were negative for circulating antigens by nine months post enrollment (p.e). One dog treated with MEL became antigen negative at three months p.e., three at four months, one at five and one at six months p.e. Evaluation of mean optical density values, showed a rapid reduction in antigen concentration in control dogs, while the decreasing was slower in moxi/doxy group. Regarding mf concentration, moxi/doxy induced a consistent reduction in mf count (P value <0.0001) with a 99.9% efficacy already after one month. At the enrollment two of the six dogs treated with Immiticide® were microfilaremic. One became negative at the third check, while the other only at eighth month. No dogs showed worsening of pulmonary patterns or criteria indicative of pulmonary hypertension. Moxi/doxy treatment was already confirmed to be efficacious in experimentally infected dogs [2] and our data are comparable. The two year trial in naturally infected dogs performed here highlights the excellent efficacy of the combined treatment. We can conclude that moxi/doxy induced a more marked adulticidal effect than the commonly used doxy+ivermectin [3], lighter side effects than Immiticide® and the rapid elimination of mf resulted in a quick break of the transmission cycle of *D. immitis*.

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PREVALENCE AND DIVERSITY OF HAEMOSPORIDIAN PARASITES IN HOODED CROWS (*CORVUS CORNIX*) FROM PIEDMONT REGION

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Haemosporidian parasites belonging to the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* form a group of vector-transmitted blood parasites inducing the avian malaria. Many recent studies on *Corvidae* family suggest that corvids can harbour a great number of Haemosporidia [1,2]. The aim of this study was to investigate the diversity of haemosporidian parasites detected in different organs of 47 hooded crows (*Corvus cornix*) from Piedmont region (Italy), identifying the involved lineages. DNA sequencing was performed on samples tested positive by nested PCR and previously reported [3], and cytochrome b gene haplotypes were identified by comparison with obtained sequences from GenBank and MalAvi.

A high prevalence of haemosporidian parasites was detected (97.9%, n=46). *Leucocytozoon* was the most prevalent genus (100%, n=46) followed by *Plasmodium* (52.2%, n=24) and *Haemoproteus* (17.4%, n=8). Five new different lineages of *Leucocytozoon* (LC2-LC6), shared with other corvid species, and a new lineage of *Haemoproteus* (HC1) were detected. Moreover, three common lineages of *Plasmodium* (PL1, PGRW6 and PSGS1) were identified for the first time in hooded crows. Mixed infections of two or more Haemosporidia lineages were common (66.0%, n=31) and 18 hooded crows (58.1%) harboured multiple haemoparasite genera.

Many studies reported a great prevalence of *Leucocytozoon* spp. in corvids [1, 2], suggesting a restriction of the host variety for the haematophagous vectors of this genus [4]. Co-infections with two or more malaria parasites are frequent in wild birds [1]. Existing parasitic infection in a host may influence the probability of a secondary infection by immunosuppressing or down-regulating the host immune system. Many researchers suggest that the infection likelihood by parasites of one or more genera is enhanced by infection with others [5, 6]. The great abundance and the wide distribution in the Piedmont Region allow to consider the hooded crows as a possible local reservoir of different avian malaria lineages, with potential effects on the transmission of the disease to other birds. Numerous migratory avian species that use the route across the Italian peninsula moving from or to Africa could be exposed to infection risk, especially for host-generalized haemoparasites.

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RETROSPECTIVE STUDY ON CANINE LEISHMANIOSIS IN SOME COSENZA MUNICIPALITIES (ITALY)

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Canine leishmaniosis (CanL) is a parasite-borne disease, caused by protozoa of the *Leishmania* genus. In the Mediterranean Basin, the disease, caused by *L. infantum*, is transmitted through the bloodmeal of the sand flies belonging to the *Phlebotomus* genus. Additionally, its not negligible zoonotic role is well known, causing human visceral and cutaneous leishmaniosis. According to the World Health Organization [1], it is considered one out of the seven tropical diseases most relevant and represents a worldwide great concern and then being an old neglected disease [2]. The survey was carried out over the three years (2013-2015) on the CanL prevalence of the dogs, hosting in the shelters across the Cosenza province during the Regional surveillance program. The presence/absence of disease was evaluated by using the IFAT according to OIE [3]. Overall, sera from 745 dogs have been obtained and tested, the total prevalence was 30.3% (226/745). During the study period the trend of the disease went up (2013-14%; 2014-35%; 2015-42%) which was significant at the Pearson's x^2 test (x^2=50.61, P<0.0001), performed by using Stata 15.1 software. In addition, 95 out of 745 (12.8%) sera had a doubt IFAT result, titer = 1:40 or 1:80, mostly belonging to the juvenile age. The obtained prevalence shows, once more, the severe endemic, if not epidemic, status of *Leishmania* in Cosenza province around 30% and a trend growing up during the studied period of about +46% from 2013 to 2015. These data can be compared only by another survey carried out in the same area 15 years back by Poglayen et al. [4], where it is reported a 6% prevalence and a growing trend of +0.8%. The national canine registry during the three-year period reported a total of 47,354 dogs present in the study area and by considering the great number of stray dogs this amount is doubtless underestimate. A greater number of dogs from the beginning of this century accompanied by an increasing trend of the CanL pose a risk also from a public health aspect. In a close future, it would be interesting to evaluate the incidence of the human cases in people coming from the same area. Furthermore, by this experience is possible to update the popular tool Scalibor®map with 19 new positive municipalities (list available to the Authors). Considering the above results, it is clear enough that what it has been made, up to now, is not adequate in order to control leishmaniosis.

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SEROLOGY AND MOLECULAR SCREENING FOR *TOXOPLASMA GONDII* IN ANIMAL AND FOOD PRODUCTS IN SICILY, ITALY

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Toxoplasma gondii is a major parasite mainly transmitted through food and water world-wide. The tissues and meat samples of many warm-blooded animals can contain cysts from chronic toxoplasmosis. Water and vegetable can be contaminated by the parasitic oocysts shed through the feces of infected felines, (mainly cats) representing the definitive host of the parasite [1].
In Sicily human cases linked to the consumption of raw sausage are sporadically reported [2]. The aim of the work was to establish the sero-prevalence of toxoplasmosis in ruminant farms from many Sicilian provinces and in farms of the autochthonous breed Black Swine of Nebrodi. A molecular screening was also performed on animal tissues and in food products. The following farms were included in the study: 57 bovine, 128 sheep, 14 goats and 94 sheep and goats mixed farms; 27 farms of the Sicilian black swine of Nebrodi were also tested for serology and 10 additional farms for both sera and brain samples of slaughtered animals. The serology screening was performed by Elisa (Toxo Id. Vet) following manufacture's instructions. Farm with a single positive animal were considered positive. The molecular analysis was performed by nested PCR targeting the ribosomal RNA locus. Common apicomplexan primers were used for first PCR followed by a *T. gondii* specific nested PCR [3]. Serology screening showed an average seroprevalence of 54% for sheep, 35% for goats, 25% for cattle. Toxoplasmosis in the autochthon breed (black swine of Nebrodi) was almost 60%. Wild boar showed an average prevalence of 55%. Positive Results by PCR were obtained on tissues of 2/112 Swine; 12/125 Black swine of Nebrodi (free and semi-free ranged) 11/96 wild boar. In food products analysis 4 /120 fresh sausages, 1/42 hamburgers resulted positive also. *T. gondii* is mainly a parasite transmitted through contaminated food and water. The high serology prevalence in livestock species in Sicily, suggests a transmission risk through the consumption of undercooked meat. This is especially true for pork sausages. Grilled pork sausage and steaks are not uniformed cooked especially in the internal parts and probably the high temperature needed to kill the parasite is not reached in these portions of meat. This can be particularly worrying for food safety. We suggest to underline that the consumption of unevenly grilled meat, is a potential risk factor for the transmission of *T. gondii* and seronegative pregnant women should be particularly aware in grilled steaks or hamburgers consumption

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SURVEY ON TICK-BORNE PATHOGENS IN TICKS REMOVED FROM HUMANS, NORTHWESTERN ITALY

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Ticks are able to transmit several pathogens to the host while feeding, and thus are considered the most important vectors of infectious agents together with mosquitos [1]. Due to increased interactions between pathogens, hosts and vectors, the global incidence of tick-borne diseases (TBDs) is rising [2]. In Italy, papers have been published on the distribution of ticks and TBDs in animals [3], while information about ticks collected from humans are limited to few regions [4,5]. As a consequence, no data are present for Piedmont, one of the largest regions of Italy hosting more than 4 million inhabitants. The present study aims to identify, through a passive surveillance system, the species of ticks biting humans in Turin province and the tick-borne pathogens they harbour. An overall number of 128 ticks from 92 patients were collected from April to October 2018, almost 98% of which belonging to the *Ixodes ricinus* species. Nymphs were most frequently collected (78.9%, 101/128), followed by adults (13.3%, 17/128) and larvae (7.8%, 10/128). Among adults, 5 females were fully engorged at the time of removal. Ticks were grouped into pools: specimens collected from the same patient and homogeneous for species, developmental stage, sex and engorgement status were pooled together. Molecular analysis showed the presence of *Babesia* spp. in 29 out of 93 analysed tick pools, with a Minimum Infection Rate (MIR) of 31.18% (29/93; CI95% 22.67-41.19%), while 1 out of 93 pools tested positive for SFG rickettsiae (MIR = 1.08%; CI95% 0.19-5.84%). No samples tested positive for *A. phagocytophilum* and *Borrelia* spp. Sequencing revealed the presence of *Babesia venatorum* (28 pools), *Theileria buffeli/orientalis* complex (1 pool) and *Rickettsia monacensis*. Two patients (2.15%) reported a local rash in the weeks after the tick bite that heal spontaneously without treatment. Ticks collected from these patients tested negative for all the analysed pathogens. Statistical analysis did not show association between tick positivity for any of the pathogens and possible risk factors as age, gender, frequented environments and activities at the time of tick bite. It is noteworthy that *B. venatorum*, the most prevalent reported species, and *R. monacensis* are zoonotic species able to cause from moderate to severe infections in humans. Although no ticks tested positive for *Borrelia* spp. in our study, up to 7% of ticks collected from humans were infected with this pathogen in Northern Italy [5]. Together, these data highlight the importance of passive surveillance to assess the epidemiology of TBDs that pose a threat to human health.

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EXPOSURE TO ZOONOTIC VECTOR-BORNE PATHOGENS IN CATS FROM ITALY

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Feline Vector-Borne Diseases (VBDs) are of growing concern in veterinary medicine and public health, for their role for animal and human health [1,2,3]. Nevertheless, there is a lack of data both on the epidemiology and clinical features. Thus, increasing the knowledge on these aspects is of great importance to raise awareness of VBDs and improve diagnostic approaches in clinical settings. This study evaluated the exposure of cats living in Central and Southern Italy to different VBDs caused by the following pathogens: *Bartonella henselae*, *Rickettsia felis*, *Rickettsia typhi*, *Anaplasma platys*, *Anaplasma phagocytophilum*, *Ehrlichia canis*, *Leishmania infantum* and *Dirofilaria immitis*. One-hundred and sixty-seven privately owned cats from Abruzzo and Apulia regions (i.e. n. 122 and n. 45 respectively) were microscopically (blood smears) and serologically (Immunofluorescence Antibody Test -IFAT) tested. Out of these, 46 were also evaluated for anti-*D. immitis* antibodies. Complete blood count (CBC), and serum chemistry were obtained from 42 and 44 animals that scored positive to at least one of microscopic or serological evaluations, respectively. All samples were microscopically negative. Overall, 52/167 cats (31.1%) were positive at IFAT for at least one VBD, specifically 35 (28.7%) from Abruzzo and 17 (37.8%) from Apulia. Thirty cats (18%) were seropositive for *B. henselae*, while 18 (10.8%) and 7 (4.2%) tested positive for *R. felis* and *R. typhi* respectively. Five cats (3%) showed seroreaction against *L. infantum*, while 4 and 4 cats each showed seropositivity for *A. phagocytophilum* and *E. canis*. No cats were positive for *A. platys*. Two (4.3%) of 46 cats were positive for antibodies against *D. immitis*. Nine (17.3%) out of the 52 positive animals had at least one clinical sign, with non-specific and respiratory manifestations being the most recorded. Different CBC abnormalities were present in 33/42 (78.6%) cats while serum chemistry alterations in 35/44 (79.5%) animals, being basophilia and increased liver enzymes predominant. Twenty-five (48.1%) out of the 52 cats seropositive to at least one VBDs were housed indoor. These data suggest that cats may be frequently exposed to VBDs in the study areas, and that also privately owned cats are at risk. Control strategies remain crucial for the prevention of feline VBDs [4] and antiparasitic drugs should be administered regularly regardless the cat lifestyle and housing, to reduce the risk of infection and to protect both animal and human health.

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HAVE YOU NEVER THOUGHT ABOUT IT? LEECH INFESTATION BY *LIMNATIS NILOTICA* IN CATTLE

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Leeches belong to the *Annelida* phylum and *Hirudinea* class and are world-wide distributed [1]. Leeches are segmented hermaphroditic blood feeding ectoparasites of man, domestic and wild animals. Among species, *Limnatis nilotica* is one of the most important; it occurs in lakes and streams in southern Europe, Middle-East countries and North Africa [2]. This species may enter the animal body through drinking from infested waters and most of them attach to the oral cavity or respiratory passages [1]. In July 2017, twenty cattle of a free-grazing herd in the province of Messina (southern Italy, 38.0239N; 14.7298E; 400 m a.s.l.) were found leech infested in the mouth. Main signs were bloody sialorrhea and/or a purple-red color of the lower lip. Leeches, in a variable number (1 to 3) per animal, were found at the lingual frenulum or on the sublingual vestibular mucosa. The leeches measured 2.5-3.0 cm in length and 0.8-1.0 cm in width with dark green-black color on the dorsal side and a lighter color on the ventral side. The well-developed posterior sucker had a diameter equal to the maximum width of the body, while the anterior sucker was featured by a median ventral furrow. Triple jaws, consisting of one dorso-medial and asymmetrical pair of ventro-lateral jaws were observed inside the oral cavity. The jaws appeared rounded, soft and light grey with clearer spots. A number of papillae were observed on both sides of jaws and a row of tooth craters, irregular in size, shape and spacing, was observed on their edge. All the collected specimens were identified as *L. nilotica* according to morphological keys reported elsewhere [1, 3]. To the best of our knowledge, this is the first report of cattle infestation by *L. nilotica* in Italy. Besides recalling the attention to leech infestation and suggesting its inclusion in the differential diagnosis of animals with suggestive signs, this short report also provides practitioners with easy-going keys for proper diagnosis and discrimination among species.

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COPROLOGICAL SURVEY OF HUNTING DOGS FROM NORTHERN ITALY

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Hunting dogs are animals with a predominant outdoor lifestyle, often frequeting environments with low anthropization and presence of wildlife that could represent a reservoir of parasites [1-2].
The present study reports the results of coprological investigations performed on hunting dogs from January 2017 to March 2019. Overall 88 fecal samples of different hunting dogs were analyzed by FLOTAC® dual technique [3]; flotation solution FS4 ($NaNO_3$, s.g.=1200) and FS7 ($ZnSO_4$, s.g=1350) were used. The following individual features were introduced in a general linear model (GLM)as independent variables to determine if they were predictors of being infected by at last one endoparasite: age, sex, neutered or not, province of origin, number of other cohabiting dogs.
Recorded individual features of tested dogs showed that samples were collected from 48 females and from 40 males; 10 dogs out of 88 were neutered. Dogs came from Bergamo (4/88) Brescia (1/88), Lodi (2/88), Milano (1/88), Pavia (61/88) Sondrio (10/88) and Varese (9/88) provinces. Eighty-seven out of 88 were purebred dogs. Dogs on average were 5.4 years old. At least one endoparasite was detected in 37 dogs out of 88 (42.05%); detected helminthic taxa were *Toxocara canis* (15/88; 17.05%), *Eucoleus bohemi* (10/88; 11.36%), *Trichuris vulpis* (8/88; 9.09%), *Toxascaris leonina* (2/88; 2.27%), Ancylostomatidae (1/88; 1.14%) *Eucoleus aerophilus* (1/88; 1.14%) and *Alaria alata* (1/88; 1.14%). Protozoan infections sustained by *Giardia duodenalis*, *Sarcocystis* sp. and *Cystoisospora* sp. were detected in nine (10.23%), four (4.55%), and three (3.41%) dogs out of 88, respectively. Final GLM obtained by backward elimination showed that the number of other cohabiting dogs was a significant risk factor for a hunting dog of being infected by at last one endoparasite ($p<0.001$; OR=1.190; 95% CI:1.085-1.304). Positive dogs cohabited with 9.2 dogs on average whereas negative ones with 4.8.
The study highlighted the exposure of parasites in hunting dogs in northern Italy. The recording of rare parasitic species (*E. bohemi, A. alata, Sarcocystis* sp.) in hunting dogs respect to companion dogs in the studied area [4] showed the hunting dogs are highly exposed to parasitic infection and harbor more different taxa.

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CLINICAL AND LABORATORY DIAGNOSIS OF LEISHMANIASIS IN DOGS

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Canine visceral leishmaniasis (CanL) is a chronic systemic disease caused by protozoans of the genus *Leishmania* and characterized by a broad spectrum of clinical manifestations in dogs with different degrees of severity [1], due to a complex interaction between the parasite and the host immune response [2]. Thus, it is very important to perform an early diagnosis and to establish the evolutionary stage of the disease, for an appropriate therapy and to prevent evolutions towards more serious phases [1]. The aim of this study was to compare two molecular and two serological techniques for the diagnosis of CanL in naturally infected dogs, specifically: i) Loop-Mediated Isothermal Amplification (LAMP) technique; ii) Real-time Polymerase Chain Reaction (Rt-PCR); iii) Immunofluorescence Antibody Test (IFAT); iv) Enzyme-Linked ImmunoSorbent Assay (ELISA). For this purpose, lymph nodes and sera samples were collected from 47 dogs with clinical signs of CanL. Dogs excluded from the study were: pregnant or lactating bitches or subjects on immunosuppressive therapy or dogs that had been treated for CanL in the previous 6 months. Before sampling, each dog was submitted to a physical examination and a clinical form was filled out. For LAMP, DNA extraction from lymph nodes and amplification were performed using the kit Leishmania Screen Glow (Enbiotech, Italy) following the producer's instructions. An aliquot of each sample was sent to the National Reference Center for Leishmaniasis (CReNaL, Palermo) to perform Rt-PCR. Serological analyses were performed by both an IFAT using slides provided by CReNaL to detect anti-*Leishmania* antibodies (cut-off titer ≥1:160) and a commercial ELISA (ID Screen Leishmaniasis, ID VET; specificity=100%, sensitivity=95.3%, accuracy=97.5% [3]). Thirty of the 47 enrolled dogs showed severe clinical signs (63.8%). The most frequent clinical signs were lymphadenopathy (80.0%) and skin lesions (60.0%). The most frequent clinicopathological signs were alterations of total proteins and low A/G ratio (73.3%), thrombocythopenia (46.6%) and non-regenerative anemia (40.0%). At molecular analysis, 21 samples of DNA extracted from lymph nodes (44.7%, 95% Confidence Interval=30.5-59.8%) resulted positive for at least one technique (LAMP or Rt-PCR). In particular, 19 samples were positive at the LAMP and Rt-PCR, whilst 2 samples resulted positive only for LAMP (Negative predictive value= 92.9, 95% CI=75.0-98.8). At serological tests, 31 samples (66.0%, 95% CI=50.6-78.7%) resulted positive for IFAT, whilst 28 (59.6%, 95% CI=44.3-73.3%) were positive and 1 doubtful (2.1%, 95% CI= 0.1-12.7%) for ELISA. All the positive samples at PCR techniques were positive also when analyzed with serological tests. However, LAMP showed a very good agreement (k=0.931; P<0.0001) with Rt-PCR, therefore although LAMP is a qualitative technique [4], it could be promising for a rapid diagnosis of leishmaniasis, because permits to obtain diagnostic results in about 1h. Nevertheless, further studies should be performed on matrices different from lymph nodes to confirm these findings.

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POSTER

P1 - PROPOSAL OF A METHOD TO INCREASE THE NUMEROUSNESS OF BIOMETRICAL PARAMETERS USEFUL FOR WILDLIFE MANAGEMENT

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The study of size (biometry) and shape (geometric morphometry) of bone structures in Ungulates are practices of extreme importance in the management of wildlife populations [1-2]. The collection of biometric data allows to characterize the populations, to evaluate their performances year after year, helping to estimate their status and trend of population features. Geometric morphometry, on the other side, plays an important role in the analysis and description of the shape of bone structures and related modifications. Unlike classical biometry, which involves the use of a calibre for measurements, geometric morphometry acquires, through software (e.g. GeoGebra), a series of reference points (landmarks) from digital photos, also providing a series of potentially linear measures, not taken by classic biometry, that could be used in assessing the relationship among body parameters and environmental features. However, the linear measurements recorded by GeoGebra represent distances between Cartesian coordinates. So, a conversion index is needed to be able to use this pool of extra-data [3-4]. We attempted to individuate the conversion index using 27 mandibles pertaining to Roe deer (*Capreolus capreolus*) of different age classes obtained by wildlife selective hunting in the Territorial Hunting Zone ATC-MC2 in Macerata Province (Central Italy). We focused the attention on two parameters: 1) Mandible Length (ML) which extends from the first incisive tooth to mandible angle for biometry, and from the first incisive tooth to the half of mandible angle for GeoGebra, and 2) Teeth Row Length (TRL) which extends from aboral limit of diastema to mesial limit of last molar tooth for both the measurement methods. The photos of the mandibles must be taken at the same time of the measurement with the calibre. The results showed that the two series of measurements are correlated (R^2>0.8) and that the GeoGebra/Calibre ratio was 0.04. This coefficient could be used as a conversion index, allowing to obtain additional data derived from GeoGebra. Obtained additional data can be used to improve the database of parameters suitable to study the relationship with environmental features and, therefore, to assess the status of the Roe deer populations.

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P2 - INCREASING DROUGHT STRESS AFFECTS THE FARM PRODUCTIVITY: STUDIES AIMED TO THE CONSERVATIVE MANAGEMENT OF THE NATURAL GRASSLANDS

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Grazing activity is fundamental to maintain grassland biodiversity and this goal can be reached only ensuring economical sustainability to the farmers. We presented 2 studies dealing with the application of anatomical competences in actions linked to conservative natural pasture management. The first study aimed to buffer the effects of increasing summer drought stress on natural grasslands evaluating food supplementation on apelin system in mammary gland and milk/cheese production (HM approval 95/2018-PR). A flock of 55 adult ewes was free to graze from June until pasture anthesis (MxF). Then until pasture maximum dryness, the flock was divided in two groups: control (Cnt), fed only on pasture, and experimental (Exp), also supplemented with cereals. Apelin (APLN) and its receptor (APLNR) were assessed by Real-Time PCR and immunohistochemistry. They were detected in alveolar and ductal epithelial cells. Immunohistochemical findings showed the major expression of APLN in MxF group and it decreases in Cnt and Exp groups. The APLNR was differently expressed among the three groups. The maximal APLN mRNA abundance was found in MxF group that showed a significant difference with the Cnt and Exp groups. Cnt and Exp groups did not show differences. APLNR mRNA was most abundant in MxF group, showing significant difference with Cnt and Exp groups. The reduced expression during parenchyma involution of APLNR could suggest its modulating role in the system control [1]. Milk production and composition showed significant difference among the three groups. Quality and peculiar features of cheese were evaluated by means of sensory panel. A consumer test associated with an experimental auction was used to evaluate consumer preference and willingness-to-pay. Collected data suggested a possible strategy to adopt by farmer to enhance farm income [2]. The second study evaluated ovine effectiveness in wood fire prevention setting the length of animal stay on *Brachipodium rupestre* high covered pasture before their well being is compromised. A flock of 50 adult ewes was divided in control and experimental group, grazing on a semi-mesophilic natural pasture and a plot covered of *Br. r.* respectively. Body weight (BW), body condition scores (BCS) and rumen epithelial keratinization degree were assessed for three weeks. Control group showed little variation in the keratinisation degree of rumen mucosa without any detrimental effects on the BCS and BW. The experimental group showed a significant increase in the epithelial keratinisation degree within 10 days and a decrease of BCS and BW within 20 days. Findings gave the opportunity to use flock for up to 12 days to clean pasture from *Br. r.* also offering an ecosystem service [3].

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P3 - THE INVOLUTION OF THE PROXIMAL SESAMOIDEAN LIGAMENT OF SHEEP: ULTRASTRUCTURAL AND MOLECULAR EVIDENCES

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In ungulates the stability of the fetlock joint is dependent on several muscles that are exposed to high stress and strain. Among those muscles, there is the proximal sesamoidean ligament (PSL); this muscle at birth is organized in layers of muscle fibres alternated with abundant connective tissue that, during the postnatal development, becomes the predominant [1]. The aim of our work was to investigate the putative mechanisms underlying the changes that lead to the shift from muscle to connective tissue at ultrastructural and molecular levels at 1, 30 and 180 days after birth.
TEM (Transmission Electron Microscopy), Immunohistochemistry, SDS-gel, Western blotting and Real Time PCR.
TEM analysis revealed a significant decrease of satellite cells already at 30 days. Concomitantly, we observed a quick proliferation of fibroblasts in the muscle connective tissue transitional area. This trend was confirmed by analysis of the gene expression of Pax7, marker of quiescent satellite cells, which showed a significant reduction at 30 and 180 days. During the first six months after birth, we observed a transition of MyHC isoforms towards the slow isoform (MyHC-1) with both Real Time PCR and Western blot analysis. Moreover, at 180 days, an altered expression of the Wnt signalling was observed (high expression of Wnt1 and absent expression of Wnt4) and this is considered as a signal of an on-going fibrosis process [2,3]. At the same time we detected a specific myogenic expression pattern (involving MyoD, Myf5 and MYOG) [4] and high mRNA levels of IGF-1 and TGF-β1. The latter is known to stimulate the growth of connective tissue, influencing mesenchymal stem cells differentiation towards a fibrogenic *lineage* [5,6]. Our study confirmed the peculiarity of the fast involution observed in PSL. This muscle undergoes a very specific active differentiation process during early development, which implies myofibres degeneration as observed at ultrastructural level. For these reasons, we suggest to consider the PSL as an excellent model of muscle fibrosis.

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P4 - PRODUCTION AND DETECTION OF AVIAN MYCOPLASMA BIOFILM: PRELIMINARY RESULTS

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Mycoplasmas are important pathogens for the poultry industry. Mycoplasma infection control is based on the evidence of horizontal and vertical transmission. Thus, the creation of Mycoplasma-free breeder groups and the application of strict biosecurity measures, such as avoiding direct contact with infected birds, avoiding closeness between personnel and equipment in different bird flocks, etc., are reported as effective against Mycoplasma transmission. It is known that the planktonic form of Mycoplasma seems to be not resistant in the environment. Also, it has been postulated that mycoplasmas are actually more virulent when growing on biofilm compared to their planktonic form in the Mycoplasma medium [1]. Despite the application of specific biosecurity measures, new outbreaks of avian mycoplasmosis, often involving Mycoplasma synoviae, are frequently reported. Thus, we decided to investigate the biofilm production ability of some avian Mycoplasma strains (MG ts-11, MG 6/85, MG S6, MS-H). Strains were inoculated in plastic test tubes containing Avian Experience® medium and incubated for several days, with and without a coverslip, at 37°C. In order to observe biofilm formation, the indirect staining method proposed by McAuliffe et al. [1] was used as follow: crystal violet staining (CV 0.5% V/V) of coverslips and absorbance measuring (560nm) of solubilized stain in ethanol. Moreover we optimized a direct method which provides for a plastic test tube bottom staining, using CV, coupled with an evaluation of the intensity and the pattern of the staining. In addition, a twin tube of each strain were processed with standard scanning electron microscopy (SEM) procedures. Basing on our preliminary results we can confirm that MG 6/85 and MG S6 are able to produce biofilm, although showing different behaviors. Despite the previous report [2], the direct method showed that MG ts-11 seems to produce biofilm. On the other hand, while evident structures were observed by SEM in tubes containing MS-H, reported as biofilm producer, the direct staining method showed no-CV-colored pattern. Lastly, the indirect staining seems to not give reproducible results. In conclusion we can say that avian mycoplasmas can produce biofilm. Biofilm detection methods should be improved since not all the methods used by the authors allow detecting the biofilm, except for SEM that allows observing the biofilm morphology.

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P5 - FIB-SEM BRAIN ANALYSIS AND 3D AXONS RECONSTRUCTION IN WHITE MATTER AREA IN SHEEP AS ANIMAL MODEL: A PILOT STUDY

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Sheep has been largely used in translational research for neurological diseases and in neuroscience field [1,2]. Despite the intensively use of sheep as an animal model for different research purposes the microscopic structure of white matter has not yet been investigated. This study is a first attempt to characterise the cytoarchitecture of Corpus Callosum as commissure with a novel method of analysis for 2D images and 3D reconstruction with particular focus on the axons. The structure of the cytological components has been analysed from samples extracted from three healthy ovine brains. The samples were obtained using a biopsy puncher of 1mm diameter and then stained with Osmium Tetroxide, embedded in resin and then observed via Focused Ion Beam Scanning Electron Microscopy (FIB-SEM). Images were acquired via a Zeiss Auriga Cross Beam featuring a Schottky field emission gun and a Gemini electron column. The FIB was set using 4nA beam current to mill at 150 nm of resolution. The SEM images have been acquired via backscattered electron detector and stored with a resolution of 1024x768 pixel. 2D areas have been imaged at random locations with different plane of cuts relative to the main directionality of the fibre tracts. The main cell structures imaged were the axons with different dimensions and different myelin bounding sizes. Artefacts composed by myelin fracture or splitting myelin have been identified. Accessory cells like Astrocytes have been occasionally imaged. After post-processing, from binarized images the white and black pixel distribution was extrapolated. Due to the nature of the Osmium Tetroxide stain that binds with lipidic structures of the samples, pixel distribution is related to the lipidic content. An indicative measure of the level of myelination via pixel counting can be post processed and qualitatively compared via MATLAB [3]. Preliminary results show a constant lipidic content of the Corpus Callosum across the subjects. To obtain the 3D reconstruction the stack images have been post processed using MIMICS software. A manual segmentation of each axon has been carried out for all the acquired areas. Preliminary data show a homogeneity and similar composition between the samples. The 3D reconstruction has been achieved using MIMICS masks method and showed a slight difference in axonal number. The 3D models show a well organised cytoarchitecture while the 2D data suggest a fairly homogeneous composition of the tissue with a constant ratio of lipid content.

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P6 - PREVALENCE OF *LISTERIA* SPP. IN SARDINIAN PORK MEAT PROCESSING PLANTS IN 2017-2018

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Listeria spp. has been detected in pork meat products and in every stage during pork processing. In meat processing plants, live animals and subsequently pig carcasses, are a source of contamination of processing environments [1]. The contamination in food from environmental sources play an important role in finished product infection [2]. The aim of the present preliminary study was to evaluate the presence of *Listeria* spp. in pork meat processing plants located in Sardinia. Between February 2017 and April 2018, 170 samples were examined, including pig meat samples originated from RTE products, pig carcasses, production intermediates and environmental samples from contact and non contact surfaces belonging to limited capacity slaughterhouse with related farm activities, slaughterhouses, cutting and sausage processing plants. The detection and enumeration of *Listeria* spp. were respectively performed according to international standard methods using ISO 11290-1:2017 and ISO 11290-2:2017. Isolated of *Listeria* spp. were characterized by multiplex PCR- according to method described by Bubert et al. (1999) [3]. *Listeria* spp. were found in 23.5% of the analyzed samples from 8 different production plants. *L. monocytogenes* (48.9%), *L. innocua* (37.8%), and *L. welshimeri* (13.3%) were identified. *L. monocytogenes* was characterized by PCR-based serotyping according to EURL Lm and as described by Kérouanton et al. (2010)[4] and Doumith et al. (2004)[5]. Four predominant serotypes were detected (1/2c, 1/2b, 1/2a, 4b). Obtained results suggest the need to improve hygienic conditions in examined meat processing plants. As already occurred in other studies [6], *Listeria* spp. obtained from processing environment can be used to design environmental controls and monitoring programs.

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P7 - ISOLATION OF ESBL E. COLI STRAINS FROM PORK MEAT PRODUCTS IN THE FOOD VALLEY

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Resistance to new generation cephalosporins is an important public health problem in terms of economic and social costs, morbidity and mortality. Extended-spectrum β-lactamase (ESBL)-producing E. coli are ubiquitous and food-producing animals represent one of the sources of antibiotic resistant bacteria, pigs included [1]. ESBL enzymes, normally plasmid encoded, confer resistance to β-lactam antimicrobials, as well as to II, III and IV generation cephalosporins and monobactams. Pork meat contamination with ESBL E. coli strains was investigated together with the evaluation of colistin susceptibility. From September 2018 to March 2019, n=164 E. coli strains were isolated from fresh pork meat products during routinely analyses by the Istituto Zooprofilattico Sperimentale della Lombardia and Emilia Romagna region following the UNI ISO 16649:2. The positive strains were tested for susceptibility to two cephalosporins: cefotaxime (5µg) and ceftazidime (10µg) using the Kirby-Bauer method. The strains resistant to one or both cephalosporins were phenotypically confirmed to be or not ESBL with the combination disk test (CDT) using cefotaxime (30µg), ceftazime (30µg), cefotaxime (30µg) + clavulanate (10µg) and ceftazidime (30µg) + clavulanate (10µg). The ESBL confirmed strains were then screened for the presence of ESBL-associated genes (blactxm, blatem e blashv). The protocol described by Roschansky [2] using a Real-Time PCR with sybrgreen was applied. Isolates with Cycle quantification (Cq) values over 31 were considered as negative. The E. coli strains were also tested for susceptibility against colistin applying the Minimum Inhibitory Concentration (MIC) test as described by EUCAST (2016). On the n=164 E. coli strains, n=7 were confirmed to be ESBL. The molecular analysis confirmed the findings, in particular, blatem gene was harboured by n=3 strains, blactxm was found in n=4 strains and none of them harboured the blashv gene. None of the E. coli strains showed resistance against colistin. These results are comparable to the EFSA report on zoonoses describing a 7% of isolation for pork meat [3]. Gene blactxm was already confirmed to be the predominant gene in Enterobacteriaceae isolates from food-producing animals [4]. The present study highlights the contamination of pork meat products with ESBL E. coli strains and the potential risk for human health associated to the consumption and manipulation of these products.

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P8 - ANTIOXIDANT AND ANTIMICROBIAL EFFECTS OF GARLIC AND SALT IN RABBIT BURGERS

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The growing attention of the consumers to avoid the use of synthetic antioxidants molecules and the negative effect of some antioxidant on the human health induced the meat industry to replace synthetic antioxidant with natural sources. Normally burgers are sold as only meat or meat mixed with other ingredients, mainly spices and salt, due to its antimicrobial properties and to increase palatability. Unfortunatelly, addition of salt could represent a starting point for lipid oxidation and the production of off-flavor compounds. Many studies have demonstrated the efficacy of natural antioxidants when used in meat products (1). Among culinary spices, garlic is one of the most commonly used ingredients with known antimicrobial and antioxydant activities (2). The aim of this study was to evaluate shelf-life parameters of rabbit meat burgers with and without addition of salt and garlic powder. Three batches of rabbit meat burgers were prepared; each one of 40 pieces (10 for the control, 10 with salt, 10 with salt and garlic and 10 with garlic). The burgers were analyzed at 0, 4 and 7 days of storage at 4°C. Regarding microbiological profile (*Escherichia coli*, *Enterobacteriaceae*, *Pseudomonas* spp., *Brochothrix thermosphacta*, lactic bacteria, total mesophilic bacterial count, yeasts and molds, and total psychophilic baterial count), no significant differences between the different types of burgers were observed; instead, significant differences for *Enterobacteriaceae*, *Pseudomonas* spp., lactic bacteria and total mesophilic bacterial count were found during storage time, with an increase up to the 7th day. Total psychrophilic bacterial count increased only up to the 4th day of storage. For the colour evaluation significant differences among different types of burger and during the storage were detected in raw products , with less brilliant colour in burgers with salt, yellow-clair meats in burgers with garlic and darkness after 7 days. Instead, after cooking, no significant differences were observed for the colour. Burgers with salt and after 7 days of storage showed the highest values of TBARS, as in raw products as well as after cooking. Garlic showed no positive effects on the microbiological and physico-chemical parameters tested during storage. No researches about antioxidant activity of garlic are available in rabbit meat, but this natural ingredient showed a good antioxidant power in other meats (3). However, the lack of antioxidant action of garlic could be linked to its low concentrations (0.25%). It would therefore be appropriate to test higher concentrations of garlic in rabbit burgers, but an increase in this ingredient could result in lower consumer acceptability of the product.

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P9 - TAIL LESIONS AT SLAUGHTERHUOSE IN ITALIAN HEAVY PIGS: DIFFERENCE BETWEEN INTACT AND NON-INTACT TAIL

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The aim of this study was to assess the prevalence of tail lesions in Italian heavy pigs (~165 kg) at slaughterhouse and the difference between pigs with intact and non-intact tail. For evaluation of tail lesions, a scoring system proposed by Vom Brocke and colleagues [1] was used. The observations were performed by 3 assessors in 4 industrial slaughterhouses on 14,601 Italian heavy pigs (4,144 intact tail; 10,457 non-intact tail) from 73 farms located in North Italy from January to April 2019. The differences between two groups were analyzed with chi square test. Fischer extract test was performed in order to analyze the difference between the two groups with presence or absence of lesions and with presence of mild (degree lesion 1) and severe lesions (sum of degree lesions 2, 3 and complete loss). All the tests were performed by GraphPad Prism 6.05 (GraphPad Software Inc. San Diego, CA, USA). The prevalence of tail lesions prevalence was significantly different ($p<0.0001$) between the two groups. Concerning intact tail pigs, the scoring system showed 47.4% for 0 degree, 36.9% for 1st degree, 13.5% for 2nd degree and 2.1% for 3rd degree. In non-intact tail pigs the scoring system showed 66.6% for 0 degree, 30.5% for 1st degree, 2.0% for 2nd degree and 0.3% for 3rd degree. Complete loss was attributed to non-intact tail group and was recorded in 0.7% of the animals. The prevalence of pigs without lesions was significantly higher ($p<0.0001$) in non-intact tail vs intact tail group (OR 2.2; 95% CI 1.6-2.4) showing respectively 66.6% vs 47.4% of the animals without lesions. Mild or severe lesions showed relevant differences between the two groups. As a matter of fact, the prevalence of severe tail lesions was significantly higher ($p<0.0001$) in intact tail vs non-intact tail (OR 4.3; 95% CI 3.7-10.4) respectively 15.7% vs 3.0%. These results were expected, since tail docking at farm reduces tail biting [2], and the odds ratio quantify the major probability in intact tail pigs. The results in non-intact tail pigs are coherent with those found in a recent study docked pigs in Germany [1]. The application of scoring system in Italian heavy pigs at slaughterhouse allowed to identify the difference between pigs with intact and non-intact tail. Unknowing the status of the farms (tail docked in all pigs, tail docked in some groups, no tail docked) was, probably, the main source of bias in this study and a further analysis could show the difference for these conditions and other risk factors. In conclusion, the prevalence of tail lesions observed at slaughterhouse, was significantly higher in intact tail pigs. According to the Italian strategy laid down by the Italian Ministry of health, to reduce tail docking in pigs, the application of scoring system at slaughterhouse could allow a permanent animal welfare indicator, especially for undocked pigs.

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P10 - HYDROXYPYRIDINONE-BASED IRON-CHELATING CO-POLYMER (DIBI) HAS ANTIBACTERIAL AND ANTIMYCOTIC ACTIVITY AGAINST PATHOGENS ASSOCIATED WITH CANINE OTITIS EXTERNA

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Otitis externa, a common inflammation of the external ear in dogs, is a multifactorial disease. Among the different aetiological agents, bacteria and yeasts represent the main microbial agents. Bacteria such as Staphylococci, in particular *Staphylococcus pseudintermedius, Streptococci, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli,* and yeasts like *Malassezia pachydermatis* are frequently isolated from ears of dogs suffering from otitis externa [1].
Antibiotic resistance is one of the most urgent threats to public' health and the increasingly limited therapeutic options both in human and veterinary medicine underline the need of new alternative therapeutic approaches, in order to limit the ever-increasing spread of multidrug-resistant strains among companion animals.
DIBI, a novel water-soluble hydroxypyridinone-containing iron chelating polymer, developed by Chelation Partners Inc. (Canada), provides a potential new antibacterial treatment by denying pathogens of iron as needed for their growth [2]. Herein, we tested DIBI against different strains isolated from dogs suffering from otitis externa.
During the years 2016-2017, canine auricular swabs were collected and processed at the Microbiology Laboratory of the Department of Veterinary Medicine and Animal Production, University of Naples Federico II (Italy). The swabs were inoculated onto different selective and differential agar plates to isolate gram-positive and gram-negative bacteria or yeasts. Isolates were identified by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) and their antibiotic resistance profiles were evaluated by disk diffusion method on Mueller Hinton agar plates, according to the Clinical and Laboratory Standards Institute guidelines.
Moreover, DIBI activity was evaluated against selected gram-positive and gram-negative bacteria (*Staphylococcus aureus*, *Staphylococcus pseudintermedius, Pseudomonas aeruginosa, Proteus mirabilis*) for a total of sixteen strains and also against two *Malassezia pachydermatis* strains.
All the selected isolates presented interesting resistance profiles to antibiotics that are frequently prescribed to dogs suffering from otitis externa. Furthermore, they were all susceptible to DIBI and precisely, all *S. aureus, S. pseudintermedius* and *M. pachydermatis* strains displayed high sensitivities to DIBI (1 to 4 µg/mL) while it was observed a wider spectrum of sensitivity for the *P. aeruginosa* strains (MICs of 2 or ≥ 128 µg/mL). Overall, bacterial sensitivities to DIBI did not correlate with their antimicrobial resistance profile.
Thus, DIBI indeed seems a promising non-antibiotic alternative therapy in veterinary medicine against canine otitis externa.

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P11 - INCREASED SPREAD OF PANTROPIC CANINE CORONAVIRUS IN DOGS

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Canine coronavirus (CCoV) infection is usually restricted to the enteric tract and generally produces only mild or asymptomatic forms of enteritis. CCoV includes two genotypes: CCoV-I and CCoV-II, the latter being further classified into two subtypes (CCoV-IIa and CCoV-IIb). Hypervirulent CCoV-IIa strains, designated as pantropic CCoV (pCCoV), able to spread to extraintestinal tissues have been detected in dogs [1, 2] and, for the first time, in 2017, also in a wolf [3]. Therefore, pCCoV has been recognised as a virulent emerging pathogen in canids. Our study was focused on the detection and molecular characterization of pCCoV strains in dogs in the period 2014-2017.
Necropsy samples collected from 352 dogs were screened for CCoV [2]. The spike protein gene (ORF2) of the detected pCCoV strains was sequenced and analysed. A phylogenetic tree was generated from MAFTT Software Version 7, using the neighbour-joining method.
CCoV RNA was detected in 76 (21.59%) out of 352 dogs and 35 (9.94%) of these animals displayed a CCoV-IIa strain in internal organs, which accounted for pCCoV infections. Fifteen of the putative pCCoV strains were sequenced in partial ORF2 gene.
The phylogenetic tree obtained by sequence analysis of the detected pCCoV strains and reference CCoV isolates showed 5 different clades. The clade including pCCoV strain 103480 is quite distant from all the others, whereas eight pCCoV strains clustered with the pantropic strain 120/10, detected in Hungary in 2010. Two other pCCoVs were grouped with historical enteric CCoV isolates. Additional three strains identified in this study belonged to the same clade including the wolf pCCoV (CCoV/wolf/2016/IT), a strain (LC190906) from a Vietnamese dog, a strains (GQ152141) detected in a Taiwanese cat and a strain (EF192155) recovered from a Chinese raccoon dog. The last pCCoV strain (31975) grouped with pantropic strains identified in Italy (185/11, 69/10) and Greece (NA/09).
Interestingly, some of the detected strains were not closely related to Italian and European CCoVs, displaying higher genetic identities to strains circulating in Asia.
CCoV infection was found to be very common in domestic dog, fox and raccoon dog populations in China [4]. It is possible that the virus was introduced into Italy through the importation of dogs or other carnivores from Asia or by the illegal trade of domestic and exotic carnivores [5].
This study demonstrates an increasing circulation of pCCoV in Italy, thus strengthening the need of intensive surveillance programs to monitor the circulation of this and other emerging viruses in domestic carnivores.

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P12 - A PRELIMINARY STUDY OF THE ANTIBACTERIAL EFFECT OF MANUKA HONEY AND PROPOLIS AGAINST *S. PSEUDINTERMEDIUS* STRAINS

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The treatment of canine bacterial skin infections and chronic wounds has become increasingly harder as multidrug resistant bacteria, such as methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) have become more common and difficult to eliminate with conventional antibacterial drugs [1]. Therefore more research should be done in the use of alternative treatments such as honeybee products. In particular honey and propolis have known antibacterial and anti-inflammatory effects which are able to accelerate wound healing and inhibit bacterial growth and biofilm formation [2, 3]. The aim of this study was to evaluate the activity of propolis and Manuka honey gel (Medihoney®, Comvita) *versus* eleven canine strains of *Staphylococcus pseudintermedius* isolated from pathologic samples and a reference strain of *Staphylococcus aureus* (ATCC 6538). For every strain, both macro- and microscopic tests were carried out (growth on blood agar and Mannitol Salt agar plates, Gram stain, biochemical tests). The antibiotic-resistance profile was done using the standardised Kirby-Bauer method testing different pharmacological classes. The minimum inhibitory concentration (MIC) was carried out for both propolis and Manuka honey using the microdilution method according with the literature [4].

Out of the twelve bacterial strains studied, only 25% were sensitive to all antibiotics tested, while 58% were found to be multi-drug resistant (MDR). The strains were first tested against propolis and subsequently against ethylic alcohol generally used as solvent for propolis. The highest dilution of ethylic alcohol had no visible effect in preventing bacterial growth in four of the tested strains, while propolis was effective against every strain with MICs ranging from 1:16 to 1:512. The effect of Manuka honey was either equal or lower compared to that of propolis, with MICs ranging from 1:2 to 1:16. The preliminary results show that both these honeybee products are able to inhibit the growth of *S. pseudintermedius* strains also MDR, even if propolis was more effective than Manuka honey gel we tested. Further studies will be performed using Gram-negative strains and Manuka oil more easily to handle will be tried.

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P13 - INVESTIGATION ON THE PRESENCE OF FELINE MORBILLIVIRUS RNA IN KIDNEY AND MESENTERIC LYMPH NODE SAMPLES OF CLIENT OWNED AND CATTERY CATS

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From the first detection of feline morbillivirus (FeMV) in 2012, the association between its infection and kidney diseases in cats has been suggested and debated. Consequently, most of the studies in the literature have investigated and compared the presence of FeMV in cats with kidney diseases and healthy cats. Nevertheless, FeMV shows tropism not only for the kidney but also for other feline tissues, in particular the lymphoid tissue [1,2]. Aim of this study was to investigate the presence of FeMV RNA in cats died due to causes of various origins and not exclusively attributable to renal diseases. Thirty-eight cats died in 2016-2017 and subjected to necropsy at the Department of Veterinary Medical Science (University of Bologna) were included in the study: 10/38 were client owned cats and 28/38 were community cats from two catteries. When available signalment and anamnestic data and clinicopathological findings were retrieved from medical records. Kidney and mesenteric lymph node tissues were sampled from each cat during necropsy. RNA was extracted from tissue samples and the presence of FeMV RNA was investigated using a one-step real-time RT-PCR assay amplifying a 155-nt fragment of the viral RNA-dependent RNA polymerase L gene [3]. Amplicons of the expected size were sequenced. The obtained nucleotide sequences were aligned with reference sequences from GenBank and translated into amino acid sequences using BioEdit 7.2.5. Phylogenetic relationships were evaluated using MEGA X version 10.0.5. FeMV RNA was detected in the mesenteric lymph node tissue of one cattery cat. The cat tested positive did not show clinical, clinicopathological and gross findings correlated to renal injury. FeMV RNA was not detected in kidney samples. The analysis of the obtained nucleotide sequence, although of short length, evidenced a correlation of the identified FeMV with the Italian strain Piuma/2015 (KT306750 and KT825132), the USA strain US1 (KR014147), and the China strains 761U and 776U (JQ411014 and JQ411015, respectively). Data obtained confirming that FeMV circulates in Italian cats, that viral infection can occurs without renal involvement and that viral sequences do not seem to be phylogenetically grouped on geographical basis.

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P14 - GENOTYPING OF HERPESVIRUSES INFECTING TORTOISE IN ITALY

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Four different species of herpesviruses have been detected so far in tortoise. They have been named *Testudinid Herpesvirus* (TeHV) because they have been shown to infect only members of the family *Testudinidae* [1]. After that a TeHV1 was found in two tortoises in Japan [3], other three different species have been found in Europe (TeHV3) [4], and in the USA (TeHV2 and TeHV4) [5,6]. Due to the limited sequences available, only TeHV3 has been officially included in the ICTV Taxonomy as *Testudinid alphaherpesvirus 3* (TeAHV3) in the sub-family *Alphaherpesvirinae*, genus *Scutavirus*. The majority of the information reported in the literature concerning TeHV-associated diseases refers to TeHV3 and consists of nasal and oral discharge and dyspnea along with conjunctivitis and is followed by the development of diphtheronecrotic plaques on the oral mucosa and the tongue [1]. The aim of this work was to genotype TeHV detected in different species of tortoises showing various clinical signs. A total of 34 archival DNA samples collected since 2009 and resulted positive for TeHV by PCR were used to amplify a 642 bp sequence of the DNA polymerase gene. The sequences were aligned with those available in GenBank and were used for phylogenetic analysis. A total of 10 TeHV1 and 24 TeHV3 were detected. These data confirm that TeHV3 is the most prevalent TeHV spread in tortoise. Although TeHV is described as generally associated with relatively low morbidity and mortality rates [2], these data show that TeHV1 are not so infrequent and that infected animals can show also severe clinical signs. It is not clear if there is a distinct, species-specific host-pathogen association, although some “preferences” appear to exist [1]. In our case, *Testudo horsfieldi* was the most prevalent species infected by TeHV1, while *Testudo hermanni* was mainly infected by TeHV3, although this latter tortoise species appeared susceptible also to TeHV1. Monitoring and genotyping of TeHV spread in Italian tortoise contribute to the knowledge of the epidemiology of these viruses and are the basis for future vaccine development.

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P15 - MOBILAB: A MOBILE ANALYSIS LABORATORY AIMED TO THE IMPROVEMENT OF THE QUALITY AND QUANTITY OF SHEEP AND GOAT PRODUCTIONS

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Mobilab is an Interreg Greece-Italy project carried out with the collaboration between Greek partners (Hellenic agriculture organisation Demeter, the Region of Ionian Islands, the University of Salonicco), the University of Bari, Department of Veterinary Medicine DiMeV, and Città Metropolitana of Bari. The two-years project (April 2018 - April 2020) aims to improve the quality of the sheep and goat productions, with particular emphasis on milk and dairy products, protecting and increasing the value of locally typical products and boosting the farms activities. The project focuses on the performance of technical and sanitary assistance to the sheep and goat farms [1] [2] [3] through an innovative approach: the presence of a mobile laboratory directly in the farms. A mobile laboratory, MOBILAB, has been totally equipped with all furnitures in order to reach all the remote farms that, for logistic reasons, do not benefit of specialized laboratories, to provide all the necessary microbiological analyses [4] [5]. A similar mobile laboratory will work in Greek Ionian Islands. A complete questionnaire form collects all the general information about farms, equipment and facilities, management, sanitary and veterinary problems. A collaboration with the Istituto Zooprofilattico Sperimentale of Puglia and Basilicata allows to perform a complete analisys of quality of milk. At this stage of the project, we have interviewed sheep and goat farmers in the territory of Città Metropolitana di Bari in order to evaluate the farming systems and to assess the mainly problems affecting the small ruminant farms. The preliminary results highlight mastitis and neonatal mortality as major problems [6]. The main farmers' concerns are related to the economic troubles caused by the discrepancy between the production and sale costs, compared to the market price of the dairy products. The final goal of the project is the development of a network with sheep and goats farms and farmers, in order to safeguard and improve the quality and quantity of sheep and goat productions.

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P18 – VIROLOGICAL SURVEY IN PIEDMONT FOR PATHOGENS INVOLVED IN BOVINE RESPIRATORY DISEASE COMPLEX: BHV1, BRSV, PI3V AND BVDV

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Bovine respiratory disease complex (BRDC) is one of the most prevalent cattle diseases. Etiologic agents, which are commonly associated with BRDC, include bovine respiratory syncytial virus (BRSV), bovine herpesvirus 1 (BHV-1) and bovine parainfluenza virus 3 (PI3V) and Bovine Viral Diarrhea Virus (BVDV). Pathogens co-infection often makes the BRDC diagnosis more difficult. Therefore the development of timely and effective diagnostic approaches could allow the adoption of biosecurity measures and minimize the economic losses especially in those provinces, such as Cuneo, with high density of livestock and large animal trade. The aim of this study was to perform a virological survey in Cuneo Province testing nasal swabs from herds with BRDC clinical symptoms and lungs samples of slaughtered cattle. BRDC infection differential diagnosis was performed using Real time PCR protocols opportunely modified and optimized. Two-hundred-eighteen nasal swabs samples from 8 herds with acute respiratory disease and 24 samples of lungs with lesions compatible with viral infection were processed individually for Nucleic acids extraction. Nucleic acids samples from lungs were tested individually while those from nasal swabs were grouped in pools of 5-6. In this study an innovative duplex RT-Real Time PCR protocol for the simultaneous detection of BRSV and PI3V was developed using primers and probes previously described [1,2]. BVDV screening was performed by RT-Real Time PCR protocol by Hoffmann et al. [3]. DNA samples were tested by an IBR Real Time PCR protocol [4] adapted for the End Point PCR kit use. BRSV and PI3V viruses were detected in 3 nasal swabs pools and in 4 lung samples. Our results showed that PI3 virus was always detected in co-infection with BRSV. BVDV was detected with higher frequency in lungs samples (4 cases) than in nasal swabs (1 case). BHV1 virus was detected prevalently in nasal swabs (6 pools), probably for the high virus' titer excretion during the acute phase, while just one lung sample was positive. Co-infections were detected in three lung samples (BVDV-VRSB, BRSV-BHV1, BVDV-PI3V-VRSB) and in one pool of nasal swabs (BRSV-PI3). Our results confirmed the main role of BRSV, PI3V, BHV1 and BVDV infection or co-infection in the BRDC etiopathogenesis.

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P19 - FIRST DETECTION OF HUNNIVIRUS IN A DOG (*CANIS LUPUS FAMILIARIS*)

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The genus Hunnivirus includes a single species (Hunnivirus A) and three different genotypes currently proposed to the International Committee on Taxonomy of Viruses [1]. To date, these viruses have been detected in sheep, cattle, rats and cats but there are no reports of Hunnivirus detection in dogs. Hunniviruses belong to the family *Picornaviridae* and they are phylogenetically related to Kobuviruses, which have been extensively described [2].

This study reports the molecular positivity for a “Hunnivirus-like genotype” in feces from a dog.

Fecal samples collected from 13 dogs (10 female and 3 male) of the Piedmontese side of the Gran Paradiso National Park were screened for Kobuvirus infection. Briefly, total RNA was extracted from 300 µl of stool suspension using the TRIzol Reagent (Invitrogen). Reverse transcription was performed and cDNA was amplified using an end-point PCR protocol described previously [3]. Amplicons were gel purified and submitted to Sanger sequencing. A PCR product of 216 bp, compatible with the target region of Kobuvirus PCR, was detected in one sample collected from a ten-year-old female German Sheperd. Unexpectedly, sanger sequencing and Blast analysis showed maximum similarity (95.6%) with Hunnivirus A**.** Sequence analysis showed that the genetic homology of the target region encoding for a portion of the 3D RNA-dependent RNA polymerase gene allowed the detection of Hunnivirus using primers for Kobuvirus routine screening [2]. Dog owner interview revealed that it is asymptomatic and lives in close contact with another dog (negative at the PCR screening) and with a cat not yet tested. Recently, a novel genotype of the species Hunnivirus A was identified in feces of a cat with diarrhea in China. Rodent Hunnivirus has also been reported [1]. The presence of a co-habitant cat and the rural habits of this dog could represent risk factors for viral infection. Our findings expand the host range of Hunnivirus.

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P20 - EVALUATION OF CAPRINE HERPESVIRUS 1 (CPHV-1) INFECTION EFFECTS ON MALIGNANT PLEURAL MESOTHELIOMA CELLS: A PRELIMINARY STUDY

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Malignant pleural mesothelioma (MPM) is an aggressive cancer correlated to asbestos exposure. Considering that there are no current therapeutic effective protocols, the prognosis remains dismal with a median survival of 16–29 months, thus it is urgent to develop new effective therapeutic strategies [1,2]. Treatment of cancer with oncolytic viruses (OVs) has been considered a promising therapeutic approach and virotherapy-based strategies have recently found a successful application in the clinical setting [3]. MPM represents an ideal candidate for virotherapy for numerous reasons including the frequently localized pattern of growth and the pleural location, which allows direct access for the intra-tumoral injection of the OVs [3]. OVs selectively replicate in and kill cancer cells with a direct lytic effect. Non-human wild type OVs show many advantages over human vectors, like the incapacity to induce infection in humans and the absence of pre-existing immunity. Caprine herpesvirus 1 (CpHV-1) is a non-pathogenic virus for humans but seems to be able to replicate, with the production of viral progeny, and kill different human cancer cell lines [4]. In our previous study we demonstrated that CpHV-1 is able to reduce cell viability, to replicate and to cause cell death in several human cancer cell lines [4]. So, we decided to test CpHV-1 on NCI-H2052 cell line, representing most aggressive MPM histotype. First, we assessed its effect on cell viability by MTT assay at 96h and 120h post infection and we determined the multiplicity of infection (MOI) for each times. Then we evaluated the CpHV-1 effects on cell cycle progression and apoptosis (by FACS and AnnexinV assays). Our preliminary data shown that this infection reduces cell viability, and consistently induces cell cycle arrest and triggers apoptosis. Overall, our findings indicate that CpHV-1 infection could be an useful strategy to treat an aggressive MPM histotype, which is usually very resistant to apoptosis-inducing treatments and for which currently only palliative therapy is possible. Although we need to further explore mechanisms underlying this infection and we need to test this virus also on MPM cell lines representing other histotypes, we hope that this study could serve as the basis for the development of new treatment options for this neoplasia.

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P22 - EVALUATION OF MULTIDRUG-RESISTANT *ESCHERICHIA COLI* IN URINARY INFECTIONS: RETROSPECTIVE STUDY AND TREND ANALYSIS IN PETS FROM TWO VETERINARY TEACHING HOSPITALS IN ITALY, 2014-2017

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Escherichia coli is the most frequent bacterium involved in uncomplicated urinary tract infections (UTIs) in pet animals. The treatment is sometimes threatened by the steady increase in the number of strains bearing concurrent resistance to various antimicrobial agents. The aim of this study was to determine multidrug-resistance patterns in uropathogen *E. coli* (UPEC) isolated from dogs and cats. A retrospective study on samples collected from January 2014 to December 2017 at two Veterinary teaching Hospitals, located in Northern (Turin, H1) and Central (Camerino, H2) Italy, was carried out . Strains were collected from dogs (H1 n=119; H2 n=96) and cats (H1 n=64; H2 n=34) with UTI. Each strain was tested to 18 antibiotics belonging to 8 categories (Aminoglycosides; Carbapenems; Folate pathway inhibitors; Not-extended spectrum Cephalosporins: 1st and 2nd generation (C1-2); Extended spectrum cephalosporins: 3rd and 4th generation (C3-4); Penicillins; Penicillins + β-lactamase inhibitors; Quinolones) by Kirby-Bauer test and interpreted according to the EUCAST guidelines [1]. Isolates were classified as MDR (Multidrug-resistant), XDR (extensively drug-resistant) and PDR (pandrug-resistant) [2]. Data were analyzed using Chi Squared or Fisher exact tests, using the STATA 13.0 software. Among 313 isolates, 25.2% were susceptible to all tested antibiotics. Comparable multiresistance profiles were observed in H1 and H2 isolates. The antimicrobials categories with highest resistance rate were: Penicillins + β-lactamase inhibitors, Quinolones, and Penicillins (43.4%, 41.8% and 39.9%, respectively), followed by Folate pathway inhibitors (37.1%), Aminoglycosides (34.5%) and Cephalosporins (30.5%). Low levels of resistance were observed for Carbapenems (4.2%). 158 strains were MDR (50.5 %), of which 29.7% were XDR and none PDR. Among MDR, a co-resistance to Aminoglycosides, C3-4, and Quinolones was observed (12.1%, n=313). The trend encompassing the years 2014-2017 showed an increase of MDR (50.4 to 58.0%). The differences in MDR resistance were not significant between H1 (45.9%) and H2 (56.9%, P=0.055). Concerning the animal species, canine E. coli showed a greater resistance to C1-2 than cats (35.8% vs 20.4%, P=0.006), while a significant percentage of resistance to Penicillins was observed in cats (50.0% vs 35.3%, P=0.014).
The UPEC isolated in this study showed high level of multidrug resistance. Moreover, 15.0% of all UPEC tested, were classified as XDR . These findings evidence serious risks for a potential zoonotic transmission of these bacteria and strongly hijack the therapeutic options left.

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P24 - EVALUATION OF SERUM PREVALENCE OF *SALMONELLA TYPHIMURIUM* IN BACKYARD CHICKENS FLOKS, IN ITALY: A PILOT STUDY

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Poultry are well recognized as possible carriers of different Salmonella species and Salmonellosis is a significant zoonotic disease which has a considerable economic impact for the poultry industry. The Italian Salmonellosis Control Plan (SCP) excluded ornamental and self-consumption poultry farms. A scientific EFSA report of 2018, reported that in chicken flocks the percentage of positivity S. typhimurium (S.T.) and S. typhimurium monophasic variant (S.T. mv) is 0.2%, while in laying hens is 0.3% [1]. Overall, according to EFSA reports 2019, the prevalence of Salmonella in poultry remains <1% [2]. However, considering the epidemiological situation for S.T. and S.T. mv in industrial poultry farming, the aim of this study was to verify the only serum prevalence for S.T. in backyard chickens, considering that these farms are not subject to official controls and exposure to zoonotic pathogens could be more frequent. Between December 2018 and February 2019, 24 rural farms of backyard chickens, numbered from "A" to "Z", located in 8 different regions of Italy, were examined. On a total of 971 backyard hens, 240 samples were taken (expected prevalence <5%). From each farm, blood samples from 10 laying hens, aged between 5 months and 5 years, asymptomatic and unvaccinated against Salmonella, were taken to verify the presence of specific antibodies against S.T. Serum analysis was performed in according with the O.I.E. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals [3], and using a commercial Antibody ELISA Kit X-Ovoflockscreen™ kit, to confirm the slow serum agglutination in micro-method (SAL) for positive or not conclusive results. Out of 240 blood samples 231 were analyzed. Nine serum were excluded for scarcity of serum, insufficient to perform the analysis. The results shows that the positive farms for S.T. were 4/24 (A,D,P,T) (16.66%). In these farms, the percentages of positive samples were 2/10 (20%), 2/8 (25%), 1/10 (10%) and 2/8 (25%) respectively. On "A" and "T" farm, 2 serum for each were not conclusive in ELISA test. The global serum prevalence was 3.03% (7/231). Our preliminary study shows the circulation of S.T. The serological prevalence obtained is >1%, not surprising aspect in consideration of the type of poultry farms. The circulation of S.T. in these farms must be an aspect not to be overlooked, whereas some amateur farmer often consider these animals as pets and consume their eggs. Further studies, including the isolation and characterization of isolates, are necessary to obtain a more precise picture of the presence and prevalence of zoonotic Salmonella species in this exclusively hobbyist breeding sector.

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P25 – MICROBIOLOGICAL HEALTH STATUS AND ANTIMICROBIAL RESISTANCE PROFILES IN FREE-RANGING AND RESCUED PSITTACINE BIRDS IN GUATEMALA AND BELIZE

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To assess the microbiological status of two free-ranging populations of Scarlet macaw (*Ara macao cyanoptera*) in Guatemala (n=10) and Belize (n=14), and Yellow-headed amazon (*Amazona oratrix belizensis*) kept in a rescue centre in Belize (n=15), blood samples (n=39), faeces (n=16), cloacal (n=34) and choanal swabs (n=6) were collected for molecular, bacteriological and mycological investigations. On blood samples, PCRs for Avian Polyomavirus, Psittacine Beak and Feather Disease virus, Pacheco's Disease virus, and *Chlamydia psittaci* were carried out. Total bacterial and mycotic counts (UFC/swab/g) were recorded [1]. To define the antibiotic resistance profiles, 11 antimicrobial and 2 antifungal categories were tested: Aminoglycosides, Penicillins, Penicillins+β-lactamase inhibitors, Cephalosporins, Quinolones, Carbapenems, Glycopeptides, Macrolides, Tetracyclines, Folate pathway inhibitors, Phosphonic acids, Polyenes, Azoles, following Kirby-Bauer and MIC methods (Oxacillin, Amoxicillin/Clavulanic acid, Vancomycin, Teicoplanin) according to the EUCAST guidelines [2]. Data were analyzed using Chi Squared and Student *t*-test (STATA 13.0).
All samples were negative to PCRs. Gram positive (63%, 8190±3495 UFC) and Gram negative bacteria (30%, *P*=0.003; 7600±4596 UFC, n=233) were cultured for a total of 218 isolates. *Staphylococcus* spp. (19%), *Bacillus* spp. (14%), *Streptococcus* spp. (13%), *E. coli* (6%), *Burkolderia cepacia* complex (6%), were the bacteria more representative. Samples resulted negative for *Salmonella* spp., *Mycoplasma* spp., and *Clostridium* spp. No significant difference was observed between captive and free-ranging birds, both for Gram +ve (63.2% *vs* 62.9%) and -ve (39.6% *vs* 31.5%). *Candida* spp. and *Rhodotorula* spp. (4%, 2228±1483 UFC; n=13), *Aspergillus* spp. and *Penicillum* spp. (0.4%, 25±21 UFC; n=2) represented the fungal flora. In free-ranging birds the mean total bacterial and fungal counts (7458±3175.5 and 1274±878.4 CFU) were lower compared to in captivity birds (8382±3175.5 and 2315.5±1636.1 CFU), but the differences were not significant (*P*>0.05). High significant resistance was observed for Penicillins (87%), Macrolides (57%), Penicillins+β-lactamase inhibitors (43%). Oxacillin Resistant Staphylococci (16%) and Glycopeptide non-Susceptible Staphylococci (20%) were isolated only from in captivity psittacine birds (*P*=0.0001), while free-ranging birds showed a high, but not significant, resistance to Macrolides (63%), Tetracyclines (39%; *P*=0.40). All fungal strains from in captivity birds resulted resistant to Polyenes.
These are the first data on the microbiological health status and on bacterial and fungal antimicrobial susceptibility of healthy Psittacine birds in Guatemala and Belize. In rescued parrots, relevant resistance profiles were observed, hypothesizing the role of captivity in the transmission of resistant strains, mainly Staphylococci.

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P26 – PORCINE CIRCOVIRUS TYPE 2 (PCV-2) INFECTION IN FERAL NEBRODI BLACK PIGS

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Porcine circovirus (PCV) is the causative agent of a severe porcine diseases, causing important economic losses [1]. PCV is a small virus, with circular, negative single-stranded DNA genome, belonging to the genus Circovirus . Nowadays, three different types of PCV have been described [2].PCV-2 was first described in Canada in 1991 [3] and has been associated with several clinical conditions, collectively named as porcine circovirus-associated disease [4]. The circulation of PCV-2 has been reported in Europe as well as in Italy, where is considered endemic. While its presence in domestic pigs of Italian farms has been described [1], PCV-2 infection in wild boars or autochthonous breeds reared in free or semi-free roaming conditions has been less documented . Aim of this study is to report PCV-2 infection in Nebrodi Black pigs in Sicily (Italy) in 2014 and 2018. Referred Pigs were of 45-120 days oldand farmed in a semi-wild breeding system with other domestic ruminants. Clinical signs were: wasting, weight loss, pale skin with diffuse and multifocal erythema, edema of the submandibular and palpebral region, respiratory and digestive symptoms, ataxia, stagger and tendency to isolation. Necropsy, hematobiochemical, histophatological exams and Real Time-PCR (RT-PCR) on lungs, liver, intestine, lymph nodes and spleen were performed. The intestine showed multifocal to locally extensive reddening located mainly in the colon, and mesenteric edema with thickening of the ileal wall were observed. Mesearic lymphnodes showed large and multifocal necrotic-hemorrhagic areas, associated microscopically with the presence of giant multinucleated cells. Lymphoid tissues were also characterized by severe lymphocyte depletion with granulomatous inflammation. Tissue samples tested positive for PCV-2 by RT-PCR. The Nebrodi's Black pig is one endangered autochthonous Italian pig breed. This preliminary results describe the evidence of PCV-2 infection in Nebrodi black pigs. These results might support further evaluation which are needed to better describe the spread of PCV infection in this autochthonous breeds.

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P27 – A 6 YEAR SURVEY ON THREE YEARS CORE VACCINATION AGAINST CPV AND CDV IN DOGS FROM NORTHEASTERN ITALY

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The recent WSAVA guidelines indicate to carry out a 3 years vaccination program against canine parvovirus infection (CPV), canine distemper (CDV) and infectious canine hepatitis (ICH, CAV), that represent 3 infectious diseases of global significance for dogs and that can be prevented by core vaccines. The use of the CAV vaccine has greatly reduced the incidence of ICH that is nowadays considered a neglected disease. The aim of this work was to assess the seropositivity in the canine population vaccinated by a 3 years revaccination against CPV and CDV. In this study, 372 owned dogs presented at the San Marco veterinary clinic of Padua (Italy) in the last 6 years and vaccinated by a 3 years vaccination program were included. Blood samples were tested with an in-clinics ELISA kit (TiterCHEK®), according to manufacturer's instruction: development of a blue colour in the sample well that is of equal or greater intensity than the colour of the positive control well is considered to be positive (CDV serum neutralization [SN] titre ≥1:16, CPV haemagglutination inhibition [HI] titre ≥1:80). Dogs were divided in groups based on age, gender, breed, size and health status. The results showed that 358 (96.2%) dogs were seropositive for canine parvovirus, while only 258 (69.3%) were seropositive for canine distemper. These results confirm the seropositivity percentages previously reported only for canine parvovirus, while a lower seropositivity for canine distemper was observed in our study compared to previously reported ones. Females showed higher seropositivity compared to males for both viruses (CPV 95.9% vs 87.7%, CDV 75.1% vs 70.6%). The highest seropositivity was observed in intact females (CPV 97.1%, CDV 76,5%) and neutered males (CPV 94.9%, CDV 82.1%). Our study confirms previous results showing a higher positivity in small size dogs only for CVD (72.9% small, 70.8% medium, 62.7% large size), whereas this difference was not observed for CPV (95.2% small, 96.9% medium, 96.4% large size). Very high seropositivity was observed in all age classes for CPV (100% puppy, 95.1% adult, 96.8% senior, 100% geriatric) while CDV seropositivity decreased in older age groups (78.9% puppy, 69.8% adult, 69.3% senior, 58.3% geriatric). Finally, considering the trend over time in dogs repeatedly tested, seropositivity remained at constant levels for CPV while was fluctuating for CDV. The low seropositivity observed for CDV in vaccinated dogs suggest that dogs may not be adequately protected against this disease. However, a lack of humoral immunity does not necessarily correspond to a lack of protection, because cell-mediated immunity was not measured. Furthermore, despite the use of TiterCHEK® has been widely reported, it does not represent the gold standard and sometimes it might not detect very low levels of seropositivity. Therefore, SN and HI should be used to confirm the high seronegative results found in our study. If the high CDV seronegativity will be confirmed, this would represent a wake-up call for the risk of a possible re-emergence of this dangerous disease and require a revision of vaccines currently on the market.

References available upon request.

P28 – CHEMOMETRIC ANALYSIS IN DEFENCE OF TRACEABILITY OF ITALIAN FOOD

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Over the last few years, due to the increasing of fraud and counterfeiting of agrochemical products, Italian and European authority have worked to establish marks as protected geographical indication (PGI) (Reg. CE N. 510/2006). The defence of these goods is based first on a documental control, but nowadays it could be supported by the recent development of instrumental physico-chemical analysis and chemometric analysis. The aim of the research is the development of an innovative tool which could not only control and verify the traceability, but also describe the natural variability of the "Red Apple of Cuneo", registered as PGI, and might help each actor of the food chain: this can be done using an integrate chemometric model, based on analytical chemistry, that considers a geo-referencing of the sample. The collected samples came from three different production zones (Z1, Z2, Z3) and they belong to different cultivar of apples: "Gala Apple" (9 lots from 9 different producers) and "Red Delicious Apple" (9 lots from 8 different producers). In order to quantify the concentration of 40 elements (metals, trace metals, transition metals, REE) a duplicate analysis was performed on each sample: after the homogenization step, 7.5 mL of HNO_3 (70% v/v) and 2 mL of H_2O_2 (30% v/v) were added before microwave digestion, performed by an ETHOS 1 Milestone S.r.l. (Sorisole, BG, Italy). The elementary analysis was carried out by an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS Xseries II, Thermo Scientific, Bremen, Germany), following the instrumental and quality assurance stablished protocols. The chemometric analysis consists of two main steps: first the pattern recognition analysis (PCA) and then PLS Discriminant Analysis (PLS-DA). First, PCA points out that samples are grouped as production areas: Z3 shows higher ratio in elements like Yb, Ni, Cu, Co, Tm, Ag, V and low values in Nd, U, Y, Rb, Mg, meanwhile Z2 shows an inverse behaviour. Later, PLS-DA was used to identify which elements and factors should have the better discriminatory ability: variable selection mode was applied to classify samples according to their production area and to verify if the model could feedback which elements are responsible for the location referencing. According to the joining of the two described steps, the design of the analysis allows good cross-calibration, and a correct classification of the 86% of samples. In the second part of the project volatile organic compound (VOC) and harvest will be added to improve the model.

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P29 – A FIRST MONITORING OF FLUORINE IN ANIMAL FEED

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Fluorine is one of the most abundant elements in the environment and in living organisms. The major natural source of fluorine is volcanic activity, resulting in an atmospheric emission, and deep well water. This element occurs in different chemical forms, but in environment, food, water and plants it is mainly present in its ionic form, fluoride. The level of fluoride in water is depending of the geological environment of provenience. In some areas such as central Asia and India, endemic fluorosis is documented, and water may contain up to 20 mg L^{-1} of fluoride [1]. Soluble fluorides can be bio-accumulated by aquatic and terrestrial biota, but it is still not clear if the element can be biomagnified through the food chain.

Excessive intake of fluorine can exert chronic toxicity in animals, resulting in skeletal and dental fluorosis, associated with nephrotoxicity. Commission regulation (EU) No 186/2015 amending Directive 2002/32/EC of the European Parliament and of the Council set the maximum levels of fluorine in animal feed. Then, the national Reference Centre for the Surveillance and Monitoring of Animal Feed (CreAA) developed a method for the detection of fluoride in animal feed following the EN 16279:2012 "Determination of fluoride content after hydrochloric acid treatment by ion-sensitive electrode method (ISE)". In the frame of the National Monitoring Plan for animal feed, 46 samples (raw material, complete and complementary feeding stuffs) were analysed in 2018. All samples were compliant to maximum limits. In all the complete feed (n=13) fluoride was found < LOQ (40 mg Kg^{-1}), while in complementary feed (n=20) 20% of samples contained fluorine in the range 60-179 mg Kg^{-1}. In raw materials (n=13) 69% of samples recorded concentration between 1169 – 1662 mg Kg^{-1} (phosphate based) and 76 -117 mg Kg^{-1} (meat, fish based). Studies regarding fluoride levels in feed are scarce but our concentrations were in the range of those found by Gikunju and coauthors [2] in Kenian feed (61-132.0 mg F/kg) and lower than the maximum value scheduled in the Commision Regulation 2015/186/UE.

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30P - RARE EARTHS ELEMENTS IN HONEY

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Rare Earth Elements (REEs) are a chemically uniform group of substances belonging to Group IIIb in the Periodic Table that possess similar physical and chemical properties. There are 17 REEs, 15 of which are named lanthanides, plus yttrium and scandium; despite their name, they are not that rare in nature, being the 15th most abundant components of the earth's crust. REEs are non-essential elements for life, they cause contamination worldwide because they are essential in several technologies (medicine, mobile communication, energy and electronics) due to their unique physical and chemical properties (high density, melting point, conductivity and thermal conductance). Nowadays the risk assessment have been scarcely investigated and remain still unregulated, not being part of environmental or safety monitoring plans, even though several in vitro and in vivo studies has highlighted inhibitory or toxic effects at high concentrations.[1] This is the first study aimed at defining the levels and patterns of REE in honey from different countries (Argentina, Brazil, Italy, Kazakhstan, Mexico, Macedonia, Montenegro, Russia, Tanzania) by ICP-MS analysis. The REE sum (REES) was found in the range 6.8 – 65 g Kg^{-1} following the increasing order: Russia< Montenegro< Kazakhstan< Macedonia< Argentina< Italy< Brazil< Mexico< Tanzania.

Regarding REE levels, concentrations were detected with the following trend: terbium (Tb), lutetium (Lu), holmium (Ho) and thulium (Tm)< europium (Eu)< ytterbium (Yb)< erbium (Er)< dysprosium (Dy)< gadolinium (Ga)< samarium (Sm)< praseodymium (Pr)< yttrium (Y)< neodymium (Nd)< lanthanum (La)< cerium (Ce)< scandium (Sc). These results highlights different order of magnitude from a country to another: however due to the low concentration it can be assumed that there is no hazard for human health. Despite the encouraging results, further studies are required to evaluate REE intake from different food matrices that could caused a cumulative effect dangerous for human health.

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31P - RUMINAL DEGRADABILITY AND INTESTINAL DIGESTIBILITY OF DRY MATTER AND PROTEIN BY A TWO-STEP *IN VITRO* ASSAY

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Rumen and intestinal degradation of dietary crude protein (CP) influences rumen fermentation and intestinal amino acid supply to ruminants. Aim of this study was to evaluate by the *in vitro* modified method [1] the ruminal degradation and the intestinal digestibility of dry matter (DM) and crude protein (CP) of soybean, sunflower and canola meals before and after an extrusion process (EP) at 130°C. Feedstuffs were analyzed for DM, CP, soluble protein (SP), as well as acid and neutral detergent insoluble protein (ADIP, NDIP). Each feed was placed in 6 flasks (0.5 g sample/flask) and was incubated for 16hrs with buffer and rumen fluid (40+10ml respectively). After that, half flasks were filtered and the residues were analyzed for DM and CP content. The remaining were immediately added with HCl and pepsin for 1hr and then with an enzyme mixture for further 2hrs. After filtration, DM and CP were evaluated. Buffered rumen fluid with (2 flasks) and without cellulose (4 flasks) was also incubated simultaneously to correct the residues respectively for microbial and buffer N contamination. The procedure was repeated twice for each feed. Soybean meal showed the highest DM rumen degradability and intestinal digestibility while EP did not influence these parameters. Surprisingly, soybean and sunflower meals showed higher CP contents than their extruded. The reason of this difference could be related to protein structure modification after extrusion or to a problem with the usual Kjeldahl extraction. Significant interactions among products and the EP were observed for ADIP, SP, ruminal protein degradation (RPD) and intestinal protein digestibility (IPD). Indeed, EP significantly reduced the SP in sunflower and soybean, but not in canola. In addition, EP increased the ADIP content in soybean. RPD's (expressed as % of CP) among feedstuffs before EP were significantly different (sunflower=73.4; soybean=58.4 and canola=47.1). EP significantly decreased RPD in sunflower and soybean (-39% and -28%, respectively), but not in canola. In addition, sunflower meal showed the lowest IPD, which significantly increased after the EP both on sunflower and on soybean products. On the contrary the EP did not affect canola IPD. Soybean meals and its extruded showed the highest total protein digestion. In conclusion, the analytical method used seems to be effective in evaluating different processes in rumen degradability and intestinal digestibility of protein in the common ruminant feeds. In comparison with the original method [1], in this study the cellulose was used to estimate rumen microbial contamination. The correction with N produced after cellulose *in vitro* fermentation significantly changed the percentage of sample RUP, in comparison with only blank correction (-5 to -10%). Further investigations are needed to validate if the use of cellulose is as an effective method to correct rumen undegraded protein from microbial contamination. As expected, the EP actually reduced the RPD and increased the protein fraction escapable from the rumen in soy and sunflower meals but not in canola, suggesting a different effect of EP in this product.

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32P - LARCH SAWDUST AS FUNCTIONAL ADDITIVE

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The larch sawdust (*Larix decidua L.*, Pinaceae) is rich in valuable physiologically active compounds and functional polymers: arabinogalactans [1], lignans (mainly secoisolariciresinol and lariciresinol) [2] flavonoids (mainly taxifolin and dihydrokaempferol) [3] and diterpenes (larixyl acetate and larixol) [4]. Arabinogalactans, abundant in the genus Larix, are an excellent source of water-soluble prebiotic fibre and in monogastric animals increase beneficial gut anaerobes [5]. Moreover, larch sawdust extracts have been suggested to enhance immune function as shown in an ex-vivo study on ovine neutrophils [6].
We investigated whether larch sawdust (by Jannach Lärchenholz GmbH) supplemented to dairy cows have effects on blood biochemical parameters. The treated group received 250 g/d of Larch sawdust (n=10) and the control group 250g/d of placebo (n=10), mixed with 1 kg of total mixed diet, for 14 d. Several biochemical changes induced by the supplementation of larch sawdust were observed. Biomarkers of liver function were influenced by the treatment. Total bilirubin was significantly lower in larch-treated animals (- 17%; $P < 0.05$). Cholesterol was lower in animals given the larch diet. VLDL, which are involved in lipid transport from the liver, were also slightly increased. A lower urea concentration was found in the larch-treated group (5.6 vs 6.5 mmol/L; $P < 0.01$). Total protein decreased significantly in animals given the larch diet, mainly due to a decrease in globulin concentration (from 35.1 to 33.5 g/L; $P < 0.01$). Because total globulin concentration can provide an indication of an animal's humoral immune status or response, the observed effect could be beneficial in animal diet during challenges such as weaning, as well as in disease states. These results confirm previous studies and indicate that larch sawdust can be a resource as an appreciable feed additive. Moreover, larch sawdust is a waste product from the wood industry and would represent an economic additive. To confirm a valuable application, we have started further studies with other animal species.

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33P - EFFECT OF RELAXIN ON CRYOPRESERVED BEEF BULL SEMEN CHARACTERISTICS

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The present study was aimed to improve the quality of cryopreserved Piedmontese beef bull semen by incorporation of different concentrations (0 [control], 25, 50 and 100 ng/mL) of Relaxin in diluted semen before cryopreservation process [1]. Semen samples were collected from 4 proven fertile bulls, once per week for 8 consecutive weeks. For each section semen was pooled, diluted with Bullxcell® extender (IMV, France) and supplemented with the different concentrations of Relaxin before to undergo to cooling, equilibration and freezing. Frozen semen was thawed at 37°C for 40 sec. and assessed for motility and velocity parameters (after incubation at 37°C for 0, 1, 2, 3 and 4 hrs), sperm viability, acrosome, plasma membrane and DNA integrities, apoptosis, mitochondrial membrane potential, mucus penetration and SOD activity. Relaxin has a significant ($P<0.02$) positive effect on all sperm motility [2] and velocity parameters (C.A.S.A. analyses) after the different periods of incubation, and the main effect was recorded with the concentrations of 25 and 100 ng/ml. Relaxin at same concentrations improved the live percentage sperm ($P<0.05$) analyzed by eosin-nigrosin staining and HOS test and numerically decreased the necrotic and apoptotic percentage (Annexin-V/PI-binding assay) of sperm without affecting the acrosome [3] and DNA integrity performed by SCSA. Relaxin increased the HMMP ($P<0.08$) determined with JC-1, without affecting neither the mucus penetration nor the SOD activity. In conclusion, Relaxin incorporation in diluted bull semen before cryopreservation has a positive effect on improving the post-thawing sperm activity mainly with the concentrations of 25 and 100 ng/ml.

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34P - INTERVAL BETWEEN REMOVAL OF 4.7MG DESLORELIN IMPLANT AND RESTORATION OF THE FUNCTION OF SEXUAL HORMONES IN CATS

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Deslorelin implants have been widely used to produce a reversible sterilization in several species [1]. A temporary suppression of reproductive activity is frequently needed in cat breeding establishments due to difficulties of managing feline reproduction. Long acting GnRH implants such as deslorelin have a prolonged duration in cats (12-15 months in tomcats and 18-22 months in queen) [2,3] which is often too much for cat breeders who frequently come back asking for early implant. However, the interval between implant removal and resumption of fertility in cats has not been studied in details yet.
Thirteen privately owned cats (7 tomcats and 6 queens) with a presenting complaint of control of reproduction were administered a 4.7 mg deslorelin implant. Cats were divided in 4 different groups; tomcats implanted for 6 or 9 months (n=4 and n=3, respectively), queens implanted for 6 or 9 months (n=4 and n=4, respectively). The study was conducted during all the year. Implants were placed in the periumbilical area and removed under light sedation at the end of the study period. All treated animals were evaluated weekly. Blood samples were taken every week and measurement of testicles, vaginal smears and penile spikes observation was performed in every visit. Testosterone levels were measured on serum samples of tomcats in order to determine testicular activity. Experimental procedures were submitted to the ethical committee of the University of Padova. Until now, 3 males and 2 females of the 6-month treatment and 2 males and 1 female of the 9-month treatment have resumed sexual hormones function after deslorelin treatment. Restoration of sexual hormones function was determined by presence of testosterone, penile spikes, 40% of keratinization of vaginal smear of $\geq$40% and by appearance of estrus behavior. Results show that in average, cats treated with deslorelin implanted for 6 months restore sexual hormones activity in 29±11 days while cats implanted for 9 months restored it in 27±15 days (p=0.355) independent of sex or season at removal. The effect of a 4.7mg deslorelin in cats has been determined in both tomcats and queens with a block of sexual hormones activity [2,3]. Our results show that removal of the 4.7mg deslorelin implant at a time when the implant is displaying its complete effects is followed by a resumption of sexual hormones functionality after about 4 weeks independent of sex, age or season. Further studies are needed to determine exact time when activity of sexual hormones is restored after implant removal.

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35P - MANAGEMENT AND RESOLUTION OF A VAGINAL LEYOMIOMA IN A BITCH. A CLINICAL CASE REPORT

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In the bitch, tumors affecting the vagina or vulva are uncommon, with the majority being leiomyomas or fibromas [1]. Ovarian hormones may be involved in the pathogenesis and medical treatment with aglepristone (AGLE) or ovariectomy are used with good results [2]. **Clinical case:** An 11-year old, intact Labrador Retriever bitch was referred to the clinic with a large vaginal mass prolapsed that appeared 3 days after proestrus onset with concomitant muco-purulent discharge. Serum progesterone (P4) concentration was 4.94 ng/mL. Observing clinical conditions, volume of the mass and age of the animal, a medical treatment was performed as treatment of choice. To prevent the mass from increasing during estrus, 10 mg/kg AGLE was administered on the day of referral, the following day and every other 7 days [2]. Eight days later the bitch showed difficulty in urinating and defecation. On ultrasound anechogenic follicles of 0.7cm were observed on both ovaries, urinary bladder and both ureteres were dilated with evidence of hydronephrosis. On day 11 a CT-scan was performed showing the presence of a 15x8x6 cm mass compressing both rectum and uretra. Due to the presence of inflammatory material in vagina, a transcutaneal biopsy was performed. Results of the biopsy showed the presence of a leyomioma. On day 18, because of the apparent lack of efficacy of AGLE and the location and volume of the vaginal mass, a prostaglandin treatment was started (alfaprostol 0.02ug/kg BID). On day 22, hydronephrosis was still present. On day 28, due to the increased values of P4 and unachieved luteolysis, ovariohysterectomy was performed on day 35 post-referral. The left ovary showed 11 corpora lutea (CL) and other 12 CL in the right ovary. A luteoma was diagnosed in the left ovary. Ten days post-surgery P4 levels were undetectable, vaginal mass was not palpable and the bitch was able to urinate without problem. Ninety days after ovariohysterectomy, a CT-scan was performed showing a complete reduction of the vaginal mass. We suspect a correlation between the ovarian tumor, high levels of progesterone and the unachieved luteolysis with prostaglandin treatment.

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36P – CRYOPRESERVATION OF OVINE PREPUBERTAL TESTICULAR TISSUE WITH A NOVEL VITRIFICATION SYSTEM (E.VIT)

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Cryopreservation of prepubertal testicular tissue can be a tool for genetic preservation of rare or high value animals or endangered species. Nowadays, vitrification is extensively used for preserving oocytes and embryos and is gaining acceptance also for gonadal tissue. Aim of this work was to evaluate the efficiency of vitrification of ovine prepubertal testicular tissue with a novel micro-device named "E.Vit" (FertileSafe LTD) recently designed to perform in-straw all cryopreservation procedures [1]. The new device consists of a 0.3 mm diameter polycarbonate grid (FertileSafe LTD Israel) with a 50 µm inserted at the open ends of straw (CBS, IMV, France). Testes collected from regularly slaughtered lambs (n=10, 40 days old) were sagittally sectioned and 1 mm^3 pieces were collected from the mid part of rete testis. Three sections were loaded into each straw and exposed to two solutions following a two-step vitrification protocol: Equilibrating Solution [ES; 7.5% dimethyl sulfoxide (DMSO) +7.5% ethylene glycol (EG) + 20% Fetal Calf Serum (FCS) in TCM-199) for 6 minutes followed by 90 seconds in the Vitrification Solution (VS: 18% DMSO +18% EG + 0.5M Trehalose + BSA 0.6% in TCM-199). ES and VS were subsequently removed and straws directly plunged into LN_2. Warming consisted in placing the straws in a solution of TCM-199 with 20% FCS and Sucrose at decreasing concentration (1 M, 0.5 M and 0.25 M) for 5 minutes each at 38.6°C. Samples were then removed from the device and transferred into culture medium (IVC: TCM-199 supplemented with 10% FCS); after 2 or 24 hour *in vitro* culture samples were evaluated in terms of cell viability by trypan blue staining or gene expression by Real Time PCR. To dissect the specific effects of IVC or cryopreservation, control (non cryopreserved) tissues cultured in vitro for 24 hours were included. Most cells survived vitrification: viability was similar immediately after warming (66%±4.73) or after 2 hours IVC (59.67%±4.18), although significantly lower compared to non cryopreserved controls (89.67%±1.45. ANOVA $p<0.05$). Extended post-warming IVC (24 hours) caused an additional decrease to 31%±3.46 ($p<0.05$). Conversely, cell vitality was not affected by IVC alone at either time point ($p>0.1$). All genes (*PLZF, TERT, OCT4, KIT, AR, FSHR, STAR, KIF11, BAX, SOD1, CIRBP* and *HSP90AB1*) were expressed in all experimental groups, with transcript specific patterns. Genes involved in cell stress response indicated moderate effects of IVC or cryopreservation on testicular cell gene expression: *BAX* was upregulated by extended IVC, while *HSP90b* was solicited by vitrification at both time points (ANOVA $p<0.05$). Importantly, post-warming survival of both spermatogonia and supporting somatic cells was shown by the expression of germ-cell- (*PLZF, TERT, OCT4* and *KIT*) or somatic- (*AR* and *FSHR*) specific markers, confirming the validity of the vitrification system to maintain spermatogenesis potential. This novel protocol for testicular tissue cryopreservation of prepubertal animals may contribute as a new approach to the development of large scale biodiversity programs.

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37P – OCCURRENCE OF SCROTAL HERNIA IN RAMS IN NORTH SARDINIA

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Scrotal hernia is commonly seen in stallions and boar but less frequently in bulls or rams. It can be hereditary or acquired and can seriously affect fertility in males for disruption of testicular thermoregulation. The objective of this retrospective study was to evaluate the occurrence of scrotal hernia in rams from centre north Sardinia (Italy) in the past 10 years, assessing age distribution of affected animals, site of hernia (right or left hemiscrotum) and content of herniary sac (omentum, intestinal loops or both). The investigation was carried out on 1927 rams (age ranging from 2 months to more than 7 years) from 234 different farms located in the centre north Sardinia, reared under traditional management. Animals with suspected unilateral scrotal hernia were admitted to the Teaching Veterinary Hospital of the Department of Veterinary Medicine of the University of Sassari. Animals underwent clinical examination followed by further examination of the reproductive tract. The scrotum was observed for abnormalities in colour and shape and for presence of skin lesions, palpated and checked for painful response. Trans-scrotal ultrasonography (MyLab One, Esaote, Italy) was carried out to confirm diagnosis of scrotal hernia. Collected data were analysed by x^2- test with significance level defined for P value <0.05. The results of this epidemiologic study showed that 30/1927 rams were affected by unilateral scrotal hernia with an incidence of 1.5%. Palpation of scrotum failed to elicit signs of pain. Scrotal hernia affected mostly animals aged more than 7 years with a rate of 23.8% (5/21; P<0.05); while it was less frequently seen in rams younger than 1 year (0.75%; 3/397). In 1-2 years old rams the incidence was 1.07% (7/651) and in subjects aged 3-4 and 5-6 years it was respectively 1.74 (11/632) and 1.77 (4/226). The most affected hemiscrotum was the left (70%; 21/30; P<0.05) and the herniary sac contained mainly intestinal loops (46.7%; 14/30) and omentum (40%; 12/30) and less frequently both of them (13.3%; 4/30; P<0.05). The high incidence of scrotal hernia in old rams reported in this study is related to the low number of observations since in common management there is a mean turnover of males every 3 years. An 8% incidence of this condition has been reported in Australian Merino rams and a genetic predisposition increased with inbreeding has been hypothesised [1]. Other studies described the left hemiscrotum as primary site of hernia in rams as a result of the weight of the rumen and of the resting habit with the left rear leg abducted [2].

[1] Carr PM. AN APPARENTLY INHERITED INGUINAL HERNIA IN THE MERINO RAM. Australian Veterinary Journal. 1972;48:126-7. [2] Al-Sobayil FA, Ahmed AF. Surgical treatment for different forms of hernias in sheep and goats. Journal of veterinary science. 2007;8:185-91.

38P – MODIFIED SURGICAL TECHNIQUE FOR INGUINOSCROTAL HERNIA RESOLUTION IN RAMS

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In domestic animals, surgical treatment of scrotal hernia commonly includes ipsilateral orchiectomy. This should be avoided in animals of genetic interest and for this reason a conservative technique was developed. However, recurrence of scrotal hernia following conservative surgery has been reported [1]. The aim of the study was to test the efficiency of a modified surgical technique for resolution of scrotal hernia and to assess its effects on resumption of semen quality parameters. The experiment was carried out on 30 rams admitted to the Teaching Veterinary Hospital of the Department of Veterinary Medicine of the University of Sassari with unilateral scrotal hernia. Anamnesis reported unilateral scrotum enlargement 3 to 4 weeks before admission to the Hospital. Following general health assessment and examination of the reproductive tract, diagnosis of scrotal hernia was confirmed by ultrasound scanning (MyLab One, Esaote, Italy). Animals were allocated to 2 treatment groups: i) control group (n=15) underwent classic conservative surgery for scrotal hernia [2]; ii) treated group (n= 15) was submitted to surgery using a modified technique. Briefly, following sedation, epidural anaesthesia and setting of the surgical field, scrotum was incised and the content (intestinal loops, *omentum* or both) was exteriorised. Hernia was reduced and the content was repositioned in the abdominal cavity. Scarification of serosa of *lamina parietalis* and *visceralis* of vaginal *tunica* was performed. The external inguinal ring was restricted using a saddle stitch suture with Nylon fitted with a curve needle on each end. An artificial ring was created with the suture surrounding the spermatic cord through the edges of the inguinal ring creating a cerclage. A mattress suture was continued approximating the edges of the inguinal ring and to strengthen the suture plan, an X introflected suture was carried out. Finally, the tissues above (external spermatic fascia, dartos and skin) were sutured. Topic and systemic antibiotic treatment was provided for the following 10 days. In order to evaluate the effects on the quality parameters of semen, rams were submitted to electroejaculation (Bayley Ejaculator mod 2-Western Instrument Company; USA) before and after the surgery (3-4 months later). The collected ejaculates were analysed for concentration (Photometer Ovin Acucell IVM Tecnologies, France), motility parameters by CASA (Computer Assisted Sperm Analysis; Hamilton-Thorn IVOS, Spain) and viability by eosin–nigrosin staining. Two months after surgery, both groups of animals were examined and checked for possible recurrence of the condition. Follow up investigations revealed that scrotal hernia recurred in 4/15 (26.6%) rams subjected to classic surgery while no recurrence was observed in the treated group. Moreover, in this group all sperm quality parameters (motility, viability and concentration) were significantly improved after surgery ($P<0.05$). In conclusion, we presented a novel modified technique for surgical resolution of inguinal hernia in rams that does not present recurrence and results in complete resumption of important parameters of sperm quality.

[1] Al-Sobayil FA, Ahmed AF. Surgical treatment for different forms of hernias in sheep and goats. Journal of veterinary science. 2007;8:185-91. [2] Braun WF, Cole WJ. Unilateral scrotal hernia repair in a ram lamb. J Am Vet Med Assoc. 1985;187:500.

39P – EVIDENCE THAT MATERNAL LACTATION MAY INFLUENCE THE SIZE OF THE OVARIAN RESERVE IN FEMALE CALVES

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Evidence indicates that the number of follicles and oocytes in ovaries of mammals (ovarian reserve) is positively associated with several measures of fertility, yet the causes of its inherently high variation are unknown [1, 2]. We hypothesized that the lactation status (lactating vs non-lactating) during pregnancy in dairy cattle may have a negative impact on the size of the ovarian reserve in their daughters. Forty-five non-pubertal, Holstein-Friesian female calves were enrolled in this study; fifteen were born to nulliparous, non-lactating mothers and thirty were born to pluriparous, lactating cows. Calves were weighed and their height at withers was recorded at birth. A single blood sample was collected between day 1 and 20 after birth to measure serum concentrations of Anti-Müllerian hormone (AMH) with a commercial ELISA kit (Ansh Labs, Texas, USA)[3]. When calves were 7 months of age, the total number of ovarian follicles ≥3 mm in diameter (antral follicle count, AFC) was assessed by ovarian ultrasonography. Daughters of nulliparous (N) and pluriparous (P) cows were similar at birth in body weight (N=40.8±0.4; P=41.14±0.3 kg) and height at withers (N=77.1±0.3; P=77.1±0.3 cm). AMH serum concentrations within 20 days after birth ranged from 35 to 4949 pg/ML and were lower in calves born to nulliparous compared to calves born to pluriparous heifers (N=154±23.5; P=844±0247.2 pg/mL; P=0.03). However, AFC at approximately 7 months of age, was similar among calves irrespectively of maternal parity. We conclude that AMH peripheral concentrations within twenty days after birth are highly variable and apparently, influenced by maternal parity, but not linked to AFC at seven months of age.

[1] Ireland JJ, Smith GW, Scheetz D, Jimenez-Krassel F, Folger JK, Ireland JLH, et al. Does size matter in females? An overview of the impact of the high variation in the ovarian reserve on ovarian function and fertility, utility of anti-Mullerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in cattle. Reproduction Fertility and Development. 2011;23:1-14. [2] Mossa F, Jimenez-Krassel F, Scheetz D, Weber-Nielsen M, Evans ACO, Ireland JJ. Anti-Müllerian Hormone (AMH) and fertility management in agricultural species. Reproduction. 2017;154:R1-R11. [3] Ireland JL, Scheetz D, Jimenez-Krassel F, Themmen AP, Ward F, Lonergan P, et al. Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle. Biol Reprod. 2008;79:1219-25.

40P – COMPARISON OF VENOUS BLOOD GAS VALUES AND ELECTROLYTES OBTAINED BY THE PUSH-PULL TECHNIQUE FROM A JUGULAR CATHETER VERSUS DIRECT VENIPUNCTURE IN SICK NEONATAL FOALS

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Blood collection by indwelling intravenous catheter (IVC) was proposed to minimize risk of infections and to reduce anxiety and pain for patients [1, 2]. Recently, Barr et al. [2] showed as the push-pull technique (PPt) from an IVC did not influence the blood gas (BG) results compared to direct venipuncture in dogs.

The aim of this prospective within-subjects study was to compare venous BG parameters obtained from blood samples collected via jugular catheter by PPt and by direct jugular venipuncture in sick neonatal foals.

Fifteen-paired blood samples were obtained from foals hospitalized at the Equine Perinatology Unit of the University of Bologna. For each foal one blood sample was collected via IVC (16G, 20 cm, LOGICATH™) by PPt: 2.4 mL of blood were aspirated and immediately reinfused into the catheter with a 10 mL syringe for 3 times, then 1 mL of venous blood was collected using a specific heparinated syringe (Monovette®). Thereafter, a blood collection was performed by direct venipuncture of the contralateral jugular vein, with a Monovette® connected with a 20G needle 1-inch. Rectal temperature (°C) was recorded for each foal. All blood samples were analysed with an automated BG analyser (Roche OptiCCA) within 10 minutes after blood sampling and BG variables were temperature corrected.

The level of agreement of BG values obtained by the two different techniques was assessed with Bland-Altman analysis. Bias (mean difference between values obtained by the two different methods) were fairly small and clinically acceptable for all variables: pH (0.01), PO2 (-1.36 mmHg), PCO2 (-1.74 mmHg), K+ (0.09 mmol/L), Na+ (0.4 mmol/L), Ca2+ (0.01 mmol/L), Cl- (-0.2 mmol/L), Anion gap (0.38 mmol/L), Glucose (-0.23 mmol/L), Lactate (0.01 mmol/L), ctHCO3 (0.65 mmol/L), Actual base excess (0.61 mmol/L), Standard base excess (0.36 mmol/L).

PPt appeared to be an acceptable method for collection of blood samples for venous BG variables as well as pH, electrolyte, glucose and lactate. This technique may reduce drastically the risk associated to venipuncture, especially in critically ill foals that usually need frequent monitoring. Moreover, the PPt can reduced the stress of necessary restraining and the number of personnel involved in the procedure.

[1] May ML et al. Comparison of hematologic and biochemical results on blood obtained by jugular venipuncture as compared with intravenous catheter in adult horses. J Vet Intern Med, 24:1462–66, 2010. [2] Barr CA et al. Effect of blood collection by the push-pull technique from an indwelling catheter versus direct venipuncture on venous blood gas values before and after administration of alfaxalone or propofol in dogs. JAVMA, 251:1166-74, 2017.

41P - EFFECTS OF A COMMON CENTRIFUGAL FORCE AND TWO DIFFERENT CENTRIFUGATION TIMES ON EQUINE SPERM QUALITY

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Conventional sperm selection techniques, such as Density Gradient Centrifugation and Swim Up, rely on centrifugation steps that might cause cell damage [1]. The aim of this study was to investigate the effects of a common centrifugal force and two different centrifugation times on equine sperm quality. Frozen semen samples (3 replicates) from one stallion of proven fertility underwent the following experimental design: 1) no centrifugation (control), 2) centrifugation at 300xg for 5 minutes (min), 3) centrifugation at 300xg for 10 min. For each sample, sperm motility was analysed by Computer-Assisted Sperm Analysis (CASA) [2]. Then, mitochondria functionality was evaluated by a polarographic assay [3]: the ratio between the rate of oxygen uptake in presence of respiratory substrates plus ADP and the rate of oxygen uptake in presence of the substrates alone allowed for the calculation of a respiratory control ratio (RCR), an index of mitochondrial respiration efficiency. All data are reported as mean value ± standard deviation and P-values (One-way ANOVA followed by Tukey post-hoc test) were considered significant if $P<0.05$. Progressive motility (reported as percentage) was impaired at both tested protocols compared to control (5.8±1.3, 6.9±2.7 vs 13.00±3.7, for 300xg for 5 min and 10 min, respectively vs control, $P<0.0001$). However, no statistical difference was found between samples centrifuged at the same centrifugal force but different times (5.8±1.3 vs 6.9±2.7, for 300xg for 5 min vs 300xg for 10 min, respectively, not significant). RCR values also significantly decreased at both tested protocols (1.4±0.2, 1.4±0.1 vs 1.9±0.2, for 300xg for 5 min and 10 min respectively vs control, $P<0.05$). However, no statistical difference was found between samples centrifuged at the same centrifugal force but different times (1.4±0.2 vs 1.4±0.1, for 300xg for 5 min vs 300xg for 10 min, respectively, $P>0.05$). In conclusion, centrifugation force, but not time, affected sperm quality in terms of both motility and mitochondrial functionality. As future perspective, “gentler” non centrifugation-based methods for sperm selection are desirable, such as the novel lab-on-chip solutions.

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42P - EFFECT OF CRONO-GEST 20 MG ON REPRODUCTIVE PARAMETERS OF DAIRY EWES IN SOUTHERN ITALY

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Intravaginal devices containing progestins are commonly used for induction of synchronization of oestrus in ewes. Recently, a 20 mg fluorogestone acetate (FGA) impregnated sponge replaced 30, 40 and 45 FGA sponges [1-3]. There are few reports on application of 20 mg FGA sponges in large flocks, excluding the registration trials. This study, approved by the local Animal Ethics Committee (014/2017), was performed in a farm in Cosenza, Italy (39°18'39"60 N, 16°15'3"60 E; altitude 238 m). A total of 1818 ewes, 1232 Lacaune (L) and 586 Sarda (S), multiparous (M) and nulliparous (N) were synchronized in November (n) and May (m). The animals were so divided into 8 groups: LMm (n=556), LNm (n=180), SMm (n=70), SNm (n=32), LMn (n=242), LNn (n=222), SMn (n=440) e SNn (n=76). The intravaginal sponge was inserted and maintained for 14 days. At sponge removal, equine chorionic gonadotropin (400 UI) was given and rams of proven fertility were joined 30 hours later into flocks with a male/female ratio of 1:8. Transrectal ultrasonography was performed for pregnancy diagnosis 30 days after mating. Ewes were monitored until delivery and reproductive parameters such as oestrus (OR), pregnancy (PR), lambing rates (LR) and prolificacy (P) were calculated. One-way ANOVA for 8 independent treatments was employed followed by Bonferroni multiple comparison test. The SMn group showed the best performances (91% OR, 88% PR, 85% LR, 1.36 P) with the exception of P that was significantly higher in the LMn group (1.68 P). SN groups had significant low rates, while LN groups had satisfactory pregnancy and lambing rates. Effect of season was more evident in S groups where SMn and SMm differed significantly for all the analysed parameters and SNm (63% OR, 56% PR, 56% LR, 1.06 P) had lower rates than SNn. The use of FGA-releasing intravaginal devices may improve productivity of the flocks. According to manufacturer registration trials, 20 mg is the minimum drug load delivering good fertility data, reporting 71% of pregnancy rate and 1.69 of prolificacy in 950 dairy ewes in breeding season in France. Other variable data in literature reflect different breed, management, regimen, season and latitude [2,3]. Lacaune breed has an optimal reproductive potential, young and adult ewes respond to progestins in breeding and non-breeding season. Sarda breed shows best adaption to environment and gives the best results in reproductive season, but young ewes have to be strictly monitored being less fertile.

[1] Letelier et al. Ovarian follicular dynamics and plasma steroid concentrations are not significantly different in ewes given intravaginal sponges containing either 20 or 40 mg of fluorogestone acetate. Theriogenology. 71:676-82, 2009. [2] Alavez Ramírez et al. Short communication: estrus synchronization using progestogens or cloprostenol in tropical hair sheep. Trop Anim Health Prod. 46:1515-8, 2014. [3] Zonturlu et al. Effect of double GnRH injections on reproductive parameters in Awassi ewes receiving long-term progesterone. J Appl Anim Res 46:1103-7, 2018.

43P - UNUSUAL GIANT CYST AS A CONSEQUENCE OF THE OVARIAN REMNANT SYNDROME IN A BITCH

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An 9 years old German shepherd bitch was presented to the Veterinary Hospital of the University of Bari "Aldo Moro" with a 1 year history of enlarged abdomen. The bitch was neutered 3 years ago and no signs of heat were seen since then. The owner claimed a progressive increase in abdominal volume that started about 1 year before and progressed faster in the last 2 months when there also was a deterioration of the general conditions with dysorexia. It worsened in anorexia for about 2 days. On physical examination the bitch was thin, depressed and reluctance to move with a distended and swollen abdomen. At superficial palpation the abdominal wall resistance was detected, the rigidity was due to an intra-abdominal fluid collection. A complete blood and urine tests showed nothing relevant. The ultrasonographic examination showed the presence of a spider-web-like structure with septa and an anechoic content that occupied most of the abdominal cavity and displace the viscera. We proceeded with an exploratory laparotomy. Laparotomy revealed the presence of a cystic formation that occupied most of the abdominal cavity (about 25 cm in diameter) developed on a remnant of the right ovary and containing about 13 lt of serum-hematic fluid and many adhesions between abdominal organs and tissues. The presence of other cysts on a further left ovarian residue was also highlighted: one with a diameter of about 10 cm and others smaller than about 5 cm. Fragments of right ovarian cysts and uterus were stored in 2% buffered formalin to be subjected to histological examination. The subject recovered from anesthesia was discharged with the following therapy: amoxicillin clavulanate (12.58 mg/kg bid for 10 days) and robenacoxib (1 mg/kg sid for 5 days). The histological examination of the cysts revealed that the wall had the internal theca replaced by fibrous tissue, several layers of degenerated granulosa cells. This finding suggest a follicular stromal cyst. Uterine tissue showed signs of cystic endometrial hyperplasia. At 10 days post-surgery check-up the bitch appeared to be in good health with no signs of depression and pain. She was completely recovering and gaining weight. The owner claimed that the bitch had started feeding again the day post surgery and had been improving day by day. Usually the subjects with ovarian remnant syndrome (ORS) manifest the typical signs of estrus, such as vulvar serohematic discharge, receptivity to the male, nymphomania, and kyphosis due to abdominal pain. Additional clinical signs are: endometrial cystic hyperplasia, pyometra and infrequently diabetes mellitus (1). The peculiarity of this clinical case is represented by the giant dimension of the follicular cyst and the presence of non-specific clinical signs due to the abdominal compression.

[1] Ball RL et all. Ovarian remnant syndrome in dogs and cats: 21 cases (2000-2007) JAVMA. 2010;236:548–553.

44P - GRANULOSA CELL TUMOR OF THE OVARY IN DOGS: CASE REPORT

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Germ cell tumors make up 6 to 12%, while Granulosa cell tumors account for 50% of all canine ovarian neoplasms. Depending on their origin, ovarian tumors are assigned into 3 groups: germ cell tumors, sex-cord stromal tumors and epithelial cell tumours (1). One of the most common neoplasms in canine ovaries is granulosa cell tumors (GCT) that originate from sex cord stromal cells. This tumor has been observed in female canines. Many ovarian tumors produce estrogen or androgen and cause various disorders of the estrus cycle, such as prolonged estrus cycle and pyometra, vulvar swelling and discharge, alopecia, etc. An 8 years old English Bulldog intact bitch was presented to the Department of Veterinary Medicine, Section of Obstetric, University of Bari “Aldo Moro" with several symptoms: hyperthermia, asthenia, hypertrophic vulva with purulent discharge and widespread small areas of dermatitis. The owner claimed nymphomaniac behavior and anorexia. On physical examination a nipple-like formation positioned on the lateral part of the thorax was detected. Blood analysis showed an increase of the white line cells. At ultrasonography the right ovary was increased in volume with a cysts. The uterus was hypertrophic and hypoechoic. An ovariohysterectomy was performed. The excised ovary was cystic and lobulated and its surface was smooth while the consistency was firm. Histologically the architecture of the ovarian parenchyma appeared totally subverted. Cystic multifocal areas were detected. It was diagnosed as a malignant granulosa cells tumor (4.80 x 3.60 cm) moderately differentiated.
The subject showed a fast post-surgery recovery, appetite returned to regular and so does the temperature. The symptoms presented at first clinical examination disappeared. The vulva returned to normal. The nipple-like formation had started to regress from the second week, as well as the widespread skin anomalies present on the entire surface of the body. Dogs with non-functional GCT usually have no clinical signs related to the reproductive tract. Instead, functional GCT can be associated with vaginal discharge, alopecia, enlarged vulva, pyometra, cystic endometrial hyperplasia, and irregular prolonged or persistent estrus (2) as in the case described here. Moreover the regression of the nipple-like formation suggests a hormone-dependent nature as many skin appendages preiously described (3). At 6 months post surgery the bitch was still in a good health.

[1] KENNEDY PC, ET AL. (1998). HISTOLOGICAL CLASSIFICATION OF TUMORS OF THE GENITAL SYSTEM OF DOMESTIC ANIMALS. IN: WORLD HEALTH ORGANIZATION, INTERNATIONAL HISTOLOGICAL CLASSIFICATION OF TUMORS OF DOMESTIC ANIMALS VOL. IV. AMERICAN REGISTRY OF PATHOLOGY, WASHINGTON, DC, SECOND SERIES: 24-27. [2] ZANGHI A, ET AL. (2007); ENDOMETRIAL POLYPOID ADENOMYOMATOSIS IN A BITCH WITH OVARIAN GRANULOSA CELL TUMOUR AND PYOMETRA. J COMP PATHOL, 136: 83-86.21. [3] MILLAN Y, ET AL. (2013). SEX STEROID HORMONES AND TUMOURS IN DOMESTIC ANIMALS. IN:PAYAN-CARREIRAR.(ED).INSIGHTS FROM VETERINARY MEDICINE. VILAREAL: INTECHOPEN, PP.191 -214.

45P – DIFFERENTIAL ABC TRANSPORTER GENE EXPRESSION IN ADULT *DIROFILARIA IMMITIS* MALES AND FEMALES FOLLOWING *IN VITRO* TREATMENT WITH IVERMECTIN, DOXYCYCLINE OR A COMBINATION OF BOTH

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Protocols combining doxycycline, which targets the bacterial endosymbiont *Wolbachia*, together with a macrocyclic lactone, are currently recommended by European Society of Dirofilariosis and Angiostrongylosis (ESDA) for treatment of heartworm disease [1].
Combinations doxycycline/ML protocols were shown to provide more rapid adulticidal and microfilaricidal effect than either Mls or doxycycline alone, although female worms were reported to have a higher tolerance to treatments compared to male worms [2,3].
Even though previous experiments have shown that ABC transporter, proteins acting as efflux pumps for various drug, are inhibited by IVM; the actual mechanism laying behind the increase in efficacy of the combination therapy is still unknown [4].
The present study was aimed at evaluating how ABC transporters may be involved in the synergic effect of the combination treatment.
Adult worms of *D. immitis* were treated in vitro for 24 hours with doxycycline (DOXY), ivermectin (IVM) and a combination of both and changes in modulation of six different genes encoding for ABC transporters' subunit were measured.
Quantitative RT-PCR analysis showed the presence of changes in modulation of ABC transporter genes taken into account in this study. In particular, in female worms, the combination treatment induced a substantial increase in gene expressions, especially of *Dim-pgp*-10 and *Dim-haf*-4; although the greatest increase in all gene expression, made exception for *Dim-haf*-4, was observed with the DOXY treatment.
These was not observed in male worms, where instead the greatest increase in gene expression was observed for *Dim-haf*-4, *Dim-pgp*-10 and *Dim-pgp*-3 when treated with DMSO+IVM and DMSO+DOXY+IVM.
Further studies are required to explain whether the modulation of cellular efflux plays a role, also partially, in the adulticide effect of doxycycline/macrocyclic lactone combinations in heartworm-infected dogs.

[1] European Society of Dirofilariosis and Angiostrongylosis (ESDA). Guidelines for clinical management of canine heartworm disease, 2017. Available at: https://www.esda.vet/guide-lines-tutorials/. [2] Bazzocchi C et al. Combined ivermectin and doxycycline treatment has microfilaricidal and adulticidal activity against *Dirofilaria immitis* in experimentally infected dogs. Int J Parasitol. 2008; 38(12):1401-1410. [3] Savadelis MD et al. Assessment of parasitological findings in heartworm-infected beagles treated with Advantage Multi® for dogs (10% imidacloprid + 2.5% moxidectin) and doxycycline. Parasit. Vectors. 2017; 10(1):245. [4] Ballent M et al.In vivo and ex vivo assessment of the interaction between ivermectin and danofloxacin in sheep. Vet. J., 2012, 192(3):422-427.

46P – THE BURDEN OF TICKS AND TICK-BORNE DISEASES IN COMPANION ANIMALS: THE PRACTITIONERS POINT OF VIEW IN EMILIA-ROMAGNA REGION

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Ticks are important vectors for numerous pathogens, including bacteria, viruses, protozoa and helminths, which threat animal and human health [1]. Agricultural and recreational activities are reducing the human-animal interface, and climate changing is also contributing for tick survival during winters, inducing the introduction of ticks into new areas and increasing the risk of tick bites [2]. Moreover, the continuous exposure of ticks to anti-ectoparasitic drugs is inducing the appearance of drug-resistant populations [3]. Although ticks and tick-borne diseases (TBDs) are important, there is no reported data about the perception of this issue by practitioners of the Emilia-Romagna region. For this reason, an electronic questionnaire with 20 questions was created and sent to all clinicians and to veterinary structures registered in the FNOVI database. Of the 340 emails sent, 168 replied to the survey with different rates among provinces (min 19%; max 91%). All participants (100%) observed infested animals during the previous two-years period, ranging from 1-10 cases (21.7%) to up than 50 (21.1%). Half of them (86/167, 51.5%) could not recognise or did not remember the genera and species of infesting ticks, while 52.7% identified *Ixodes* spp. and 35.3% *Rhipicephalus* spp. Interestingly, 8.4% recognised *Dermacentor* spp. and 0.6% *Haemaphysalis* spp. About 60.7% of interviewed reported TBDs in their own patients, in particular anaplasmosis, babesiosis, borreliosis, ehrlichiosis, and rickettsiosis, although only 3 (1.8%) of them carried out a molecular analysis for TBDs, probably because the 70.2% of practitioners considers the risk for TBD transmission as null, or lower than 5%. The description of three cases of tick-borne encephalitis (TBE) was of concern, even if no more information was available. Concerning the control and prevention of tick infestations, practitioners recommended treatment from January-May up to September-December, with a mean coverage of 8 months, using isoxazolines (78.6%), pyrethrins (56%), phenylpyrazoles (42.3%), and natural compounds (17.3%). However, 139 practitioners (82.7%) observed a lower efficacy of drug treatments, in particular with fipronil (73%) and permethrin (24%). This survey showed that ticks harbouring pathogens are present in Emilia-Romagna region, and the observation of reduced sensitivity to different molecules have to be investigated, because these results might suggest the presence of a real risk for animal and human health.

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47P – *PEARSONEMA* SP. INFECTION IN DOMESTIC AND WILD CARNIVORES

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Pearsonema plica and *Pearsonema feliscati* are two nematode species that colonize the urinary bladder and sometimes the ureters and renal pelvis of domestic and wild carnivores, in which they are responsible for urinary capillariosis [1-5]. In domestic carnivores, the prevalence of this infection is still poorly known [6,7]. This study was aimed to assess the prevalence of *Pearsonema* infection in 83 privately owned dogs and 26 privately owned cats from various areas of central and northern Italy and in 42 necropsied free-ranging foxes of the province of Pisa. Among domestic carnivores, asymptomatic and symptomatic animals were included. Urine samples were collected from each dog and cat and examined for capillariids first under a stereoscope, then centrifuged at 1000 rpm for 10 minutes and the obtained sediment observed under a light microscope after flotation test with saturated NaCl solution. From all foxes, the urinary bladder was carefully opened, urine collected and processed as above. If urine was absent, the bladder was washed with saline and the lavage fluid was examined. Moreover, all opened urinary bladders were observed under a stereomicroscope for presence and collection of capillariid nematodes, that were identified at the species level under a light microscope [4]. Clinical signs and some urinary parameters were evaluated in symptomatic dogs and cats. Among examined animals, 2/26 cats (7.7%), 1/83 dogs (1.2%) and 38/42 foxes (90.5%) were found positive. In positive cats, only immature eggs or parasite fragments were identified. Moreover, recurrent cystitis and hematuria were evidenced as the main clinical signs associated with symptomatic urinary capillariosis in examined dogs and cats. Obtained results confirm the role of the red fox as a reservoir host for *P. plica* [3,4] and show a high prevalence of *Pearsonema* infection in domestic cats of Italy.

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48P – GOOD PRACTICES=GOOD RESULTS: ANTHELMINTICS STILL WORK ALSO IN LIONS

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In a previous SISVet abstract [1], we reported own parasitological activities in a Zoo Safari. According to the Italian Law (D.Lgs 73/2005), zoos housing domestic, wild and exotic animals are required to address both animal health and welfare. The last two concepts perfectly fit in a strategy for parasite control. If in ruminants we should be satisfied by the control of gastrointestinal helminths in felids, the approach should be similar to pets and the eradication could be reached. This topic is widely discussed among zoo vet community and one emerging doubt is the potential risk of chemoresistance of ascarids to the routine treatments. The present experience tries to apply the WAAVP guidelines for domestic animals [2] to lions constantly affected by *Toxascaris leonina* after the strategic treatments. Five lions resulted positive for *T. leonina*; recently the drug utilized was febantel powder put into rabbit and poultry carcasses. According to Enigk and Dey-Hazra [3], we prepared and administrated five meatballs (300g) mixed 30g of Rintal®10%, considering an average weight of 300kg per animal. Using the following formula ($\%E = \frac{Mean\ of\ S\ in\ C - Mean\ of\ S\ in\ T}{Mean\ of\ S\ in\ C} * 100$), Fecal Eggs Count Reduction (FECR) was evaluated at T0, T7, T14 and T21, where S was the eggs shed, C the pre-treatment, T post-treatment. The FECR resulted of 98.4% at T7, 98.4 at T14 and finally 100% after three weeks. No adverse reactions were observed immediately and in the days after treatment. We have demonstrated that a single treatment, when well administrated, is able to stop the *T. leonina* eggs dropping, without any drug resistance effect. Probably, previous treatments administrated by animal carcasses did not allow the right drug intake amount. Inadequate information on diseases and parasites of zoo animals is a major limiting factor in the management of zoological gardens. Elimination of *T. leonina* from the zoo environment is defined difficult [4], in fact after twelve months lions were still positive. The next step after another treatment is to seek out the reservoir of infection: paratenic-hosts or environment?

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49P – *ONCHOCERCA JAKUTENSIS* IN RED DEER: AN EMERGING PARASITE

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Filaroid Nematodes of the genus *Onchocerca* are cause of a vector-borne parasitic disease called onchocercosis. *Onchocerca* species are present worldwide, infecting wild and domestic ungulates, dogs and humans and are transmitted by blackflies (Simuliids) or biting midges (Ceratopogonids) [1]. Species of zoonotic interest are *O. volvulus* which is endemic of tropical Africa and South America and is the etiological agent of river blindness, *O. lupi* which has been recently described as cause of ocular infection in Europe [2] and *O. jakutensis* that was reported for the first time to cause subcutaneous nodules in a human patient with impaired immunity in Austria [3]. The European red deer *Cervus elaphus* is host to four species of Onchocerca including *O. jakutensis*, *O. flexuosa O. skrjabini* and *O. garmsi* [4]. Little is known about the pathogenicity of the different species of *Onchocerca* in red deer with symptoms ranging from chronic sclerosing to microfilaria-induced myositis and dystrophic change of the hypodermal tissue [1]. Recently *O. jakutensis* has been described for the first time in Italy, in Red deer from Tuscan-Emilian Apennines (Pistoia Province) [5] and in Switzerland [4]. Here we report the first detection of *O. jakutensis* in a Red deer from the Italian Alps (Susa Valley, Torino Province). The deer, culled in December 2017, presented multiple nodular lesions on external thighs and in the caudal part of the back. Nematodes collected from the nodules, were fixed in 70% ethanol and examined by clearing in lactophenol and observed with a light microscope. Total genomic DNA was extracted and a specific region of 12s rRNA gene was amplified to confirm morphological identification using primers 12SOvC and 12SOvB [3]. Filaroid nematodes were morphologically identified as *O. jakutensis* and PCR amplification of the 12s rDNA showed 100% homology with deposited sequences (Genbank Accession n. HQ717719). This is the first report of this parasite in the Italian Alps. In the last years *O. jakutensis* has been expanding its range of presence from Jakutia in Eastern Russia, to Caucasus, Germany, Switzerland and Central Italy. Further studies are needed to assess the origin of parasitic infection, the possible role of Red deer reintroductions in parasite expansion, and the role of *O. jakutensis* as zoonotic parasite.

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50P - GENOTYPING OF *TOXOPLASMA GONDII* IN WILDLIFE FROM NORTHWESTERN ITALY

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Toxoplasma gondii is an obligate intracellular protozoan belonging to the phylum *Apicomplexa*, able to infect all warm-blooded species including humans. Human toxoplasmosis is one of the leading causes of death among foodborne pathogens and consumption of raw/under-cooked meat and meat products containing *T. gondii* tissue cysts is a major route of zoonotic infection [1]. In Northwestern Italy *T. gondii* cysts were detected in muscles of free-ranging wildlife with a prevalence of 10.99% [2]. In the same study area, atypical genotypes are reported more frequently in wildlife compared to sympatric livestock [3]. The aim of this project was to assess *T. gondii* prevalence in skeletal muscle (SM) and in Central Nervous System (CNS) of wild herbivores and carnivores from Alessandria Province (Piedmont Region, Italy), to compare SM and CNS positivity rate and to determine the circulating GRA6 genotypes of *T. gondii*. We tested by PCR (targeting a specific fragment of the GRA6 gene of *T. gondii*), 245 animals of 10 species (Roe deer, Wild boar, Red deer, Fallow deer, Badger, Hystrix, Red fox, Hare, Cotton-tail and Coypu) [2], all animals were regularly culled or road-killed between 2014 and 2018. Positive amplicons were sequenced, and in-silica digested for RFLP genotyping as previously described [3]. The overall prevalence of *T. gondii* was P=35.92% (88/245; CI95% 30.17-42.10%). Significantly higher prevalence was recorded in Wild boar *Sus scrofa* P=59.52% (CI95% 44.49-72.96) ($p<0.001$) compared to other species. The highest prevalence of infection was recorded in Fallow deer *Dama dama* P=75.00% (CI95% 30.01-95.44%), but due to the limited sample size (total tested animals n=4) the value is not significantly higher than that of other species ($p<0.1$). Genotype was determined for 52 of the 88 positive animals. Genotype I was the most prevalent P=92.31% (CI95% 81.83%-96.97%) ($p<0.05$), followed by genotype III P=7.69% (CI95% 3.03-18.17%). Genotype II was determined only in the CNS of 1 Roe deer. No differences were recorded on genotype prevalence among different species. Our data show the higher prevalence of type I-alleles in *T. gondii* infecting free-ranging wildlife in Northern Italy. In humans, genotype I has been associated to a more severe symptomatology in immunocompromised patients [4] and in newborns [5]. It is important to note the high prevalence of genotype I in sylvatic species may play an important role in increasing the clinical burden of *T. gondii*.

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51P - TICKS AND TICK-BORNE PATHOGENS IN MIGRATORY BIRDS

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Migratory birds can act as biological and mechanical carriers of several pathogens such as viruses, bacteria, protozoa and arthropods, allowing them to spread into new areas. This is the case of ticks of the genus *Hyalomma* spp., which are widely distributed in the Mediterranean and African regions but accidentally found in some Northern European countries as UK [1]. In particular, migratory birds are carriers of immature stages of the ticks which, soon after the blood meal, drop off from the host. In case of suitable weather conditions in the new spot, those ticks are able to moult to the next stage (e.g. adult) to seek for another host. Given the fact that more than 2 billion passerine birds migrate annually from Africa to Europe, limited information are available on the bird species involved in this migration and on the ticks and tick-borne pathogens that they carry. Therefore, we chose Ponza, an Italian island in the Tyrrhenian sea known to be a resting place for migratory birds along their route, as the site of investigation. Ticks collected from the birds during the standard ringing procedure in spring 2016 and 2017 were firstly identified using the morphological keys and the amplification of the ITS region [2]. Then, they were analysed for the presence of several pathogens such as Crimean-Congo Hemorrhagic Fever virus (CCHFv) and *Rickettsia* spp. using PCR amplification [3]. Totally, 728 birds belonging to 17 different species were captured and 231 ticks were collected, with 104 birds carrying at least one tick. The majority of the ticks belonged to the genus *Hyalomma* spp. or *Hyalomma marginatum* complex (comprising *H. m. marginatum* and *H. m.* rufipes) and 20.1% tested positive for *Rickettsia* spp., while none of the ticks were positive for CCHFv. Sequencing analysis showed the presence of *Rickettsia aeschlimannii*, *R. africae* and *R. raoultii*. Our results confirm the role of migratory birds as carriers of exotic tick species (e.g. *H. m. rufipes*) into new areas, thus leading to the spread of zoonotic pathogens like *R. aeschlimanni* into Europe. Even though all the ticks tested negative for CCHFv, the risk of introduction of this virus in Europe is not negligible, and the current climate change could lead to the establishment of autochthonous population of *H. marginatum* ticks, the well-known vector of CCHFv, in this continent.

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52P - NON-INTESTINAL CANINE AND FELINE NEMATODES IN ITALY: OCCURRENCE, RISK FACTORS AND CLINICAL FEATURES

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Different epidemiological drivers have led to an emergence of non-intestinal nematodes affecting dogs and cats in Europe [1]. Given the merit to keep high our attention, this survey has evaluated occurrence, possible risk factors and clinical aspects of infections caused by major non-intestinal nematodes in dogs and cats from selected Italian regions. A total of 1055 dogs were sampled for faeces and blood in Abruzzo (site A, n=218), Marche (site B, n=116), Molise (site C, n=69), Puglia (site D, n=210) Lazio (site E, n=171), Campania (site F, n=83), Veneto (site G, n=68), Friuli-Venezia Giulia (site H, n=66) and San Pietro Island (site I, n=54). Also, 1000 cat faecal samples were collected in sites A (n=380), B (n=103), C (n=111), E (n=172), G (n=45), H (n=32), I (n=94) and Piemonte (site J, n=63). Samples were examined using conventional and molecular methods to identify parasite elements at the species level. Associations between prevalence and epidemiological data, and between clinical manifestations and positivity to parasites were statistically evaluated. *Aelurostrongylus abstrusus* (cats) and *Angiostrongylus vasorum* (dogs) were recorded with an overall prevalence of 10.3% and 3.4%. *Capillaria aerophila* was found in 3.4% (cats) and 2% (dogs). The infection rate of other species were *Troglostrongylus brevior* 3.2% in cats, and *Capillaria boehmi* 1.2%, *Dirofilaria immitis* 1.7%, *Dirofilaria repens* 1.6%, *Crenosoma vulpis* 0.2% in dogs. *Angiostrongylus vasorum* was found in sites A (4.1%), C (5.8%), E (4.1%) and F (19.3%), *C. boehmi* in sites A (1.4%), D (1.4%), E (3.5%), H (1.5%) and *C. vulpis* in site D (0.9%). Microfilariae of *D. immitis* were found in dogs from sites G (2.9%), H (1.5%) and I (27.3%), while those of *D. repens* in sites A (2.8%), D (0.5%), and I (18.5%). *Aelurostrongylus abstrusus* was detected in sites A (10%), B (3.9%), C (3.6%), E (4.7%), H (3.1%), I (38.3%), J (20.6%) while *T. brevior* in sites A (5.8%), C (1.8%), E (4.7%). *Capillaria aerophila* was found in sites A (cats 4.7%; dogs 3.7%), B (cats 0.9%), C (cats 5.4%), D (dogs 0.5%), E (cats 1.7%; dogs 3.5%), G (cats 2.2%; dogs 2.9%), H (cats 6.2%; dogs 6.1%), I (cats 2.1%), J (cats 1.6%). Outdoor access was associated with higher prevalence of *A. vasorum* in dogs and *C. aerophila* and *A. abstrusus* in cats. Cats ageing <1 year resulted more prone to be infected with *T. brevior* and *A. abstrusus*. Cardio-respiratory signs were associated with higher prevalence of *A. vasorum*, *C. aerophila* (in dogs), *T. brevior* and *A. abstrusus*, while “other clinical signs” (i.e. except cardio-respiratory, gastrointestinal and coagulopathies-related), were found more frequently in dogs with angiostrongylosis than in dogs with other parasitoses. No other difference was found. These data underline that a parasitic cardio-pulmonary disease should be always suspected in the presence of compatible signs in dogs and cats in Italy, and that parasitological vigilance and increased awareness of veterinarians are of overriding importance under both epidemiological and clinical standpoints.

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53P - STRONGYLE INFECTION IN HORSES UNDER DIFFERENT MANAGEMENT SYSTEMS

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Strongyle infection (SI) represent an important issue affecting the sanitary status of horses, with clinical outcomes varying from a decrement of host fitness, to colic. The difficulty in control SI is due to their complex mechanisms involving both management and sanitary aspects. The possibility for the animals to pasture may facilitate the maintenance of their biological cycle, although strongyle may infect also stabled horses [1,2]. Indeed, horses may be reared in several ways, depending on their attitude and use. Horses may be kept in stable, isolated, or may be housed in paddock or at pasture, with the possibility to have social interactions. Furthermore, systems of "natural" housing of animals are emerging in last years: horses are kept in quite stable social groups, allowing the specie-specific social behaviors, in a wide pasture surface area. A longitudinal study was planned with the aim of evaluating the impact of horse management on SI and thus on their sanitary status. Three independent groups of animals having different management systems were included in the study: in stable A (A) horses were kept at pasture in small social groups, without a fixed stabulation and with irregular anthelmintic treatment; in stable B (B) horses were kept in paddocks and stabled during night, regularly treated; in stable C (C) horses were managed under a "natural" housing system, never treated. Fecal samples were collected from 71 animals (A=22 horses, B=22 horses, C=27 horses) every three months during a year, before any scheduled anthelmintic treatment, with an overall of 227 observations. Quali-quantitative analysis was performed by the FLOTAC® double technique [3] using NaCl (s.g. 1200) flotation solution, recording values of eggs per gram of feces (EPG). Obtained data were analysed though a generalized linear mixed model (GLMM) to evaluate differences among season and stables (SPSS v.19, IBM, USA). Considering all horses, a prevalence of 62.9% (P), an abundance (a) of 289.4 EPG and an intensity (i) of 390 were recorded. Stable C showed higher values (P=78.6%; a=366.6; i=466.1), if compared to stables A (P=70.9%; a=244.9; i=345.1) and B (P=38.1%; a=177.3; i=277.6). GLMM showed that EPG values were associated to the interaction between the variables "stables" and "season" (F=6.24, p=0.0001), with significant differences in autumn among stable C and the other ones (p=0.038 and p=0.0001, respectively). The study confirmed stable management as a risk factor for SI in horses. However, further studies are ongoing to rule out the role of other factors affecting SI, including the possibility of social interactions, since an increased level of stress may lead to a reduction of the immune defense of the host, exacerbating SI.

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54P - THE MINI-FLOTAC TECHNIQUE: A NEW FIELD DIAGNOSTIC TOOL FOR NOSEMOSIS?

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Nosema spp. are gastrointestinal microsporidia, that attack all adult forms of honey bees [1] and are causative agents of nosemosis, one of the most common diseases of honey bees [1, 2, 3]. *Nosema apis* and *N. ceranae* can both infect *Apis mellifera*. Although for many years, European nosemosis was exclusively attributed to *N. apis,* to date *N. ceranae* is considered a more common agent than *N. apis*. Conversely to *N. apis* which is characterized by clinical symptoms such as swollen abdomens and diarrhea, *N. ceranae* silently impairs honeybees' health, leading to reduction of colony population and potentially causing colony collapse [2]. As the parasite represents a major hazard for beekeepers, a sensitive, rapid and affordable technique for a proper diagnosis is necessary [4]. Usually, the field diagnosis of nosemosis is performed using microscopic examination of macerated abdomens suspension taken from clinically or subclinically affected bees, to evaluate the presence of spores [5]. In this study, for the first time the Mini-FLOTAC technique, a new quantitative very sensitive, accurate and precise copromicroscopic method [6] has been used for the diagnosis of *Nosema* spp. For this purpose, 20 forager bees were collected from the entrance of 5 different hives. The abdomens of the bees were separate from the head and thorax and were observed under a stereomicroscope for identification of anatomical changes. Ten abdomens from each colony were analyzed with the standard method [5] while the remaining abdomens were analyzed using the Mini-FLOTAC technique. For the latter, 10 abdomens were homogenized in the Fill-FLOTAC [6] with 10 ml of sodium chloride flotation solution (specific gravity 1200); then the two chambers of the Mini-FLOTAC apparatus were filled. After 10 minutes, the Mini-FLOTAC was translated and examined under a microscope. The reading field was clear and allowed to identify the presence even of few spores that appeared bright and refractive, while in standard microscopic examination spores were often covered by debris. Our results suggest that the Mini-FLOTAC technique could be useful for the diagnosis of nosemosis in endemic areas, as quantification seems to be more accurate than with traditional method of detection. Furthermore, the Mini-FLOTAC simplicity and cost effectiveness allows it to be used with light microscopes in any laboratory or field conditions.

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P55 - EVALUATION OF ALTERNATIVE TREATMENTS FOR *NOSEMA CERANAE* INFECTION IN HONEY BEES (*APIS MELLIFERA*)

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Nosema ceranae is a microsporidian parasite able to infect epithelial cells of the ventriculus of adult honey bee *Apis mellifera* and *Apis ceranae*, causing gut tissue degeneration [1]. It has been implicated in Colony Collapse Disorder [2] and it is usually transmitted between bees by oral-faecal route or by honey, pollen and beekeeping materials contaminated with environmental spores of the parasite. The only effective treatment against nosemosis is the antibiotic fumagillin [3], which is however forbidden in Europe. In order to find alternative treatments, we tested the efficacy against *Nosema ceranae* of the supplementary feed ApiHerb® and of acetic acid (used for sucrose inversion) on naturally infected honey bee (*Apis mellifera*) colonies. For this study, 140 honey-bee colonies from one apiary, were divided into 4 homogeneous groups of 35 colonies each, based on initial *Nosema* spore number: one treated with ApiHerb®, one with acetic acid 6 ml/l, one with acetic acid 9 ml/l, and one control group. Each treatment was provided twice in the period between autumn 2017 and spring 2018. Before and after the treatments, 20 bees were randomly sampled from each colony and analysed (total analyzed bees n=2800) in order to quantify *Nosema* spores with light microscopy. Statistical analysis (ANOVA test) was performed using the software R. Results showed a common trend in all the groups, with a reduction in the number of spores after the first treatment. Spore number decreased variably among colonies from 55.38% to 91.49% after the first treatment, and it was followed by a marked increase, common to all treatment groups, after the second treatment. None of the colonies treated with ApiHerb® died during the study, while colonies treated with acetic acid 9 ml/l showed a higher mortality compared to control colonies. Our results suggest a possible usefulness, as a supplementary feeding, of ApiHerb® as it reduced the winter mortality. All applied treatments showed a lack of efficacy in reducing *Nosema ceranae* spore burden in spring.

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P56 - CYSTIC ECHINOCOCCOSIS IN DOMESTIC AND WILD ANIMALS IN CALABRIA REGION OF SOUTHERN ITALY

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Cystic echinococcosis (CE) caused by the larval stages of the small tapeworm *Echinococcus granulosus* is one of the most important zoonosis in the world. Its lifecycle involves carnivorous (mainly dogs) as definitive hosts and a broad spectrum of mammalians, including humans, as intermediate hosts. Over the past two decades, numerous studies have addressed the epidemiology and distribution of *E. granulosus* worldwide, resulting in better-defined boundaries of the endemic areas [1]. In some Italian regions surveillance programs, based on organ/meat inspection and sheepdogs management, were developed [2,3,4], but in Calabria region (southern Italy), data of CE distribution are very few and underestimated. The aim of this study was to perform an abattoir survey to collect data on CE distribution in livestock (cattle, sheep and goats) and wild boars regularly slaughtered in Calabria region, using a Geographical Information System (GIS) to develop a control approach of *E. granulosus* in this region. According to the Manual of World Organization for Animal Health (OIE) [5], the detection of CE was performed in three slaughterhouses situated in the Reggio Calabria province by visual inspection, palpation and incision of organs. Between January and December 2018 a total of 6,909 cattle, 3,314 sheep and 1,528 goats were slaughtered and inspected for CE, moreover, between December 2018 and February 2019 a total of 131 wild boar were analyzed for CE. The results showed a CE prevalence of 18.8 % (622/3,314) in sheep, 7.4 % (113/1,528) in goats, 0.8% (54/6,909) in cattle and 17.5% (23/131) in wild boar. This study showed that EC is widespread in Calabria region, especially in sheep, probably due to the lack of diagnosis and treatment of the sheepdogs and to the bad habit of farmers to feed sheepdogs with parasitized organs. The prevalence of CE in cattle was low, probably largely influenced by the origin of the slaughtered animals and their management, in fact the most part of these animals was of foreign origin with a very low period in Italy (about 1 month). In conclusion, the data from this study showed the presence of *E. granulosus* in Calabria, mainly in sheep and wild boar. However, further studies are needed to better clarify the diffusion of this parasite in animals and humans in this region and to perform appropriate control strategies.

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P59 - MELATONIN TREATMENT IN MARCH AND REPRODUCTIVE PERFORMANCE IN SHEEP WITH DIFFERENT MILK PRODUCTION LEVELS

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Photoperiod plays a key role in control of the reproductive seasonality in small ruminant. The light signal is transmitted to the reproductive neuroendocrine system by circadian secretion of melatonin from the pineal gland [1]. Melatonin treatments have shown a significant reduction in the length of the lambing period in treated sheep, resulting in improved management of the flock [2]. The aim of the research is to highlight if the reproductive response to melatonin treatment can be influenced by milk yield in Sarda breed sheep. For this research, 4 ovine farms located in North Sardinia were chosen, with the same management and feeding conditions. In each farm 200 sheep 3-5 years old were selected, that had lambed only one lamb between November 20th and December 10th. From the beginning of lactation the individual daily milk production and BCS every 15 days were registered. In each farm, on March 10th, the chosen animals were divided in 2 homogenous groups, one treated with melatonin and the other as control. The animals were included in the groups according to the individual daily milk production, to obtain homogeneous treated and control groups. On March 20th the animals were treated with a single slow release subcutaneous implant containing 18 mg melatonin. After treatment the groups were separated. On April 23rd the rams were introduced (ratio 1/20), and removed after 90 days (July 23rd). Starting on September 20th the lambing date and numbers of newborn lambs were recorded. The data were analyzed with R statistical software, and the multivariate linear regression was used to evaluate if the reproductive response to melatonin treatment can be influenced by the yield milk production. The mean difference in days between male introduction to lambing was lower in the treated groups (about 8 days less, $P<0.05$). Furthermore, the treated animals showed a higher fertility rate compared to the controls ($P<0.05$). Within the groups, the yield milk did not influence the reproductive activity of treated animals, indeed, in the control group the yield milk improved the reproductive efficiency. The results show that melatonin has improved reproductive efficiency in the Sarda sheep breed. In this study, the yield milk showed that it was able to enhance the reproductive response.

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P60 - EFFECT OF THE PHOTOPERIOD IN THE EXPRESSION OF THE GENES INVOLVED IN THE PARACRINE CONTROL OF THE OVARIAN FOLLICLE IN THE PREPUBERTAL AND ADULT OVINE

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In sheep, follicular growth and ovulation are modulated by the photoperiodic control of melatonin. Follicular growth and ovulation of healthy oocytes are a complicated process that is regulated by several endocrine and paracrine factors as well as by cross-talk between the oocyte and its surrounding somatic cells. It has been shown that bone morphogenetic proteins (BMP) modulate the maturation of the cumulus-oocyte complex and its ovulation. The aim of this work was to study the influence of photoperiod before (prepubertal) or after (adult) the activation of the hypothalamic-hypophysis-ovarian axis in the expression of genes involved in the paracrine control of the follicle. The antral follicles were recovered from ovaries of adult and 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 [1] using specific primers for FSHr, BMP4, BMP7, BMP15, GDF9, BMPR1A and 1B, SMAD9 and Stat 5. The differences were calculated using the Delta-Delta(Ct) method [2]. Our data showed that some genes were expressed differentially between the antral follicles during the breeding and non-breeding season in both adult and prepubertal sheep. In particular, in adult follicles the expression of genes FSHr, BMP4, BMPR1A and 1B, GDF9 and SMAD9 was more than 100 times higher during the breeding season compared to the non-breeding season ($P < 0.01$). In prepubertal antral follicles, the expression of FSHr, BMP4, BMP15, GDF9, BMPR1B and SMAD9 was significantly slightly higher (3-5 times) in the breeding season compared to that of the non-breeding season ($p<0.01$). In conclusion, our results showed that the photoperiod plays an important role in the modulation of follicle development, improving the expression of FSH receptors and different factors of the BMP family during the reproductive season in both adults and prepubertal sheep. Its effect is much higher in adults than in prepubertal animals, which suggests that its action is mediated by the active hypothalamus/pituitary/ovary axis factors.

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P61 - EFFICIENCY OF DNA EXTRACTION FROM FEATHERS OF GYPS FULVUS WITH TWO DIFFERENT METHODS

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The genetic analyses involve the extraction of DNA usually obtained with highly invasive and stressful methods. It is usually extracted from blood samples, which require the capture and containment of the animal, with objective difficulty in ensuring a reasonable number of samples necessary for the characterization of the population, colonies or family groups. The aim of our work was to test non-invasive methods for collecting DNA necessary to study the genetics of the Sardinian griffon population. To this aim, the possibility of extracting efficiently DNA from griffon feathers taken in the field was evaluated. We compared two different techniques for extraction: the classic phenol/chloroform/isoamylalcohol organic extraction [1] and a technique based on the use of paramagnetic beads (ChargeSwitch® Forensic DNA Purification Kit). 55 griffon feathers have been collected near nests in the griffon colonies of Bosa in the N/W of Sardinia and classified as flight (n=16) or duster (n=39) feathers. Each class was divided into two groups, the tips of the scapes have been cut and lysed with proteinase K. Twenty-seven samples were extracted with the organic method and 28 with the Forensic kit. DNA quantity and purity was determined by nanodrop spectrophotometer. Quality of the extracted DNA was determined using an amplification test using primers for sex determination [2]. The quantitative analysis showed a statistical difference in the total amount of extracted DNA ($p=0.0147$). By extraction with the Forensic Kit, an average of 2760±729 ng of DNA was obtained while organic extraction resulted in an average of 855.4± 55 ng. Five samples out of 27 (18.5%) extracted with the forensic kit, all belonging to the flight feather group, did not reach the detection limit (10 ng/ml) of the nanodrop. The ratio of A260/A280 nm wavelength between 1.5 and 1.8 indicate the purity of DNA related to protein and phenol contamination. Data showed a higher ratio ($p=0.0035$) for the organic extraction (1.49±0.025) compared to the forensic kit (1.15±0.11). The test for DNA amplification showed a higher number($p=0.003$) of positive amplification with organic extracts (88.9%) than with Forensic kit (53.6%). In conclusion, extraction from duster feathers is more efficient than from flight feathers. The Forensic kit offers a better yield than the classical organic method even if the purity is scarce and the amplification of the DNA presents a lower efficiency.

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P62 - DETERMINATION OF BETAMETHASONE RESIDUES IN RAT LIVER BY USING A NMR SPECTROSCOPY APPROACH

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Betamethasone (BETA) is a synthetic corticosteroid authorized in animal breeding for the treatment of metabolic disorders and inflammatory diseases. Beside legal pharmacological treatments, in Europe, it is illicitly used, at lower doses as growth-promoting agent, to improve productive performances, constituting a health risk for meat consumers [1]. To date, although it requires extended times for sample preparation and analysis execution, LC-MS/MS is the official control method of veterinary drug residues in food of animal origin [2, 3]. Therefore, an experimental study was developed to evaluate the applicability of NMR spectroscopy [4] as an alternative simple and fast screening tool to identify and quantify the presence of BETA residues in different livestock edible tissues, in order to check eventual unauthorized growth-promoting administration of corticosteroids in cattle. Eight rat liver samples collected from treated (experimental group received i.p. 0.009 mg/rat of BETA in saline solution) and untreated (control group received i.p. only saline solution) animals were investigated for the determination of BETA by NMR spectroscopy. All ex-vivo MR imaging and spectroscopy were performed using a 7.0T/16 mm Pharmascan small animal magnetic resonance scanner (Bruker Biospin, Ettlingen, Germany), BETA and other metabolite were quantified with the QUEST algorithm in jMRUI v5.2 using a simulated metabolite basis set. To ensure reliable detection and quantification of BETA in samples, NMR reference spectra were recorded using phantoms with increasing concentrations of BETA (Bentelan 4mg, BETA 5mM, 10mM and 100mM in DMSO). Therefore, considering spectroscopy data analysis both of the experimental and control group against reference samples with the HLSVD algorithm, no betamethasone peak is evident in Bentelan 4mg, in BETA 5mM, in liver samples containing BETA and in untreated ones, respectively. Overall, this preliminary research has proposed the determination of betamethasone residues in foodstuffs of animal origin from cattle by NMR spectroscopy, but our results suggested that higher concentrations of BETA in tissue samples (≥10 mM) are necessary in order that this synthetic corticosteroid can be detected through the investigated approach.

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P63 - THE EFFECT OF CURCUMIN ON OCHRATOXIN-A INDUCED NEPHROTOXICITY IN RATS

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Ochratoxin-A (OTA) is a metabolite produced by *Aspergillus ochraceus* and *Penicillium verrucosum* that accumulates in the food chain because of its long half-life. OTA has been shown to induce a tubulointerstitial nephropathy in animals (1) and to be probably involved in the Balkan endemic nephropathy in humans (2). In addition, OTA disrupts blood coagulation and glucose metabolism (3). Anyway, its mechanisms of toxicity remain unclear. Therefore, the focus of this work was to investigate the antioxidant and anti-inflammatory effects of curcumin on OTA-induced nephrotoxicity. Therefore, we have analyzed, on 24 adult Sprague Dawley rats, oxidative stress by the measurement of malondialdehyde production, renal inflammation by western blot of IL12 and IL 6 and renal function by clearance of inulin. Statistical analyses were performed using the GraphPad Software. We found that the animals treated with OTA (0.5 mg/Kg b.w.) by gavage for 14 days reduced the glomerular filtration rate (GFR) measured by clearance of inulin (0.41±0.15 vs 0.98±0.11 ml/min) and this effect is related to the alteration of oxidative stress. In fact, we have found a severe increase in malondialdehyde production (368±15 vs 128±14 pmol/mg of proteins) and a decrease of SOD (23.2±10 vs 42.5±12 U/mg), CAT (263.4±11 vs 352.8±18 U/mg) and GPx (18.6±6 vs 28.2±5 U/mg) enzymatic activity in OTA-treated rats. Moreover, our results have shown that OTA do not induced a significant increase of IL12 (1.02±0.05 vs 0.98±0.08 arbitrary unit) and IL6 (2.08±0.8 vs 2.12±1.1 arbitrary unit) in the kidney of exposed animals for 14 days of OTA. Curcumin (100 mg/Kg b.w.) is able to partially prevent the GFR decrease (0.78±0.8 ml/min) and several effects was found on anti-oxidant (38.5±8 SOD, 304±18 CAT, GPx (25.5 ± 7) and anti-inflammatory (1.12±0.9 IL12 and 2.08±0.8 IL6) pathways. Finally, histopathological examinations revealed a severe tubular vacuolization and atrophy associated with interstitial infiltrate in OTA-treated groups and co-treatment with OTA plus curcumin is associated with a modest restore of these histological parameters. In conclusion, this study demonstrated that there is a strong link between oxidative stress and OTA-induced renal injury in a chronic toxicity rat model and curcumin is able, in part, to prevent this renal injury.

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P64 - MULTI-RESIDUE ANALYSIS OF 52 ANTIBIOTICS IN MILK AND MUSCLE BY LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY ON A QTRAP 6500+

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Herein we describe multi-class multi-residue test methods for screening and confirmatory analysis of tetracyclines, sulphonamides, quinolones, macrolides, penicillins, cephalosporins, amphenicols, pleuromutilins, trimetoprim, virginiamicin in milk and muscle from many animal species. After simple clean up procedures, samples were analyzed by UHPLC coupled to a new hybrid QTRAP 6500+ mass spectrometer (ABSciex), ensuring higher sensitivity and selectivity. Up to 52 drugs belonging to ten antibiotic groups can be detected in about 15 minutes. The aim of the present study was to introduce innovative analysis methodology to detect a wide panel of antibiotics, belonging to ten different groups, in bovine, buffalo and ovi-caprine raw milk, and bovine, swine, chicken and horse meat, and to increase official control potency. Milk and meat were cleaned up by extraction with a buffer/acetonitrile mixture, sonication, and centrifugation; a part of the extract was dried, then solubilised in water/methanol/acetonitrile mixture. About meat samples, a step with *n*-hexane liquid partition was introduced prior to drying. Then, 2 µl of sample were analyzed using an UHPLC system coupled to a hybrid QTRAP 6500+ mass spectrometer (ABSciex). Chromatography was run on a Kinetex XB-C18 column at 0.50 ml/min. Analyses were carried out in multiple reaction monitoring (+MRM) for quantitative confirmation and EPI SCAN IDA mode for qualitative screening and qualitative confirmation. The methods we developed to analyze 52 antibiotics are highly reliable, selective and sensitive. They were validated according to the Decision 2002/657/EC [1] and Regulation 882/2004/EC [2], evaluating specificity, linearity, trueness, precision, the CCβ, the limits of quantification (LOQs) and ruggedness for slight changes for all drugs, at levels below the maximum residue limits (MRL) set by the EU law [3]. The high sensitivity of QTRAP mass spectrometer allowed for simple and rapid clean up of samples, reducing matrix effects by dilution, thus improving analysis. The methods are specific for raw milk from bovine, buffalo, ovi-caprine milk, and for bovine, swine, horse and chicken meat. All the LOQs measured are well below the MRLs. The quantitative confirmatory method in +MRM mode allows for quantification and unambiguous identification of each antibiotic. Moreover, a method for qualitative screening and confirmation was developed in the EPI SCAN IDA mode; this way, if a characteristic product ion from an antibiotic is detected by the quadrupole, then a full scan mass spectrum is registered to get unambiguous identification based on its characteristic product ions pattern. The possibility to observe false positive and false negative results is highly reduced, performing analyses in a shorter time.

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P65 - MODULATION OF AQUAGLYCEROPORIN EXPRESSION BY NON DIOXIN-LIKE PCBS: POTENTIAL ROLE FOR THE ONSET OF METABOLIC IMPAIRMENT IN MATURE 3T3-L1 ADIPOCYTES

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PCBs are lipophilic pollutants that accumulate in adipose tissues and induce several toxic effects, such as immune and reproductive disorders. PCBs also contribute to the obesity and its related disorders like diabetes [1]. The aquaglyceroporins belong to the family of those proteins, namely the aquaporins (AQPs), that regulate the flux of water and small solutes, mainly glycerol, through cell membranes [2]. The AQPs play a pivotal role in the control of fat accumulation and glucose homeostasis, in adipocytes and hepatocytes; some of them also contribute to the adipocyte differentiation. Their impairment was also shown in obese subjects [3]. To investigate an additional mechanism of the PCB toxicity, we studied the effects of three Non Dioxin-Like (NDL) and indicator PCBs (101, 153 and 180) on the AQPs expression in 3T3-L1 mouse fibroblast cells. In particular, the preadipocytes were differentiated through a standard procedure, and the mature adipocytes were treated for 48 hours with: 1) the PCBs alone, 2) a combination of two PCBs, 3) or all PCBs together (1µM final concentration). Then, cell lysate was subjected to western blot analysis to determine AQP3, AQP7 and AQP9 protein expression. Our results clearly demonstrate that the exposure to PCBs modulates the expression of all the considered AQPs. In particular, PCB 153 and 180 alone or in combination significantly reduced AQP3 and AQP7 (for AQP3: PCB 153=-42%, PCB 180=-48%, all PCBs=-86%; for AQP7: PCB 153=-40%, PCB 180=-39%, PCB 153+180=-25% vs control). At the same time, all PCB congeners, both alone and in association, significantly reduced AQP9 expression (PCB 101=-46%, PCB 153=-32%, PCB 180=-36%, PCB 153+180=-34%, all PCBs=-48% vs control). As previously demonstrated, glycerol efflux is facilitated by AQP3 and AQP7 preventing the excessive lipid accumulation in adipocytes and this effect is mediated by insulin and leptin via the PI3K/Akt/mTOR pathway [4]. Interestingly, in our experiments a reduction of Akt phosphorylation was also observed. To the best of our knowledge, this *in vitro* study is the first demonstration of the impairment of AQP expression caused by NDL-PCB treatment. The investigated PCB indicators are still of great interest because they are the predominant congeners in biotic matrices and food items of animal origin, posing a serious risk for the consumer health. We believe that our results represent an original contribution to gain new insights on the mechanisms by which PCBs participate in the induction of obesity and related metabolic disorders.

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P66 - A NEW BLEND OF ESSENTIAL OILS TO TREAT EXTERNAL OTITIS IN DOGS

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External otitis is one of the most frequent and annoying pathologies in dogs. It could be part of more widespread dermatological disorders. It represents a challenge for veterinarians because underlying causes are often difficult to individuate and correct. Therapeutic approaches include the use of anti-inflammatory, antibacterial and antifungal agents. The interest for "natural" products in the treatment of canine otitis has grown in recent years, also to avoid the extensive use of antibacterial drugs in light of the concern for possible phenomena of antimicrobial resistance [1].

The aim of the present study was to evaluate a new phytotherapic topical treatment for canine otitis, which main ingredients are essential oils *of Malaleuca alternifolia*, *Thymus serpillum*, *Salvia officinalis* (off.), Eucalipto off., *Rosmarinum* off., *Lavandula* off. and *Heliantus annus*.

In a preliminary *in vitro* evaluation, *Malassezia pachydermatis*, *Candida albicans*, *Proteus* sp. and *Staphylococcus intermedius* strains were cultured in presence of the formulation. The substance was added to 1-5 x 10^7 units forming colonies (UFC) using the test suspension method, with a contact of 5 and 15 minutes and 1 hour. Then the samples were seeded and checked after 24 and 48 hours for the growth of microorganisms [2]. All the experiments were performed in duplicate. Then an *in vivo* trial was performed. After a complete physical examination, 12 dogs presenting only acute external otitis were enrolled. Each ear was considered as a separate case. Before the treatment, a score ranging from 0 to 3 was assigned to evaluate pruritus, erythema, exudates, bad smell and earwax content, and an auricular swab was collected for a cytological evaluation. The same procedure was repeated at the end of the treatment. The product was administered once a day for 7 consecutive days.

In vitro experiments showed a 99,9% reduction as regards *M. pachydermatis* for all experimental time point starting from 5 minutes of contact, and from 1 hour for *C. albicans* and for *Proteus* spp. The formulation was able to inhibit only 50% of the growth of *S. intermedius* from 1 hour of contact. The *in vivo* trial compared the scores collected before and after the treatment. All the clinical signs were improved: decrease of pruritus, reduction of earwax content and bad smell, and absence of erythema. The owners appreciated the pleasant fragrance of the formulation. The cytological evaluation demonstrated a significant decrease of the presence of bacteria and *M. pachydermatis* in all samples after the treatment.

Both *in vitro* and *in vivo* evaluations seem to suggest the efficacy of this new phytotherapic formulation in the treatment of acute external otitis in dogs. Further analyses are necessary to evaluate the formulation also in case of chronic otitis.

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P67 - AFLATOXIN M1 EFFECTS ON THE METABOLOMIC AND CYTOKINOMIC PROFILING OF A HEPATOBLASTOMA CELL LINE

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The metabolism of AFB1 occurs in the liver in the presence of enzymes belonging to the cytochrome P450 superfamily [1]. On the other hand, AFB1 hydroxylation produces aflatoxin M1 (AFM1), present in mammalian milk when the producing female is fed on contaminated feedstuff. Since AFM1 is also found in the milk of lactating human mothers, it is indicated as a human carcinogen of Group 2B type and the maximum residue limits have been defined in milk. Regarding the effects of AFM1 on hepatoblastoma cells, there are only data related to its capacity to induce cytotoxicity and DNA damage in HepG2 cells [1]. Therefore, considering that hepatoblastoma develops in infants and children and AFM1 can be present in mother's milk and in marketed milk products, in this study we decided to test the effects of AFM1 on a hepatoblastoma cell line (HepG2) [2]. In detail, cell viability was assessed by colorimetric sulforhodamine B assay (SRB, Sigma Aldrich). Apoptosis and cell cycle were assessed after 48h treatment with AFM1, using the Annexin V and Dead Cell Assay kit and the Cell Cycle Assay Kit (Merck Millipore, Darmstadt, Germany), respectively. ^{1}H spectra of the cellular polar and lipidic fractions were acquired at 300 K by a 600 MHz Bruker spectrometer equipped with a TCI cryoprobe. Cytokine levels on supernatants of HepG2 cells were evaluated by Bio-Plex Pro Human Cytokine 27-Plex Immunoassay and a Bio-Plex array reader (Luminex, Austin, TX, USA). Incubation of HepG2 cells with AFM1 decreased the viability of HepG2 cells even if no increase in the number of apoptotic cells was observed. In fact, AFM1 resulted to be able to block the cell cycle in the G0/G1 phase. Then, a metabolomic analysis was conducted on HepG2 cells treated with AFM1, and compared to untreated cells. It evidenced that the levels of acyl groups of fatty acids, cholesterol, lactate, glycine, choline, phosphocholine, glycerophosphocholine, betaine, trimethylamine N-oxide, hydroxyproline, branched-chain amino acids, and glutamate were increased in treated HepG2 cells, whereas the levels of formate were decreased after AFM1 treatment. Finally, considering that cytokines are involved in all inflammatory processes, and in cancer initiation and progression, we decided to evaluate the levels of a panel of 27 cytokines in HepG2 cellular supernatants after AFM1 treatment using the untreated cells as control. This evaluation demonstrated that the levels of IL-6, IL-8, and TNF-α increased after treatment, whereas those of IL-4 decreased. Therefore, overall data evidenced that AFM1 was able to induce in HepG2 cells an increased synthesis of lipids and amino acids, membrane damage, and the enhancement of the glycolytic pathway and inflammatory status. Hence, we can conclude that the possible presence of AFM1 in mother's milk or in marketed milk products represents a topic to consider during hepatoblastoma progression and treatment.

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69P - BUFFALO MEAT AND DIETETIC MANAGEMENT OF THE OBESITY IN DOGS

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Obesity can have serious consequences for the health of the dog by reducing the expectation and quality of life, increasing the risk of severe diseases, in addition to the worsening pre-existing conditions (1). The aim of this study was to evaluate the efficacy of buffalo meat whose organoleptic qualities are different from other red meats widely used in the dog's diet, mainly in fatty acid profile and for the presence of bioactive molecules. It was evaluated the use buffalo meat in the management of diseases in which the lipid intake must be reduced (2). Nine obese dogs were enrolled. At time 0 (T0) dogs were submitted to: determination Body Condition Score system with a 5-point scale (3); clinical examination, hematological and biochemical profile; blood pressure measurement; electrocardiographic and echocardiographic examination. To each subject was prescribed a personalized diet based on buffalo meat for 3 months. In function of the overweight degree a caloric restriction (from 30 to 35%) was applied. In order to nutrients balance a commercial supplement (vitamin A, D3, Ca, P, Na) was added. At the end of the treatment (T1) dogs were monitored as in T0. Dogs were female, 5 mongrel and 4 Shih Tzu; 3 dogs were classified with BCS 5, 2 dogs BCS 4.5 and the other 4 with BCS 4. Dogs presented loss of waist and need to loosen the collar as well as fatigability, breathlessness and exercise intolerance. At T0, 5 dogs showed clinicopathological alteration of hepatic enzymes and lipidic metabolism. Cardiological examination revealed a mild systolic heart murmur only in two dogs, without irradiation on the thorax. The following echocardiographic exam showed a mild mitral insufficiency, without hemodynamic consequences. No dogs had blood arterial hypertension at T0. Regarding standard ECG, at T0 all animals showed sinusal rhythm and mean electrical axis in the normal range. Sinusal respiratory arrhythmia was present in seven dogs (7/9), and in only one animal intramural microinfarcts were found. Clinical examination performed at T1, pointed out a reduction of BCS in all subjects, a reduction in waist and neck measurements, breathlessness and movement difficulties. Laboratory data showed an improvement of cholesterol, triglycerides, alkaline phosphatase and liver enzymes in most subjects. At cardiological examination no rhythm disturbances or significant changes in heart rate were observed. From the comparison between the two observation times no statistically significant differences for ecocardiographic parameters and blood arterial pressure values were found. Based on our results, buffalo meat could represent a valid dietary alternative in diseases characterized by obesity, dyslipidemia and cardio-vascular alterations; representing a new protein source it could also be administered in allergic pathologies. It is important to underline the excellent compliance by the owners. The diet was easily administered considered highly palatable. This study could be considered a preliminary study on the evaluation of the use of buffalo meat which has been shown to have considerable potential for use in veterinary medicine.

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P70 - RETROSPECTIVE INVESTIGATION OF THE RETICULOCYTE HEMOGLOBIN CONTENT IN THE DOG

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Reticulocyte hemoglobin content (RETIC-HGB) as part of complete blood count (CBC) in dogs is considered as an early diagnostic marker of iron deficiency. [1,2] A retrospective evaluation of the RETIC-HGB and the relation with the main parameters of the CBC was carried out in samples managed at the Veterinary Teaching Hospital, Clinical Pathology Laboratory. In addition, the trend monitoring of RETIC-HGB values in few patients and its comparison with the main markers of inflammation was done. All CBCs (Procyte, IDEXX®) performed from May 2018-January 2019 were collected and divided into 2 groups according to the RETIC-HGB values: <22.3 pg (RHr) and >22.3 pg (RHn). The main RBC and RETIC variables (HCT, MCV, MCH, MCHC, RETIC absolute value and RETIC-HGB) of the two groups were compared by Mann-Whitney or Chi-Square tests, and also the Spearman test (rho) was used to investigate their correlation. Six sick patients were monitored over time for RETIC-HGB, MCV, neutrophilia, C-Reactive Protein, and Fibrinogen. CBCs with reduced RHr were 21.5% (262/1,218). CBCs with RHr had significantly lower HCT and MCV values than CBCs with RHn (P=0.0001). Significant differences between the two groups for all the RBC and RETIC variables were also found. Anemia was observed in CBCs with RHn (23.9%; 229/956) and in CBCs with RHr (40.5%; 106/262). Normocytic normochromic anemia was assessed in CBCs with RHn (69.9%; 160/229) and in CBCs with RHr (52.8%; 56/106) (P=0.0036). Microcytic normochromic anemia was assessed in CBCs with RHn (17.0%; 39/229) and in CBCs with RHr (31.1%; 33/106) (P=0.0054). In both groups the most represented anemia was non-regenerative [RHn 71.6% (164/229), RHr 50% (53/106) (P=0.0002)] or moderately regenerative [RHn 4.8% (11/229), RHr 13.2% (14/106) (P = 0.013)]. There was no correlation between the RHr and the RBC and RETIC variables studied (rho range between -0.01/+0.29). During the monitoring of six patients the reduction of RETIC-HGB values was observed when there was neutrophilia and/or an increase in C-reactive protein and/or fibrinogen. CBCs with RHr values showed higher rate of occurrence and more severe form of anemia. [1] The clinical condition of dogs related to the CBCs collected was unavailable as well as parameters related to inflammatory markers inducing a study limitation except for 6 patients monitored over the time. In our study the reduction of RETIC-HGB was unable to indicate iron deficiency sooner than the reduction of MCV or MCHC. In accordance with previous studies, RETIC-HGB values appear to be influenced by inflammatory conditions in both anemic and non-anemic dogs [3].

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P71 – ASSESSMENT OF TRACE ELEMENTS IN SERUM DOGS AFTER EXPOSURE TO FLEA AND TICK PRODUCTS: PRELIMINARY DATA

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Insecticides are extensively used for the control of fleas, ticks on companion animals. Very limited data representing the use of pet care products are available. Pets are serving as involuntary sentinels of the widespread chemical contamination that scientists increasingly link to a growing array of human and animal health problems. In sharing their living environment with pets, humans are exposed to many of the same environmental contaminants as their animals, and the other way around. The majority of dangerous chemical pollutants, considered particularly harmful for humans, especially children, are heavy metals. The World Health Organization estimates that about a quarter of all diseases are due to prolonged exposure to environmental pollution. Certain heavy metals have been reported to seriously affect the immune system potentially resulting in a broad range of harmful health 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. The flea dog and cat collars can spew droplets of insecticide and children living with pets might be exposed to high concentrations of chemicals substantial concentrations resulting in cumulative exposures with unknown health risks.

The study was performed on 50 dogs divided in three groups. Group 1(G1) was composed of 20 dogs wearing Neem oil collars; group 2(G2) was composed of 20 dogs wearing conventional anti-parasite flea collar or other chemical substances used to fight parasites; group 3 (G3) was composed of 10 dogs that did not use anti-parasite flea conventional collar or other chemical substances used to fight parasites.

The present study was aimed at determining trace element concentration in dog serum to evaluated levels of heavy metals differences between dogs using Neem oil collars and dogs using conventional anti-parasite flea collar or other chemical substances used to fight parasites.

Clinical evaluation of the dogs and their hemato-biochemical profiles were recorded. The determination of 16 trace elements (As, Hg, Sb, Pb, Cd, As, Sr, V, Ni, Se, Cr, Mo, Li, Cu, Zn Mn and Fe) was carried out by using a validated analytical method based on inductively coupled plasma mass spectrometry (ICP-MS-Perkin Elmer, Waltham, MA-USA). Trace element concentrations were calculated by using calibration curves. Statistical Analysis of Variance (Anova) results showed that three elements trace concentrations (Li, Sb, Se) were statistically significant in the Neem oil dog group in T0 vs T2 and T0 vs T3. No haemato-biochemical alterations were observed in Neem oil collars group. The study suggest that chemical substances used to fight pet parasites may be responsible of the pet pollution.

P73 - PLATELET ALTERATIONS AND PLATELET-TO-LYMPHOCYTE RATIO (PLR) IN 41 DOGS WITH IMMUNOSUPPRESSANT-RESPONSIVE ENTEROPATHY (IRE)

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In humans, risk of thrombotic events is a major concern in people affected by ulcerative colitis and Crohn's disease [1]. Platelets number (PLTs), increased PLTs activation, mean platelet volume (MPV) reduction and immune-mediated thrombocytopenia are reported in patients with chronic enteropathies [1]. In dogs with inflammatory bowel disease, thrombocytopenia or thrombocytosis are reported to be 2.5-13% or 31% respectively [2,3]. Platelet-to-lymphocyte ratio (PLR) has been suggested as marker of active disease in humans with Crohn's disease [4].

This study investigated PLTs, MPV and PLR in dogs with immunosuppressant-responsive enteropathy (IRE). Forty-one dogs with IRE were retrospectively enrolled in a one-year study period. Food and antibiotic responsive enteropathies were previously excluded with therapeutical trials. All patients underwent a full staging including CBC, serum biochemical profile, fecal exam and endoscopy with histological study. Results from PLTs, MPV, PLR, CCECAI score, serum albumin and histopathological score were considered and analyzed. Evaluating follow up at 1 month (T1), dogs were divided into improved and unimproved: improved group had dogs with a T1 CCECAI <4 and dogs with CCECAI ≥4 were in the unimproved group. Continuous and categorical variables were analyzed to compare data between variables with t-test, ANOVA, correlation and Fisher's exact tests. Odds ratio (OR) was calculated. Median age was 4 years (range 1-15 years). The most common breeds were German shepherd (n=7), Boxer (n=2), Dachshund (n=2), Rottweiler (n=2) and Jack Russel (n=2). The remaining 26 dogs were mixed (n=12) and other breeds. Five dogs (12%) showed thrombocytopenia and 9 (22%) had thrombocytosis. Seven (17%) dogs showed decreased MPV. PLTs were negatively correlated with MPV (p=0.001, r=-0.500). PLTs were significantly higher in dogs with low albumin (median 420 vs 210K/uL, p=0.008). Decreased MPV was associated with low albumin (p=0.023, OR 13.8, 95% CI=1.46-130.1). Median CCECAI was higher in dogs with thrombocytosis (10 vs 6, p=0.014) and in dogs with decreased MPV (10 vs 6, p=0.001). PLR was positively correlated with CCECAI (p=0.005, r=0.429) and negatively correlated with albumin (p=0.029, r=-0.348). Lastly, improved group had lower PLTs (P=0.047), MPV (P=0.029) and higher PLR (P= 0.046) than unimproved dogs.

PLTs, MPV and PLR should be considered in the evaluation of severity and follow up of IRE dogs, along with other markers already known to be useful, as serum albumin. PLR has been applied for the first time and could add interesting view of the PLTs and lymphocytes involvement in IRE dogs.

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P74 - APPLICABILITY OF CAPSULE ENDOSCOPY PILLCAM SB2 ® IN VETERINARY MEDICINE AND PRELIMINARY STUDY OF INTESTINAL TRANSIT TIMES IN DOGS

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Capsule endoscopy (CE) is a technique to explore the gastrointestinal tract (GIT), that can provide high quality images of the mucosa and information about gastrointestinal transit times (GET- Gastric empting time and SITT- Small intestine transit time). In human medicine it is useful to detect obscure GIT bleeding [1], while in dogs it has been used to determine the efficacy of anthelmintic treatment [2], to identify mucosal lesions causing GIT bleeding [3] and to record GIT motility and pH [4]. The aim of this study was to create a standardized protocol useful to provide information about the upper GIT in dogs, especially the macroscopic appearance of the mucosa, the GET and the SITT. Ten private owned female adult dogs (mean age 6.4±2.6; mean weight 22.8±3.0kg) with no signs of GIT diseases were used in the study. Informed consent was obtained from the owners. PillCam SB2® (already distributed by Covidien Italia SpA, Segrate) was administered per os to each dog according to one of the three following protocols: 12hrs fasting with access to water and dry feed 4hrs after administration of the capsule, 24hrs fasting with access to water and dry feed after the capsule was seen in the duodenum through the monitor of the equipment, 24hrs fasting and monitoring of the progression of the capsule using serial abdominal X-rays. The recording equipment was attached to the animal with a chest support used for Holter monitoring. Because of this system, only female dogs were selected because it was easier to manage bulking and size of the chest support. After expulsion of the capsule, the recordings were downloaded on a computer and the videos analysed using Rapid Software. PillCam® was well tolerated by all animals, with no adverse effects or signs of discomfort shown. High quality images were recorded in all animals for the upper GIT. Seven out of 10 (70%) CE were useful to detect GET and SITT, but the transit time was extremely variable in the animals evaluated: mean GET was 250.0±216.8 minutes (min 66.0 minutes and max more than 12hrs), mean SITT was 106.3±41.6 minutes (min 81.0 minutes and max 196.0 minutes). In the other 3 dogs, the capsule was submersed in the ingesta and it was not possible to visualize the mucosa and identify the different portions of GIT. The main limitation of capsule endoscopy is the impossibility to collect biopsy samples, that are considered the gold standard for the diagnosis of GIT diseases in small animals, nevertheless, this technique could be used as a screening tool when anaesthesia could be dangerous for the animal. Further studies are needed to evaluate different protocols and possible applications of this technique to males and animals of different sizes.

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P75 - A NEW HETEROLOGUS TURBIDIMETRIC IMMUNOASSAY FOR THE MEASUREMENT OF C-REACTIVE PROTEIN IN THE DOG

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C-reactive protein (CRP) is one of the major acute phase proteins produced by the liver following many pro-inflammatory stimuli. In dogs, as well as in humans, CRP is the most sensitive biomarker to quantitatively assess the presence of an inflammatory status [1]. For years, the lack of adequate laboratory methods to detect CRP has limited its clinical use. Because human and canine CRP (cCRP) shares a similar molecular structure [2], the same assay used for human CRP detection has been equally applied for measuring cCRP concentrations. The aim of this study was to evaluate a new and cheaper heterologous immunoturbidimetric method for determining the serum concentration of CRP in dogs and to compare this method with an already validated cCRP assay. A total of 91 canine serum samples, obtained from clinically healthy and dogs with different diseases, were analysed using the standard cCRP test (Canine CRP randox) and the new heterologous method (CRP Biotecnica) in the same analytic run with the analyser BT 1500 (Biotecnica Instruments spa). Serial dilution with NaCl 0.9% were obtained from canine serum pool with a level of CRP of 60.1 mg/L to achieve a final concentration that was 1.0, 0.5, 0.25, 0.125 and 0.0625 parts of the original solution, then each point was measured five times. The descriptive statistics reported intra-assay coefficient of variation (CV), recovery rate (%) and bias (%). Accuracy was assessed by evaluation of linearity under dilution. Normal distribution of the data was tested using the Shapiro-Wilk's test. Differences between methods were studied using the Mann Whitney test and ROC curve analysis. A Bland-Altman plot was used to detect percentile bias. A value of $P<0.05$ was considered to be statistically significant. Statistical analyses were carried out using statistical software (SAS version 9.3, SAS Institute Inc., Cary, NC, USA; MedCalc® version 12.6.1.0, MedCalc Software, Ostend, Belgium). Recovery rate and CVs varied between 87.1 and 109.2% and between 3.3% and 7.6%, respectively. Bias ranged from -12.9 to 9.2%. Linearity study revealed that the assay measured proportionally in the analytic range up to 60 mg/L. There were no statistically significant differences between the two methods. ROC curve analysis of cCRP measured with the tested method resulted in an AUC of 0.994 (95% confidence interval, 0.948-1). At the cut-off value of 4.63 mg/L for cCRP, the sensitivity and specificity were 100% and 93.75%, respectively. Bland-Altman analysis revealed a mean constant bias of -0.6%. with the 95% limits of agreement ranging from -3.7% to 2.5%. In conclusion, the new turbidimetric immunoassay shows good agreement with the validated method and may represent an alternative assay for routine analysis of the cCRP with BT 1500 analyser.

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P77 – A CASE OF TRANSIENT MYOCARDIAL THICKENING IN A DOG AFFECTED BY ACUTE MYOCARDITIS

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Diagnosis of myocarditis is difficult to achieve in dogs during life and its direct cause can rarely be determined [1]. The aim of this report is to highlight the possibility of a transitory severe myocardial thickening associated to acute myocarditis in dogs. A 10-year-old male rottweiler was presented to the D.E.T.O. Veterinary Clinics, University of Bari, in cardiogenic shock associated with polymorphic ventricular tachycardia. Anamnestic data did not indicate the possibility of toxic intake, routine laboratory tests did not report alterations worthy of note (WBC 13.9 k/µl, HGB 15.8 g/dl, PLT 114 k/µl), except for slight hyperazotemia, and electrolytic panel was normal. Transthoracic echocardiography revealed a severe concentric hypertrophy of the left ventricle, in absence of aortic stenosis, with mild left atrial dilation. Based on clinical and echocardiographic findings, hypertrophic myocardial disease associated with ventricular arrhythmias or myocarditis were considered in the differential diagnosis. Cardiac troponin, blood culture and serology for the most common pathogens associated to myocarditis (i.e. VBD pathogens, *Toxoplasma gondii, Neospora caninum*) were performed. Intravenous and antibiotic support therapy were established (Ampicillin Sulbactam 25mg/kg, Enrofloxacin 5mg/kg). Lidocaine EV in bolus 2mg/kg and CRI 50 µg/kg/min followed by Esmolol EV in bolus 0.1 mg/kg and CRI 25 µg/kg/min were administered without effects on the ventricular tachycardia. Cardiac troponin was 180 ng/ml (range 0.05-0.24). Methylprednisolone 1mg/kg was added to the therapy and the dog was kept under constant electrocardiographic monitoring. Twenty-four hours after presentation the dog reverted to a normal sinus rhythm. Blood culture and serology were negative. The patient was discharged after 72 h hospitalization in good clinical conditions, sinus rhythm, MAP 148 mm/Hg and without medication instructions. At 3-months follow-up, the owner reported that the dog was in good general condition despite a chaotic rhythm due to atrial fibrillation was documented. The echocardiogram revealed that myocardial thickening had completely reverted, and the cardiac troponin level measured 0.2 ng/ml. Clinical presentation of myocarditis in dogs includes rhythm disturbances and is commonly associated to generalized heart chamber dilation [1]. Differently, myocardial thickening associated with myocarditis is a poorly documented condition both in humans [2], cats [3] and dogs [1]. The gradual return to normality of the myocardial thickening associated with the normalization of troponin values suggests that, although not common, myocardial thickening may be the result of the inflammatory process (edema or cellular infiltration) at the base of myocarditis and conduction abnormalities [4]. The rapid response of severe ventricular arrhythmias to steroids could be due to the anti-inflammatory effects exiting in improvement of conduction. The absence of endomyocardial biopsy (not allowed) represent one of the limitations of this case report.

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P78 - SELECTIVE USE OF ANTIBIOTICS IN CALVES WITH NEONATAL DIARRHEA – PRELIMINARY DATA

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This study investigates the role of antibiotics in the treatment of calves with Neonatal Calf Diarrhea (NCD), widely employed in the field although their controversial efficacy [1]. The Ministry of Health approved this study (approval number 14/2018). Twenty-four Friesian calves with NCD, aged from 1 to 28 days were enrolled, excluding those with other concurrent neonatal diseases. Upon admission, dehydration was estimated as body weight percentage (b.w.) and the acid-base imbalance was assessed by venous blood-gas analysis. Fecal antibiotic susceptibility tests were also done. Treatment consisted of natrium bicarbonate, 0.9% natrium chloride saline solution and glucose infusion, NSAID (flunixin meglumine, 2.2 mg/kg b.w. IV) and vitamin E-selenium, as previously reported [2]. After emergency therapy, calves were randomly assigned to Group A (antibiotic-treatment group) or Group B (antibiotic-free group). Group A received ampicillin (10 mg/kg IV q12h for 5 days), a wide spectrum antibiotic, as described by Constable [1], as the antibiogram results were delayed. Each calf was monitored for 28 days. Calf Health Scoring Chart (CHSC), average daily gain (ADG) and sepsis score [3] were recorded daily. Calves of both groups whose general conditions deteriorated (sepsis score >60%) were given an antibiotic based on antibiotic susceptibility tests. Descriptive statistics, chi-square test for categorical variables, T-test and general linear model for repeated measures for continuous variables were performed using SPSS. In the general linear model, treatment with antibiotics in group B and changes in active substances in group A were considered for statistical analysis. The ADG in calves A and B was 11±0.4 kg and 13±5 kg, respectively (p=0.146). The average sepsis score in group A was 29%; 4 of 12 calves developed a sepsis score >60% so their antibiotic was changed. The average sepsis score in group B was 24%; 4 of 12 calves developed a high risk of sepsis (>60%) and so received antibiotics. Mortality rate in group A and B was 33.3% and 25%, respectively (p=0.202). The CHSC revealed no statistical differences between groups. Our data suggests antibiotic treatment should be given to calves based on the high probability of developing sepsis and avoided in cases of uncomplicated NCD.

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P80 - USE OF ELASTOSONOGRAPHY IN THE CHARACTERISATION OF SUBCUTANEOUS SOFT TISSUE LESIONS IN DOGS

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Elastosonography is an imaging technique based on ultrasound, which measures mechanical properties of tissues. The only study on elastosonography of subcutaneous lesions in dogs compares lipomas and malignant subcutaneous neoplasm.[1] Aim of the study: to characterize superficial soft tissue lesions in dogs with elastosonography. Medical records were searched for dogs with subcutaneous soft tissue lesions, which underwent elastosonography and cytology or histology from January 2017 to January 2019. All examinations were performed by the same operator with the same equipment (Esaote MyLab Class C with ElaXto software), using a linear array (8-18 MHz). Images were considered only when the visual indicator provided by the software determined an adequate degree of correlation of the relative hardness over time (green ElaXto spring function) and the distribution of colours in the elastogram compared with the underlying B-mode image was coherent. Two values were measured for each region of interest (ROI): Elax-t%SFT (the percentage of tissue softness) and Elax-t%HRD (the percentage of tissue hardness). Semi-quantitative analysis were performed to create an elasticity score on the basis of the Elax-t%SFT values: 1) >70%: soft; 2) <70% >50%: mostly soft; 3) <50% >30%: intermediate; 4) <30% >20%: mostly hard; 5) <20%: hard. Elax-t%SFT and Elax-t%HRD values were compared between benign and malignant lesions with Student t test. Elax-t%SFT and Elax-t%HRD values were then compared with ANOVA and post-hoc Tukey test, considering 3 groups: tumours, non-tumours, lipomas. Receiver operating characteristic (ROC) curves of the sensitivity and specificity were obtained. Wilcoxon rank sum test was used to compare the semi-quantitative score with the nature of lesion (benign vs. malignant). A P value <0.05 was considered significant. Eighty dogs of a variety of breeds (53 females, 27 males), aged between 3 months and 18 years (mean 8.75±3.41 years), met the inclusion criteria. Overall, 85 lesions were considered, 53 benign and 32 malignant (14 mast cell tumours, 14 sarcomas, 4 carcinomas). Among benign lesions, 12 were benign neoplasms, 20 were lipomas and 21 were non-neoplastic lesions. Mean Elax-t%HRD value was higher in malignant lesions. Mean Elax-t%SFT value was higher in benign lesions. There were significant differences in Elax-t%SFT and Elax-t%HRD between tumours and non-tumours, tumours and lipomas. No significant difference was found between lipomas and non-tumours. ROC curves identified a hardness cut-off of 52.2% (sensitivity: 83%, specificity 75%), and an elasticity score cut-off of 2.5 (sensitivity 79.2%, specificity 75%). Malignant lesions were harder than benign lesions. Non-neoplastic lesions and lipomas were softer than neoplastic lesions. Elastosonography may be useful in the diagnosis of subcutaneous soft tissue lesions in dogs.

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P81 - MRI-CT STEREOTACTIC HEAD FRAME VALIDATION FOR SHEEP AS ANIMAL MODEL

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Sheep as animal model has been largely used in research fields [1,2]. The large size of the sheep brain allows to be an ideal model for the development of neurosurgical techniques using standard clinical computer tomography (CT) and magnetic resonance imaging (MRI). Despite all characteristics limited stereotactic head frame systems for sheep as animal model have been used in research. The aim of the current study was to validate the sheep head frame system made by Renishaw® with integrated fiducial marker for MRI and CT studies for surgical planning. The head frame system is compatible with Cosman-Roberts-Wells (CRW) stereotactic frame. This study was composed by an *ex vivo* and an *in vivo* phase. A total of eight sheep head *Ovis aries* and four female sheep *Ovis aries* were used. Four spherical CT skin markers (BrainLab, Germany) were used and placed in four different and asymmetrical points on the skull surface. After the fiducial spheres placement a CT scan was made and neurosurgical trajectory planned with Renishaw® surgical planning software Neuroinspire™. For each head two targets points were identified as corticospinaltracts. Once obtained the coordinates the CRW stereotactic system the Anspach® drill system have been assembled and two burr hole ports placed. Then the patient was moved from the surgical room to the CT scan room and the second imaging dataset was acquired. The datasets have been uploaded and used in 3D slicer software. The images have been segmented and edited used a threshold window to identify and clean the 3D structure of the fiducials arc spheres and the fiducials spheres on the skull surface. The 3D has been uploaded in MeshLab software, cleaned from artifacts and then analysed in MATLAB software in order to compare the movement for each spheres in X,Y,Z planes.

The accuracy has been studied in mean and standard deviation and presented in *ex vivo* as 0.815 mm and 0.4325 mm and *in vivo* as 0.8275 mm and 0.29 mm respectively.

In literature other studies like Oheim et al. [3] used a Kopf stereotactic frame which is an experimental frame without clinical translational purpose. The measurement in our case has been analyzed on the skull surface and not on the catheter tip which can be influenced by the catheter variability. In conclusion this study describes a stereotactic head frame validation in sheep as animal model. The head frame is MRI and CT compatible. The compatibility with CRW stereotactic frame increases the translational purpose of the experimental method.

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83P - PIEZOSURGERY TOUCH® A NEW USEFUL TOOL FOR THE PIEZOELECTRIC OSTEOTOMY

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Since 2001, Piezosurgery represents a useful device for the bone surgery, due to its capacity to cut only mineralized tissues. Selective cutting is possible for different ultrasonic frequencies acting only in hard tissues saving other anatomical structures. These ultrasonic frequencies generate mechanical microvibration that compared to other osteotomy devices (drills, saw, etc) give to Piezosurgery greater cutting precision and safe. Piezosurgery touch ® is the last evolution of this device and it is characterized by a digital platform with a black touch-screen that made this tool more intuitive for the user and more suitable to the operating room environment.
The cavitation effect, due to the NaCl solution irrigation, leads to a free-blood surgical site.
The handpiece presents new LED light to illuminate the tip of the insert. Furthemore, the new longer tips make the Piezosurgery touch more suitable also for the spinal surgery.
The aim of this study is to present the results of use of this new instrument, technical and operative advantage compared to the old generation Piezoelectric instruments.

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84P - STRANGULATING OBSTRUCTION OF THE SMALL INTESTINE BY A FIBROUS BAND ORIGINATING ON THE NEPHROSPLENIC LIGAMENT

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Nephrosplenic entrapment is a relatively common disease, mostly involving the large colon although non strangulating entrapment of the small intestine has been described. Aim of this report is to describe, for the first time, the features of a strangulating lesion of the small intestine in the nephrosplenic ligament.

A 2-years-old male Thoroughbred was referred for acute abdominal pain. At the presentation the horse was depressed, PCV was 43%, TP 5.2 g/dl, HR 100 beats/min, RR 24 breaths/min, temperature 37.8°C. Rectal palpation demonstrated a distended small intestine. Exploratory laparotomy was performed and a tract of jejunum was found strangled by a fibrous band in the caudal portion of the nephrosplenic ligament. The band was blindly cut with scissors deep in the abdomen. Seven metres of small intestine resulted necrotic and were resected. The horse recovered uneventfully. Laparoscopy closure of the nephrosplenic space was performed days later. During laparoscopy several fibrous bands bridging from the renal capsule to the splenic capsule and a fibrous plaque on the spleen were found, as well as the resected ends of the band that caused the entrapment. Closure of the nephrosplenic space was obtained with intracorporeal suturing.

The fibrous bands were presumably consequence of inflammation caused by multiple previous nephrosplenic entrapments not clinically evident.

Multiple large colon dislocations could result in fibrous bands formation across the nephrosplenic space that ultimately could lead to small intestine strangulation. Laparoscopic closure of the nephrosplenic space is recommended to prevent this occurrence.

85P - COMPARISON BETWEEN DEXMEDETOMIDINE AND XILAZYNE IN COMBINATION WITH ISOFLURANE FOR BALANCED ANESTHESIA IN HORSES

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The aim of this study was to compare the use of a constant rate infusion (CRI) of dexmedetomidine (DEX), Xylazine (XYL) or saline solution (CTR) in combination with isoflurane in healthy horses undergoing general anesthesia for elective orthopedic surgery. Our hypothesis was that both drugs would have a sparing effect on isoflurane dose, ensuring at the same time an intra-operative hemodynamic and analgesic support. Moreover, the residual sedative effects of the alpha 2 agonists would ensure a better quality of recovery. To test our hypothesis we have monitored: invasive mean arterial pressure (MAP) and the amount of dobutamine required to maintain MAP between 70 and 80 mmHg, the end-tidal concentration of isoflurane (EtISO) required to maintain an adequate plane of anesthesia and the quality of recovery in horses receiving the three treatments. The study included 40 horses undergoing elective orthopedic surgery, premedicated with acepromazine at 0.02 mg/kg IV and after 30 minutes XYL at 0.4 mg/kg IV (XYL and CTR group) or DEX at 0.004 mg/kg IV (DEX group), general anesthesia was induced with midazolam (0.1 mg/kg IV) and ketamine (2.2 mg/kg IV). All horses were orotracheally intubated and anesthesia was maintained with a mixture of isoflurane in pure O_2. All horses were mechanically ventilated during the procedure. After horses were placed on the surgical table they were randomly divided into three treatment groups DEX (n=15) which received a CRI of DEX at 0.002 mg/kg/h IV[1]; XYL (n=15) which received a CRI of XYL at 1 mg/kg/h IV[2]; CTR (n=10) which received a CRI of saline solution. At the end of surgery: time, quality of recovery and the number of additional xylazine boluses required during recovery were recorded. Quality of recovery was assessed based on a validated score going from 1 (excellent) to 5 (very poor).[1] Data were analyzed with the two ways ANOVA for repeated measurements ($P<0.05$). The mean values of EtISO were lower in the XYL (1.30±0.11 %) and DEX (1.31±0.10 %) groups as compared to the CTR (1.72±0.13 %) group at all times during anesthesia. The average consumption of dobutamine was lower in the XYL (0.11±0.15 µg/kg/min) compared to DEX (0.28±0.49 µg/kg/min) and CTR group (2.55±0.46 µg/kg/min). During recovery 2 horses in the group XYL, 4 in DEX and all in the CTR group required additional doses of XYL. Quality of recovery was better in the XYL and DEX than CTR group. The results of this study demonstrated that both XYL and DEX have a sparing effect on the dose of isoflurane required to maintain general anesthesia in horses, moreover, both drugs improved the quality of recovery, however DEX required a larger amount of dobutamine to maintain an adequate MAP.

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86P- CERVICAL CYSTIC LYMPHANGIOMA IN A YOUNG DOG: CT FINDINGS

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Lymphangioma is a rare lymphatic disorder; in veterinary medicine it is still considered a benign tumour, while in human medicine has been recently classified as a Lymphatic Malformation (LM), in fact it probably origins from a failure in development of connections between lympatic and venous system. Reported localizations of canine lymphangioma include skin, subcutaneous and fascial tissue of axilla, limbs, inguinal and mammary regions; lymph nodes; retroperitoneal space; nasopharynx. In the present report we describe the computed tomographic (CT) features of a cervical cystic lymphangioma in a young dog. A 1-year-old intact male Italian Shepherd dog was referred to the primary care veterinarian with a 1-month history of left ventrolateral neck swelling. No other clinical signs were present. Ultrasonographic (US) examination revealed a mass with hyperechoic thick wall, hypoechoic content with hyperechoic fluctuating areas. Fine needle aspiration biopsy (FNAB) revealed a cloudy pinkish fluid, citologically referable to serous-hematic fluid with chronic inflamation. For better assessment of the morphology and of the margins of the lesion, the dog was referred for CT examination. Pre- and post-contrast CT scan of head, neck and thorax were made. A mass located between the muscles of the caudal neck and thoracic/axillary regions (from the level of C4 to the level of T2) was found, which partially occupied the left visceral space of the neck and bulged into the thoracic inlet. The mass was ellipsoid-shaped (40x45x140 mm), with well-defined margins and heterogeneous soft tissue attenuation. It was apparently capsulated, with fluid-like content and soft tissue attenuating septa and small areas within the fluid. Adjacent to the mass three areas of soft tissue mineralization, smoothly marginated, were found. It was responsible for mild mass effect, without significant compression on the surrounding structures. Mild left axillary and left medial retropharyngeal lymphadenomegaly was noted, with normal shape and attenuation of the limph nodes. Post-contrast images showed moderate enhancement of the mass wall and the soft-tissue-attenuating areas/septa within it; no enhancement of the fluid was noted. No other abnormalities were found. The mass was completely surgically excised and submitted for histopathologic analysis, with a definitive diagnosis of cystic lymphangioma. In human literature CT features of lymphangioma are widely described, while in veterinary literature there are no reports about its CT appearance; moreover, human lymphangioma is described as preferentially located in the neck and axillary regions, while, to our knowledge, cervical localization has never been reported before for canine lymphangioma.

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87P- THE DEFORMITY REDUCTION DEVICE (DRD) IN THE SURGERY FOR CORRECTION OF MULTIPLANAR ANGULAR DEFORMITIES AND NONUNION AT THE TIBIAL DIAPHYSIS FROM INVETERATE FRACTURE IN A DOG

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Correction of angular deformities of long bones often involves multiplanar osteotomies [1]. After planning angulations and sites of osteotomies, the main difficulties are those of executing them in exact manner and of aligning the obtained fragments while holding them firmly in place while performing osteosynthesis [2,3,4]. Aim of the work is to verify the usefulness of the DRD in the surgery for correction of multiplanar angular deformities and of nonunion at the tibial diaphysis from inveterate fracture in dogs. A cross-bred 8 months 17 kg female dog presented a nonunion with angular deformities on frontal and sagittal planes in the middle third of the left tibial diaphysis. On plantaro-dorsal and medio-lateral radiograms of the tibia, a 25° varus and a 7° procurvatum deformities were respectively observed. Two tibial osteotomies were planned, one proximal and one distal to the nonunion site for obtaining a closed wedge osteotomy: the first one perpendicular to the axis of the distal fragment, and the second one bi-oblique on the proximal fragment, the latter for correction of both 25° varus and 7° procurvatum angular deformities. Under general anesthesia, with the dog on dorsal recumbency, a standard medial approach to the tibia was performed. The DRD was preo-peratively set according to 25° varus deformity. A 1.5 Kirschner wire was inserted through the hinge of the DRD dorsally on the distal fragment of the tibia and perpendicular to its axis. The arch and the rod of the DRD were fixed with Kirschner wires on the distal and the proximal tibia respectively according to the manufacturer's description. After performing the above mentioned osteotomies, the two fragments were moved each other until DRD set was reported to zero. The clamps on DRD rod were slightly loosened for bringing the proximal tibial fragment towards the distal one, while alignment of the fragments was refined by the DRD translation screws and the clamps were tightened again. Osteosynthesis was performed with a 3.5 Synthes locking compression plate and 1 cortex and 6 locking screws. A compressive Robert Jones bandage was applied for 24 hours. The dog was allowed to walk on a leash for the next 30 days before returning to normal activity. Clinical and radiographic follow-up at one month showed a good correction of the deformity and a relatively slow formation of the bone callus. Three screws were removed after 2 months for dynamization of the implant, before its complete removal. The DRD, a device planned for correction of angular deformities of distal femur and proximal tibia [3], is helpful where a bone segment has to be removed and the two consequently free bone fragments obtained must be firmly maintained in place during osteosynthesis. Further, it helps in bi-oblique osteotomy for one-step correction of multiplanar deformities.

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P88 - SURGICAL REPAIR OF BILATERAL FRACTURED MANDIBLE IN A FOAL USING THE "FIXIN" SYSTEM

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Repairing bilateral mandibular fractures is highly problematic because of the limited space on the rostral fragments for application of plates or placement of pins or screws. In foals or young horses, the soft bone reduces the implant stability with process failure. Both internal and external fixation methods are reported. This clinical case discusses the repair of a bilateral exposed mandibular fracture using the "FIXIN" system (Intrauma).
A 14 days old foal was admitted to the ODVU (UniSS) three days after the damage was discovered. Clinical signs included swelling of the jowl and surrounding area, dysphagia, ptyalism and pain on palpation. Displaced fractures were diagnosed through clinical examination and radiography. Surgical anaesthesia was performed using Acepromazine (0.03 mg kg^{-1}), Medetomidine (0.006 mg kg^{-1}) and Butorphanol (0.05 mg kg^{-1}) in premedication. After 3 minutes induction was obtained with Diazepam (0.3 mg kg^{-1}) and Ketamine (0.1 mg kg^{-1}). Maintenance was achieved using Sevoflurane (1.8%) with FGF 0,5 l min^{-1} (75% O_2). The bilateral rostral mandibular fractures has been reduced and stabilised using the "FIXIN" plate (Series 3.5, T-shaped, four holes). A good maxillary-mandibular alignment was seen and the foal ate immediately normally. During the post-op recovery, the foal was treated with Amikacin sulphate (1 ml/10 kg) and Procaine Benzylpenicillin/Dihydrostreptomycin sulphate association (4.5-6 ml/100 kg), administered via IM once-daily for 10 days. Moreover, the foal was exposed to a daily clinical evaluation and an assessment of the surgical wound in order to check the healing process.
Adequate immobilization is the major problem in the repair of some mandibular fractures because the horse mandible is exposed to a continuous force during mastication: so an inappropriate osteosynthesis could cause pain and inability to take food, as well as excessive callus formation. Several techniques have been reported despite the risk due to infections or bone necrosis along with tooth loss and malocclusion. "FIXIN" is a fixation system with stable angle, which can be described as an internal fixation system with conical coupling of bone-plates and screws and more efficiently preserves perivascular structures of the osteal tissue, allowing for a better and faster healing process. In the clinical case reported the prothesys remained stable and the occlusion of the incisor teeth was good without infection or significant complications. The foal recovered the sucking reflex immediately and managed to eat easily without apparent pain or difficulty. The repair method chosen in this foal proved to be excellent for the configuration of the fracture and confirmed that dynamic compression plating was an excellent technical option.

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P89 - FREQUENCY OF DEA 1 ANTIGEN IN 71 FONNESE DOGS: A PRELIMINARY STUDY

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The Fonnese dog is one of the 14 dog breeds of Italian origin officially recognised by the National Agency of the Italian Kennel Club (ENCI). The Fonnese dog is an interesting example of regional population of dogs which share distinct features and represent a closed population, but which have never been recognised as true breeds. Typically, these closed populations live within well demarcated geographic regions, they are often used as guard dogs for sheep flocks or country homes. Even today, Fonnese dogs are trained by shepherds using ancient methods with the goal of forming an unshakeable bond between the herd and the dog.The first archaeological documents that attest to the presence of similar dogs date to the Bronze Age (19th century B.C. - 2nd century A.D.).The discovery of earthenware pieces, depicting both molosser (mastiff-like) and levrieroid (sighthound-like) dogs confirms the presence of these two distinct dog populations in the region during the Phoenician-Punic age. The combination of these two ancestors is believed to be present in the modern Fonnese dog [1].
The FCI nomenclature splits purebred dog into ten groups based on morphology, current use and historical criteria. Recently, a correspondence between genetic and phenotypical/functional breed grouping was observed. Dogs belonging to FCI groups 1 and 2 resulted statistically more likely to be DEA 1-. The Fonnese dog has been classified as group 2. Aim of this study was to investigate the frequency of dog erythrocyte antigen (DEA 1) in Fonnese dog population. The prevalence of DEA 1 antigen was statistically related to gender, coat color and eyes. From September 2018 to March 2019, during Fonnese dog breed recognition gatherings blood samples were collected from Fonnese dog officially recognised by ENCI. Data on gender, coat and eyes color were also collected for each dog sampled. Blood was collected from cephalic or jugular vein into K3EDTA tubes, stored at 4–6°C and processed within 24 h of collection. DEA 1 blood group was determined using an immunochromatographic strip typing kit (LabtestDEA 1, Alvedia, Limonest, France) according to the manufacturer's instructions. Based on the test results, the dogs were classified as DEA 1+ and DEA 1–. In the period of the study 71 dogs were evaluated: 41 female and 30 male, coat and eye color were described. Seventy-seven percent dogs resulted DEA1 + and 23% DEA 1-. Among gender 68.3% of female (28) and 90% of males (27) were DEA 1+. Any statistical correlation was observed between coat or eyes color and DEA antigen status. To the author knowledge this is the first study about DEA 1 antigen frequency in Fonnese dog population. Unlike the majority of the breeds enrolled on FCI group 2, data obtained by this study highlight a high prevalence of the DEA1 + antigen. Additional studies are needed to clarify the distribution of DEA antigens in this breed.

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P90 - ULTRASOUND EVALUATION OF THE OPTIC NERVE SHEATH DIAMETER IN 40 HEALTHY CATS. PRELIMINARY STUDY

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The optic nerve sheath is contiguous with the dura mater, and its contents are contiguous with the subarachnoid space. Thus, raised intracranial pressure (ICP) leads to an increase in the optic nerve sheath diameter (ONSD). When ICP rises, cerebrospinal fluid is pushed towards the tiny rim of subarachnoid space between the sheath and the nerve, causing an expansion of the dural covering. These changes are more marked in the anterior part of the nerve sheath behind the globe. As with any physiological change, the ONSD changes dynamically with changes in ICP. There is a growing body of evidence, also in veterinary medicine, stating a positive correlation between the increase in the sonographic ONSD and raised ICP. In human being, ONSD is used in emergency room to distinguish increase of ICP; it is considered a rapid, safe, noninvasive and bedside procedure. Recently, analogous applications have been proposed also in dog [1] and horse. To the best knowledge of the authors, there are no studies describing the technique of measuring the ONSD-US nor the normal values of the ONSD in cat. Aims of this study was to measure ONSD in a population of healthy cats through standardized ultrasonographic transpalpebral approach. Healthy, skeletally mature cats were recruited during clinical activities. Brachycephalic cats were excluded. Data on age, weight and sex were collected. Animals were randomly divided in two groups: a) awake; b) anesthetized. ONSD was performed bilaterally in all the cats by two investigators, separately, through a 3-13 MHz linear array ultrasound transducer. The transducer was placed on the upper eyelid in a slightly temporal position. Measurement was performed approximately 3 mm posterior to the retina; a total of 6 measurements for each eye was performed. Data were statistically evaluated. Forty domestic shorthair cats were recruited, 20 were female, mean age 3.6 y (range 1–8 years), mean weight 3.55 (range 2-6.7 kg). Twenty-eight cats were sedated (16 females and 12 males). ONSD-US has been identified and measured in all the cats. Excellent intra-and inter-observer agreement was observed. When considering all the cats, ONSD ranged between 1.1-1.4 mm; mean±standard deviation (SD) was 1.26±0.09, 1.25±0.08 for right and left respectively. No significant difference was found between left and right ONSD. Any significant correlation was registered between ONSD and age, sex or weight. When considering the two groups, mean±SD was 1.25±0.09, 1.26±0.08 mm in group a) and b) respectively; no significant difference was found among groups. The measurement of ONSD through transpalpebral approach requires practice to accurately identify the optic nerve and obtain a longitudinal image at the maximum diameter. Color Doppler may be useful for identifying neighboring vascular structures. Future studies are warranted to determine normal range in feline population and its correlation with raise in ICP.

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P91 - A CASE OF PROSTATIC LEIOMYOSARCOMA IN A DOG

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Prostatic tumors are relatively uncommon in dogs [1]. Most common are adenocarcinoma, while sarcoma of the prostate (fibrosarcoma, leiomyosarcoma, osteosarcoma and hemangiosarcoma) are extremely rare (0.1-0.2%) [2]. This case report describes the diagnosis, the surgical treatment and the follow-up of a primary prostatic leiomyosarcoma in a dog. A 12 years old intact male, Labrador retriever, was referred to the Veterinary teaching hospital of Naples with a history of lethargy, stranguria and hematuria from two weeks. Complete blood count (CBC), serum biochemical profile and urinalysis were performed and no clinicopathological alterations were recorded, except for the presence of red blood cells in urine. The neurological and cardiological examination were normal and endocrine disorders were excluded. Physical examination revealed a mass in the caudal abdomen and the rectal examination suggested a large asymmetric prostate. The ultrasonography and the computed tomography (TC) confirmed the presence of a large mass in the left prostatic lobe. A TC scan of chest was also performed to rule out metastatic lesions. A celiotomic approach allowed the excision of the left prostatic lobe. Intraoperative complication was a urethral lesion, that was reconstructed by an interrupted suture pattern in PDS 4-0. No bleeding or urine leakage were noticed after the procedure and the abdomen was reconstructed in routine fashion. A Foley probe, placed during surgery, was let in place to enhance the urethra healing for 17 days after surgery. No complication was noticed at the weekly follow-up. Twenty days after surgery, the micturition was considered normal and the owner didn't report about stranguria or hematuria after probe retraction. The histological exam of the mass showed a proliferation of spindle cells arranged in interlacing fascicles and the immunohistochemical staining revealed that cells were immunoreactive for smooth muscle actin, confirming a prostatic leiomyosarcoma. The dog survived for 11 months after the surgery and he died from causes unrelated to the tumor. Leiomyosarcoma is a rare neoplasia; in literature only 3 cases are described but no one of that underwent surgical treatment [2,3]. This is the first case, to our knowledge, with a 11 months follow-up. Prostatic neoplasia carries a poor prognosis in dogs because of its aggressive local invasion and high rate of metastasis [3], for this reason it is important to obtain early identification of prostatic neoplasia in order to give a chance to the surgical treatment.

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P92 - ANASTOMOSIS OF THE PENILE TO THE PRE-PROSTATIC URETHRA AFTER URETHRAL STENT FRACTURE IN A DOG

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Several surgical or interventional tecniques have been proposed in dogs to restore intrapelvic urethral patency in case of benign obstruction, including baloon dilation, stenting, uretrostomy [1]. This report describes a new intrapelvic urethral anastomosis tecnique in a dog with urethral stricture. A 7 y o male castrated Cocker Spaniel was presented with 1-year history of urinary overflow incontinence and inability to voluntary urinate. Six years before the dog had a urethral self-expanding metallic stent placed as treatment of pelvic urethral stricture developed after a previous total prostatectomy and pubectomy because of traumatic pelvic injuries. On physical examination an overdistended urinary bladder and continuous urine dripping were detected. Blood works were within normal limits while urinalisys and urine culture revealed bacteriuria and growth of Staphylococcus spp. Contrast retrograde urethro-cystography indicated a urethral obstruction due to urethral stent rupture and tissue in growth through the stent. An attempt to pass retrogradely a hydrophilic .035" guide wire through the urethra under fluoroscopic guidance failed and surgery was scheduled. A ventral midline caudal abdominal approach was performed exposing the urinary bladder and cranial urethra. Retrograde and antegrade urethral catheterization were performed until the site of the obstruction was encountered. The obstructed urethral tract, containing the stent, extended from 1 cm caudal the trigone to the ischiatic urethra. This tract was isolated, double ligated and excised. The root of the penis was then isolated and both ischiocavernosus and ischiourethralis muscles were transected close to their ischiatic origin. The dorsal penile vessels were blunty dissected from the underling penile tissue preserving their integrity, the retractor penis muscle was transected and the remaining penile attachments freed from the ischium. The mobilized penis and the related urethra were tunnelized into the abdominal cavity, through the abdominal wall muscles, at inguinal area. An intrapelvic direct end-to-end tension-free apposition urethral anastomosis was performed. Urine diversion was obtained with both urethral and cystostomy catheters. The urethral catheter was removed after 10 days while the cystostomy one was maintaned for 28 days. No post-operative complications were observed and at 5 months follow-up, the dog remained unable to voluntary urinate but urinary bladder was easily emptied by manual expression. In this case, the length and the localization of the obstructed portion of the urethra prevented direct anastomosis. Prepubic urethrostomy has been recommended in case of insufficient urethral length available but it can be associated with significant postoperative complications [2]. In this dog, the previous pubectomy facilitated the penis transposition and the intrapelvic urethral anastomosis.

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P93 - A CASE OF SEVERE HEAMORRHAGIC CYSTITIS AND PROCTITIS IN A LEOPARD (*PANTHERA PARDUS*)

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Large felids diseases pose particular challenges in clinical diagnosis because of lack of information on species-specific illnesses and their etiopathology. A 14-year-old female leopard (*Panthera pardus*) weighing 55.6 kg was presented to the Veterinary Teaching Hospital of the University of Padova, Italy, after 3 days of progressively severe obtundation, anorexia, and signs of abdominal pain.
The animal was chemically restrained and maintenance of anaesthesia was achieved by volatile anaesthetic agent. At clinical examination, the leopard appeared overweight, dehydrated, and abdominal distension as well as bloody vaginal discharge were noticed. Blood sampled was drown from the femoral vein and an intravenous catheter was inserted into the caudal vein to administer intravenous rehydration therapy. The vaginal smear showed high numbers of neutrophils and bacteria. Haematological alterations showed leukocytosis with neutrofilia, lymphopenia and mild thrombocytosis. Serum biochemistry revealed an increase in creatinine (9.15 mg/dL), urea (540 mg/dL) and phosphorus (22.9 mg/dL). Abdominal ultrasonography showed a significantly enlarged bladder and 1.5 L of urine was collected by catheterization. Urinalysis resulted in marked haemoglobinuria. Extremely dilated bladder due to the high urinary volume prevented to perform an accurate ultrasonography of urogenital and gastroenteric apparatus, thus making difficult to express a diagnostic suspect. Prolonged intensive care and therapeutic long-term management were considered not feasible and the leopard was humanely euthanized. At necropsy, a diffuse and severe haemorrhagic cystitis was revealed while kidneys and ureters showed moderate to severe congestion. Similar findings were also noted on rectal mucosa with multifocal to coalescent pattern. All organs were sampled for further histological examinations, which are currently under assessment; nevertheless clinical presentation shows similarity to a previously described extraintestinal *Escherichia coli* infection in a melanistic leopard [1]. Both cases presented indeed the same hyperacute onset and necropsy results of the urinary tract were common but intestinal involvement was not previously observed. Considering that the prevalence of this condition among feline species is still unknown, this report aims to improve the general knowledge on large felids diseases.

[1] Carvalho et al. Characterization of extraintestinal pathogenic Escherichia coli isolated from captive wild felids with bacteraemia, Journal of Veterinary Diagnostic Investigation, 24: 1014-1016, 2012.

P94 - CLINICOPATHOLOGIC FEATURES OF TWO CASES OF UNUSUAL DACRYOPS IN TWO BRACHYCEPHALIC DOGS

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Cysts involving lacrimal tissue are an uncommon condition reported in humans [1], dogs, cats, horses [2], and in a red-eared slider [3]. The exact pathogenesis of these anomalies remains obscure [1]. In this report, we describe the clinicopathologic features of two cases of unusual dacryops in two brachycephalic dogs. First case. A three-year old male Corso dog was referred with a 1 month history of swelling ventromedial to the left eye associated with ocular pain, blepharospasm, and epiphora. Furthermore, a severe lower and upper eyelids entropion and a deep corneal ulcer were present. B-mode ultrasonography revealed a subcutaneous cyst measuring 25x15 mm, characterized by an anechoic content lined by a thin wall. Left Computed Tomography dacryocystography delineated a normal nasolacrimal structure without any communication with the cystic lesion that was closely adherent to maxillary bone. A viscous and transparent fluid was aspirated from the lesion. Surgical removal of the lesion was performed and correction of entropion was achieved using the Hotz-Celsus modified technique. Eyelids and cornea healed without complications, and there was no recurrence of the cyst 12 months later. Cytology of the cystic fluid showed abundant proteinaceous material with numerous crystals and rare cuboidal epithelial cells. Histopathology revealed a cystic structure with single to double cell-layered, non-ciliated, cuboidal epithelia. Alcian blue stain revealed rare disseminated goblet cells admixed with epithelial cells. The epithelium was strongly Cytokeratin-positive by immunohistochemistry and appeared lined by several layers of smooth muscle actin (SMA)-positive myoepithelial cells. Second case. A 1-year-old male French Bulldog, with a lesion of 3 months duration of the third eyelid of the right eye. The lesion was located on the anterior aspect, beneath the conjunctiva, and appeared pale-pink, smooth and multilobulated. Excision was performed by blunt dissection through the conjunctiva on the palpebral surface of the nictitans. The cyst was filled by clear fluid, and measured 15x7 mm. Recovery was uncomplicated and no recurrence has been noted in the 6 months after cyst removal. Cytology of the cystic fluid, histopathology and immunohistochemistry of the cyst wall showed similar findings to those described for the previous case. We report the first case of canine dacryops associated with unilateral entropion, although in humans these cysts lead to eyelid conformational abnormalities such as entropion [3]. The histological findings in our cases are consistent with previous reports of canine dacryops. Myoepithelial cells are normally present around the ducts of the lacrimal glands in dogs and can be used as a marker for the diagnosis of dacryops [4]. The presence of myoepithelial cells directly beneath the epithelium of dacryops is consistent with previous reports in dogs [4] and horse [5].

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P95 - INTRAVENTRICULAR GLIOMAS IN THE DIFFERENTIAL DIAGNOSIS OF INTRAVENTRICULAR MASSES OF DOGS

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Gliomas are one of the most common primary tumors of brain in both dogs and humans. Although they primarily consist in intra-axial tumors, exceptionally they have been reported as intraventricular tumors (IVTs), mainly as oligodendrogliomas (IVOs)[1,2,3,4].
In this study we report four cases of canine IVTs of suspected glial origin. Cases were from 3 to 7-year-old, female, Bulldog (2), Jack Russell Terrier (1) and Labrador (1) dogs. They consisted in three brains coming from necropsy, and a biopsy sample removed during surgery. In the first three cases, we identified the presence of an intraventricular soft mass, from grayish to brownish in color, in all cases associated with severe shift of the middle line and prominent infiltration of septum pellucidum or periventricular white matter.
Routine histological evaluation was performed with H&E followed by IHC investigation for GFAP, Olig-2, NSE, SYN, CD133, DCX, and Ki-67 on FFPE 5 µm sections. Histologically, sheets of roundish cells supported by fine fibrovascular stroma, and glomeruloid vascular proliferation characterized necropsy cases. The cells had well defined cellular borders, perinuclear halo and round hyperchromatic nucleus. The mitotic index (MI) was from 20 to 46/10 HPF. For the biopsy case, roundish to polygonal neoplastic cells arranged in sheets and cords, showing moderate amount of homogeneous eosinophilic cytoplasm and occasionally perinuclear halo. The MI was 15/10 HPF.
As for IHC all cases showed diffuse nuclear Olig-2 reaction. GFAP-positivity involved <30% of tumor area. SYN- and NSE-immunoreaction was occasionally found only in undifferentiated cells of the biopsy sample. All tumors did not express CD133 while a considerable number of neoplastic cells were DCX positive. Anti Ki-67 immunolabelling was from 13 to 25 positive-cells/10 HPF in necropsy cases and 200 positive cells/10 HPF in the biopsy sample.
All intraventricular tumors were diagnosed as oligodendrogliomas, three of them being anaplastic (high grade) with undifferentiated neuronal precursor cells (DCX+). Considering all the canine IVTs reported in our database from 2008 to 2017, choroid plexus tumors, oligodendrogliomas, and ependymomas accounted for 62%, 25% and 13%, respectively. These results strongly support oligodendroglioma should be included in the differential diagnosis for canine intraventricular mass lesions along with neurocytoma [2]. To date, glial cell precursors of the Subventricular Zone (SVZ) are considered as the most accredited primary source of origin [4]. However, stem cells were not found in our study (CD133-).

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P97 - SERUM METABOLOME CHANGES IN BOVINE PARATUBERCULOSIS INFECTION BY AMBIENT MASS SPECTROMETRY

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Paratuberculosis (Johne's disease, JD) is a chronic enteritis of ruminants caused by Mycobacterium avium subsp. paratuberculosis (MAP). JD is responsible for considerable economic losses to the dairy industry and is spread mainly via fecal shedding. The control of JD is hampered by inadequate diagnostic tools that fail to promptly reveal infected animals (1). The Metabolomic approach is gaining importance in the study of infectious diseases. Mass spectrometry methods demonstrated their capability in quantifying specific metabolic changes in experimental MAP infection (2). On the other hand, Ambient Mass Spectrometry (AMS) allows direct, rapid, and high-throughput analyses with little or no sample pretreatment. These systems are becoming popular in food technology and safety and may be applied in the health area.

Age-cohorts from JD-affected farms were studied over a 3-year time period during which each animal were periodically tested for MAP infection by ELISA and faecal culture/PCR. Sera were stored at -80°C. For this study 25 animals testing positive in faeces and ELISA were selected. Two control animals for each case were designated from sex- and age-matched, MAP-free herds. Each case was also matched to 2 animals from the same herd and sampling time that tested negative along the whole study. After thawing, samples were diluted and deposited in triplicate on a disposable support (DIP Stick). The analysis was performed with DART (IonSense) coupled with HRMS based on Orbitrap technology (Thermo Exactive) applying two extraction protocols, with ethyl acetate and methanol and two settings: positive and negative ionization.

We compared the spectra of the 25 sera from JD-infected animals with both 50 healthy animals from uninfected herds and from cohorts sampled in the same herd. The differential analysis allowed us to identify the two modes with greater discriminating power. The number of identified molecules was high and their molecular weight was determined at the time. This approach allowed us to discriminate sera from JD infected and healthy cattle from both uninfected and infected herds. Metabolites identification and characterization is undergoing. The identification of biomarkers associated with paratuberculosis will allow us to determine the diagnostic potential of these molecules, through a high-capacity and low-cost methodology as DART-HRMS.

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P99 - LYMPHOCYTE SUBPOPULATIONS (CD3/CD20) IN THE ENDOMETRIUM OF SUBFERTILE MARES WITH CHRONIC ENDOMETRITIS

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This study aimed at evaluating the variations induced by chronic endometritis (CE) on endometrial lymphocyte (CD3/CD20) subsets in subfertile mares. CE is a common reaction in response to semen introduction into the uterus, or it follows repeated artificial inseminations (AI) or intrauterine treatments. CE consists in the protraction of an inflammatory condition, characterized by an abnormal pattern of lymphocyte subsets. Endometrial leukocytes have been shown to be involved in the regulation of the immune response during embryo implantation and trophoblast growth.
The interpretative value of immunohistochemical staining of CD3/CD20 of endometrial biopsies in subfertile mares was assessed in 15 animals that had undergone obstetrical examination with cytological and histological uterine sampling. The mares had no clinical signs of endometritis and were negative for uterine cytology [1]. The uterine biopsies, collected using sterilized uterine biopsy forceps (Equivet, Kruuse, Marselv, Denmark), had been classified as belonging to group 2 a/b of the histological classification by Kenney (1986) [2]. Histological observation had been mainly focused on evidence of increased stromal density, mononuclear inflammatory infiltrate dominated by lymphocytes and plasma cells, superficial stromal edema. According to the degree of clinical infertility the mares were divided into Group A) 9 mares that were empty after more than 3 AIs with refrigerated semen and/or had shown abortion or embryo resorption; Group B) 6 mares that were empty after no more than 2 AIs with refrigerated or frozen semen, at the end of the breeding season. Immunohistochemistry was performed on the uterine biopsies by use of CD3 (DAKO, Denmark; AR Tris-EDTA buffer pH 9, dilution 1:50) and CD20 (Thermo Fisher Scientific, U.S.A; AR Tris-EDTA buffer pH 9, dilution 1:800). Positive cells were counted in 4 randomly selected high-power fields (HPF; 400x; 2.37mm^2). Group A samples showed about twice number of CD3 positive cells than Group B samples [80 cells/HPF (range 48/157) vs. 35 cells/HPF (range20-80)], with a prominent periglandular localization. The CD20 positive cell, were almost absent in group B while were in number of 3-4/field in group A. Immunohistochemical staining with CD3/CD20 can help in the interpretation of the Kenney's (1986) category 2 a/b in which our samples fall into. Mares with higher number of CD3 aggregates showed surprisingly higher, although vaccinated, EHV titers (data not shown).

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P100 - CHARACTERIZATION BY SANGER SEQUENCING OF THE CXCR-4 RECEPTOR IN DOG CELL LINES

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C-X-C motif chemokine receptor type 4 (CXCR4) is trans-membrane protein located on the cell surface involved in homing and chemotaxis, in the hematopoietic and immune systems, and capable of directing cell movements according to chemotaxis gradients [1]. The specific ligand of CXCR4 is a stromal cell-derived factor-1 (SDF-1/CXCL12); this molecule is highly expressed in bone marrow, brain, heart, liver, and lung. In addition to cell migration, CXCR4/SDF-1 axis has been demonstrated to participate in physiologic and pathophysiologic processes such as embryogenesis, hematopoiesis, angiogenesis, inflammation, wound repair and most recently tumorigenesis and metastases both in human and animals [1-2]. In particular, recent studies demonstrated the expression of CXCR4 both in animal cancer than in canine cell cultures [2-3]. The important roles of CXCR4 in multiple diseases have encouraged the development of clinically viable CXCR4 antagonists. Many studies employed cell lines for *in vitro* test to evaluate anticancer therapies; however no data are available on CXCR4 expression and sequence in cell lines. This gap makes difficult to test *in vitro* anti-cancer therapy, that have as target the CXCR4 axis. In this conceptual framework, the aim of our study was to investigate the presence of mutations on CXCR4 gene in the canine tumoral cell lines. This work was focused on the biomolecular characterization by Sanger sequencing of CXCR4 gene in cell lines originated from canine neoplastic cells: D17 (osteosarcoma), A72 (soft tissue) and CF33 (mammary cancer). Cells were grown until confluence at 37°C with 5% of CO_2 in BME enriched with 10% (v/v) of fetal calf serum and mixture of antibiotics. DNA was extracted from cellular pellets, each of about 1 million cells. D17 were tested at 302 and 255 passages; A72 were analyzed at 108 and 98 passages; finally CF33 were tested at 58 and 48 passages. Each experiment was repeated three times. DNA yields were quantified and each DNA preparation was amplified by end point PCR. In domestic dogs (*Canis lupus familiaris*) CXCR4 gene is located within chromosome 19 [NC_006601.3 (38874650..38877740, complement)] and is involved in the transduction for the synthesis of a 1129 bp mRNA [DQ182699.1]. Specific primers were selected on a 902 bp segment of a conserved encoding region. Amplicons were sequenced by using internal primers and BigDye Terminator v3.1 kit Sequences alignment (BioEdit software) and following comparison enlighten both the absence of mutations in the CXCR4 gene in different tumor cell lines and its stability over serial passages. Moreover no differences were showed with geneBanck data. Our results suggest the possibility to employed these cell line to evaluate *in vitro* the activity of the antagonist of CXCR4/SDF1 axis.

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P102 - *ASPERGILLUS TERREUS* A RARE PATHOGEN IN DOGS

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Fungi of the *Aspergillus* genus include more than 300 species of saprophytic organisms, ubiquitous in the environment and opportunistic pathogens [1]. Among the species *A. terreus*, *A. fumigatus* and *A. deflectus* are the most frequent involved in disseminated aspergillosis (DA) [1,3]. DA affects primarily German Shepherd dogs and it has been suggested that hereditary immune defect might exist [1,2]. In September 2018, a 2 years-old female crossbreed dog was presented with a joint pain at the left forelimb. Corticosteroid treatment was administered. A month later the dog showed right vestibular abnormalities due to otitis and an antibiotic therapy was administered for 3 weeks. On January, vision impairment at the right eye, due to glaucoma and retinal detachment, was observed. By Computerized Tomography (CT) severe multifocal diskospondylitis with myelopathy and monolateral uveitis were detected. Urinary sediment analysis and culture were negative. Furthermore, invasive diagnostic steps, including ocular globe enucleation were neglected. Due to progressive worsening of clinical conditions, the dog was euthanized on March. At necropsy bilateral ophtalmitis, multifocal hepatic necrosis and severe purulent pyelonephritis and cystitis were observed. Histologically multiple granulomatous lesions, containing fungal hyphae were detected in pancreas, liver, kidney, and meninges. Free hyphae were also present in the ocular chambers, CNS, within lung vessels and in the ureter. *A. terreus* was molecularly identified from tissue samples and a diagnosis of DA was done.

In animals various risk factors have been suggested such as unhygienic management, trauma or suspected immunological deficiencies [2]. In our case we supposed that the joint injury and the corticosteroid treatment may have contributed to the pathogen's spreading. Since clinical signs are often non-specific and could mimic other infectious diseases, diagnosis of DA is often made after prolonged periods of therapeutic attempts, with advanced dissemination by the time the correct etiology is identified [4]. A differential diagnosis of systemic aspergillosis should be considered in young to middle-aged dogs with non-specific clinical signs including diskospondylitis, ocular abnormalities and/or neurologic signs.

The identification of the fungal species is strongly recommended to better define the prognosis and to choose the appropriate therapy, based on the differences in drugs susceptibility among the species.

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P103 - GONADAL TUMORS IN KOI CARP (*CYPRINUS CARPIO KOI*): IMMUNOHISTOCHEMICAL APPROACH THROUGH TISSUE MICROARRAY TECHNIQUE

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Due to a long life span and a high economical and emotional value koi carp (*Cyprinus carpio koi)* is considered as a pet worldwide [1]. In this species descriptions of gonadal tumors exist since years [2], however their occurrence only recently represents a regular finding [1]. Given the difficulty to fit into histological classification and to assess the incidence of these tumours in koi populations, the present work aims to describe and classify gonadal tumours by using an immunohistochemical tissue microarray (TMA) technique. Seventeen cases of koi gonadal tumors collected in Belgium by a veterinary practitioner from 2014 to 2015 were included. They were evaluated according to the veterinary WHO classification [3] and characterized with an antibody panel (vimentin, CD117, AE1/AE3 cytokeratin, E-cadherin) by using TMA technique. The koi carps were held in private ponds, their age ranged between 3 and 12 years and they were mostly females. The affected fish exhibited abdominal enlargement, lethargy and anorexia. Grossly, the neoplasms were white-yellowish with soft cystic areas and multifocal haemorrhages. Twelve tumors were histologically diagnosed as sex cord-stromal tumors (SCST), three were mixed germ cell sex cord-stromal tumors (MGCSCST), two were germ cell tumors (GCT), and one was a carcinoma. AE1/AE3 cytokeratin strongly labelled the carcinoma and several SCST, but rarely GCT; E-cadherin strongly labelled almost all SCST, MGCSCST and GCT. Vimentin and CD117 were only expressed in one SCST and one MGCSCST, respectively. Our results indicate sex cord-stromal cells as the main cells of origin in accordance with a recent paper [1]. The diagnosis of gonadal tumours in fish as in mammals is still difficult due to the variety of histological patterns of SCST and the occurrence of GCT and SCST that mimic epithelial gonadal tumours [3,4]. Furthermore, none of the immunohistochemical markers can be considered specific for a tumor type and the use of a wide panel of antibodies is suggested [4,5]. Nonetheless, the antibodies here tested confirmed to be immunoreactive in fish tissues [6] and therefore useful for refining the histological diagnosis.

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P104 - PATHOLOGICAL FINDINGS IN SEA TURTLES STRANDED IN NORTH -WESTERN ADRIATIC SEA (VENETO COASTLINE) BETWEEN 2013 AND 2018

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The Northern Adriatic Sea is one of the most critical areas in the Mediterranean for human activity and sea turtles interaction, due to high population density, high fishery effort and commercial and touristic marine traffic [1]. In this area, only a few studies have focused on post-mortem analysis of sea turtles and the link with human interaction [2,3]. Between 2013 and 2018, 407 sea turtles were collected along the Veneto coast. They stranded dead or died during recovery in rescue centers. For all animals, anamnestic and morphometric data were recorded and post-mortem examinations were performed by veterinarians of the University of Padua, according to standard protocols [4]. Appropriate samples were collected, depending on carcasses conservation status [4], while all evidence of human interaction, including lesions attributable to boat strikes or bycatch, and the presence of marine litter in the gastrointestinal tract, were registered for animals in every decomposition code. All animals were loggerhead sea turtles (*Caretta caretta*), with the exception of one leatherback sea turtle (*Dermochelys coriacea*). Twenty-seven percent (n=109) of the carcasses had a decomposition code between good and moderate, making them at least histologically evaluable. Multifocal or diffused edematous and/or hemorrhagic lesions were found in the majority of evaluable animals (75%, n=95): this is most likely attributable to endotoxic or septicemic mechanisms, even if due to decomposition code only in a small number of sea turtles it was confirmed by bacteriological analysis. 1% (n=5) of all specimens presented lines or hooks in the gastrointestinal tract and 4% (n=17) showed carapace or plastron lesions attributable to ship strikes. One percent presented plastic in the gastrointestinal tract. No signs attributable to bycatch were found. For 12% of evaluable sea turtles (n=15), it was not possible to determine the cause of death. Innovative forensic approaches are needed to assess the human impact on this species in all decomposition statuses. For this reason the animals are being tested for the presence of diatom algae in bone marrow, to diagnose death by drowning and to contribute to the assessment of bycatch-linked death. It's important to point out that carcasses floating at sea are subsequently subjected to winds and currents, therefore the stranding site doesn't directly correspond to the marine area where the animal died. Carcasses drifting studies are necessary to improve the understanding of hotspots for humans-sea turtle interactions.

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P105 - INTERPLAY BETWEEN E-CADHERIN EXPRESSION, NEOPLASTIC CELL PIGMENTATION AND OUTCOME: A PRELIMINARY INVESTIGATION IN CANINE MELANOCYTIC TUMOURS

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E-cadherin, a glycoprotein involved in cell-cell adhesion, has a pivotal role in epithelial-mesenchymal transition, a process through which cells get an invasive phenotype [1]. While in human cutaneous melanomas, a decrease of E-cadherin expression is associated to a shorter survival [2], in dogs its role is poorly understood [3,4]. Pigmentation is considered a positive prognostic feature in canine melanocytic tumours. Recently, the role of E-cadherin in melanin transfer from normal melanocytes to keratinocytes was highlighted: the loss of its expression lead to an accumulation of melanosomes within melanocyte dendrites [5]. However, whether E-cadherin function is retained in neoplastic melanocytes has to be determined. Our aim was to investigate the linkage between E-cadherin expression, pigmentation and outcome. We evaluated E-cadherin expression by immunohistochemistry in 81 formalin-fixed, paraffin-embedded primary canine oral and cutaneous melanocytic tumours. The expression was assessed as present or absent, depending on both percentage of neoplastic E-cadherin positive neoplastic cells and staining intensity [6]. E-cadherin expression was more frequently present in cutaneous melanocytomas than in cutaneous melanomas ($P<0.05$). The absence of expression was associated both to tumor-related death and to presence of recurrence/metastasis ($P<0.05$), suggesting its usefulness in prognosis assessment. E-cadherin expression was also associated to pigmentation: as the protein is involved in melanin transfer, a decreased pigmentation would have been presumed in cells with high E-cadherin expression [5]. Unexpectedly, it was more often present in highly pigmented tumours. Although further focused studies are needed, this finding support the hypothesis of an alteration of functionality of E-cadherin in tumour cells. Finally, we showed that amelanotic tumours with absent E-cadherin expression had more frequently an unfavourable clinical outcome, while pigmented tumours with present E-cadherin expression do not ($P<0.05$). Our preliminary results reveal the potentiality of E-cadherin as prognostic marker in canine melanocytic tumours, especially when considered together with pigmentation, and would be worth of further validation.

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P106 - ISOLATION AND CHARACTERIZATION OF NORMAL AND NEOPLASTIC CANINE MELANOCYTES

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Melanoma is one of the most aggressive neoplasia in dogs and humans. Different animal models largely failed to predict the human response due to differences in skin physiology and immunity [1]. Moreover, international guidelines recommend replacing the use of animals for experiments with alternative methods [1]. We aimed to obtain cell cultures of normal and neoplastic melanocytes in order to compare their morphologic and molecular characteristics and eventually point out the differences. When possible, neoplastic and normal melanocytes were selected from the same animal. We isolated neoplastic melanocytes from five dogs (1 culture derived from melanocytoma, 3 from oral melanomas and 1 from metastatic oral melanoma). From the same dogs, we obtained 2 normal melanocyte cultures from oral mucosa and 2 from epidermis. The normal mucosa and the epidermis were digested with dispase II, while neoplastic samples were treated with collagenase. Obtained normal cells were cultured respectively in medium M254 supplemented with HMSG (human melanocyte growth supplement) and cholera toxin for normal melanocytes and in medium DMEM/F12 supplemented with EGF (epithelial growth factor), insulin, hydrocortisone, cholera toxin and horse serum for neoplastic cells. Primary culture of melanocytic tumors were characterized using histochemistry (H-E; DOPA reaction) and immunohistochemistry (Melan-A, PNL-2, S-100). Primarily spindle-shaped cells and multi-dendritic cells with occasional ovoidal cells, with centrally located oval nucleus and moderate to abundant cytoplasm, frequently containing multiple granules, composed primary cultures from normal and neoplastic melanocytes. Normal melanocytes were constantly associate with keratinocytes proliferation, which, in a few days, accounted for most of the cells present in culture. Neoplastic melanocytes resulted positive to DOPA reaction and Melan-A, PNL-2 and S-100 immunostaining. The canine model of melanoma plays an increasing role in comparative oncology. In human medicine melanoma cell lines models are widely used to study the cancer biology and efficacy of potential therapeutics [3]. In veterinary medicine, still few studies describe the application of canine melanoma cell lines to evaluate biological and molecular aspects of canine melanoma [2] and none, to our knowledge, using the normal melanocytes of the same animal to compare differences. This study represent a preliminary step to realize canine cell lines to investigate molecular pathways involved in the pathogenesis of canine melanocytic tumors and, as a future step, to develop canine melanoma 3D models.

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P107 – MOLECULAR AND HISTOPATHOLOGICAL STUDY OF MASS MORTALITY IN PACIFIC OYSTERS, *CRASSOSTREA GIGAS*, IN SARDINIA

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Farming of Pacific Oysters (*Crassostrea gigas*) represents a promising aquaculture sector with an estimate production of about 5,600,000 tons/2016 (FAO, 2018). Over the last 5 decades loss of Pacific Oysters due to physiological and environmental factors occurred globally with heavy impact on mollusks aquaculture [1]. Moreover, specific pathogens belonging to the genus *Vibrio*, above all *Vibrio aestuarianus*, were suspected as causative agents of these mortality episodes [2,3]. Affected oysters showed weakness of the adductor muscle and a moribund appearance [1].

Limited histological descriptions associated to *V. aestuarianus* have been reported in oysters, mainly consisting of necrosis of the mantle subepithelial connective tissue, atrophy of the digestive diverticula, and hemocytes infiltration in the hemolymphatic vessels [4].

Aim of this study was to investigate the presence of *Vibrio aestuarianus* in 78 oyster samples during a mass mortality episode in the main Sardinian oyster's aquaculture site (San Teodoro's lagoon) by molecular and histological techniques.

Polymerase chain reaction (PCR) and real time PCR were performed using in-house-designed *V. aestuarianus* primers (VesTox F and VesTox R) on gill's and mantle's tissues. Histological sections of PCR positive oyster samples were stained in hematoxylin and eosin (H&E) and evaluated by light microscopy. *V. aestuarianus* have been detected in 23 out of 78 samples (29%) by PCR, with bacterial concentrations ranging from $5x10^2$ copies/µl to $7x10^6$ copies/µl. Histologically, 18 out of 23 samples (78%) showed a moderate to severe inflammatory reaction mainly located in the gills (78%), in the mantle (100%) and in haemolymph vessels (33%) and characterized by nodular aggregates of haemocytes.

This study is consistent with the hypothesis that *V. aestuarianus* caused severe inflammatory lesions in different tissues and is responsible for mortality episodes in Sardinian oysters.

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P108 - A CASE OF EQUINE DYSAUTONOMIA

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Equine dysautonomia (ED, also known as equine grass sickness) is a polyneuropathy affecting both the central and peripheral nervous systems of, almost exclusively, grazing horses. It has been detected mostly in Northern Europe and South America (1). Enteric nervous system is the more consistently and severely affected. The severity of disease and gross pathologic findings can largely be attributed to the extent of enteric neuronal loss. It has been hypothesized the potential role of either *Clostridium botulinum* neurotoxins or ingested pasture derived mycotoxins (2).

Ten years old female quarter horse was referred for recurrent oesophageal obstruction, anorexia, nasal discharge (alimentary), dyspnea, in the previous month. Upper GI endoscopy revealed oesphageal dilation and atony of cranial-median-caudal portions, associated to severe mucosal ulceration. Ecography revealed interstitial pneumonia compatible with *ab-ingestis* pneumonia.

Due to the poor condition, the animal was humanly euthanized. Necropsy was performed.

Gross pathology revealed numerous severe linear ulceration of the esophagus mucosa, fluid distension of the stomach and small intestine, firm corrugated impactions of the large colon and cecum, with a black coating on the surface of the firm ingesta were observed. Fibrino-purulent pneumonia, pleuritis and dilated cardiomyopathy were observed. During necropsy samples of intestine, mesenteric ganglia and all other organs were collected, formalin fixed in 10% neutral-buffered formalin, paraffine embedded , processed and stained with haematoxylin & eosin, PAS, Toluidin blue, Von Kossa.

Histologically, oesophageal mucosa was extensively ulcerated and the muscular layer was severely degenerated. Axonal degeneration was also observed. The small intestine showed muscular layer severe degeneration. Submucous plexus and myenteric plexus showed a reduced number of enteric neurons, affected by extensive central chromatolysis, eccentricity or pyknosis of the nuclei, neuronal cytoplasm swelling and vacuolation, accumulation of intracytoplasmic eosinophilic spheroids. The myenteric plexus of caecum, large colon, small colon showed marked neuronal loss. Frequently medium and small arteries showed muscular layer hypertrophy and intimal mineralization.

ED is a multisystemic neuropathy with an extremely high mortality rate and significant welfare, emotional, and financial consequences (4). Nowadays, histopathological examination of myenteric plexus is still the golden standard for ED diagnosis.

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P109 - SQUAMOUS CELL CARCINOMA OF THE EPIGLOTTIS AND LARYNGEAL MUCOSA IN A HORSE

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Squamous cell carcinoma (SCC) is the most frequent tumor of mucocutaneos junctions in horse (1,3), rarely reported in mucous membrane of larynx, oropharyngeal, esophagus and non-glandular stomach (2).
A 17 year old female, 550 kg, Maremmano Horse was referred for post-mortem examination. Clinical signs, during the previous 5 days, were severe dyspnea, anorexia and anxiety. An endoscopy transnasal/transoropharyngeal investigation of upper respiratory tract was performed. Due to the poor condition, the horse was humanly euthanized.
Endoscopy showed 70% reduction of laryngeal lumen, due to a dorso-cranial anatomical alteration and position of the epiglottis. The epiglottis showed a firm, cauliflower-like, neoformations and smaller multi-lobulated nodules on the oropharynx. Endoscopic lesions were confirmed during necropsy. Gross pathology showed a white multilobulated firm mass, partially ulcerated, with irregular margins and central areas of necrosis at the base of the larynx, locally expansive to epiglottis and surrounding tissues. Lymphoadenomegaly was recognized in the regional nodes. Tissue samples collected from epiglottis, larynx, lungs and regional lymph-nodes were formalin fixed, paraffin embedded and 5µm thick sections were prepared for routine histology and for ABC-immunohistochemistry (IHC) for cytokeratin AE1/AE3, Vimentin, CD3, CD79α, foxp3.
Histologically, non-encapsulated islands of anastomosing bands of neoplastic epithelial cells were separated by and had invaded the abundant surrounding collagenous mature connective tissue of submucosa of epiglottis, larynx and amigdala. Clusters of lymphocytes surrounded and lightly infiltrated the neoplastic cells. Epithelial cells were large, polygonal/round, with abundant eosinophilic/amphophilic cytoplasm containing large vesicular nucleus. Small clusters of neoplastic epithelial cells were also found in lymph and blood vessels. Cranial cervical lymph nodes showed activated follicles and scattered micrometastasis in paracortex area.
IHC revealed strong positivity for cytokeratin on neoplastic cells and for vimentin on fibroblasts of connective tissue. CD3+ cells were present in tumor periphery and scattered inside the neoplasia, while CD79α+ cells were less numerous. Foxp3+ cells were present in most of the infiltrates.
Few laryngeal SCC have been previuosly described in the horse. The immunological response to SCC in the horse is still not well investigated yet, but according to other authors (4), CD3+ cells predominate in the cellular infiltrate in the periphery and inside of the tumor, but this seems to be uneffective against tumor spread or local expansion. The presence of abundant stromal cells such as fibroblasts and foxp3+ cells within tumors cells may suppress immunological response to tumor enhancing its growth.

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P110 - TRANSFERRIN RECEPTOR 1 (TFR1) IN FELINE MAMMARY TUMORS, PRELIMINARY IMMUNOHYSTOCHEMISTRY RESULTS AND SCORES EVALUATION

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The Transferrin receptor (TFR) 1 is one of the most interesting receptor for future cancer therapies in veterinary medicine . TFR1 is a transmembrane protein whose goal is to transport inside the cell the iron that's needed. In human medicine it has already been demonstrated that the expression of this receptor is more abundant in some types of cancers compared to the correspective healthy tissues. The same has been demonstrated for some dog's cancers(1). There is lack of information about TfR1 expression in feline mammary cancer. Most of the feline mammary tumors are very similar from a molecular point of view to human triple negative breast cancer (2).
The aim of the study was to test the TFR1 expression by immunohistochemistry (IHC) in feline mammary tumors and to test different scoring systems to find the the best score to evaluate the IHC results. Twenty eight samples of mammary tissue were analysed including: 7 samples of healthy mammary tissue; 7 samples of non metastatic mammary carcinoma; 7 samples of metastatic carcinoma; 7 samples of lymph node metastasis. Cell count for number of positive cells and intensity of staining, counting 100 cells per 10 HPF for every sample was performed. Commonly used scoring systems (Allred, H score, Quick scrore) were assesed. Statistical analysis was performed using the software Prism 8. There was a statistically significant difference in the expression of TfR1 from healthy mammary tissue to neoplastic mammary tissue and there was also an augmented expression between lymph node metastasis and healthy tissue, moreover there was a trend of increased TFR1 expression from non-metastatic to metastatic primary tumors. The best IHC score was the H score. The IHC analysis showed how TfR1 expression in feline mammary cells was both citoplasmatic and membranous and that there were different intensities of staining between healthy and neoplastic tissue but also within the same sample in different areas indicating the need for standardization in evaluation.
The other scores examinated (Quick score, Allred score) were not ideal to describe this heterogeneity , because they're specific for nuclear staining and for samples with omogeneous staining of the cells (3). In conclusion, we evalutaed IHC expression of TFR1 in feline mammary tissue for the first time and assessed the best scoring system for standardization.

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P111 - TIME DEPENDENT IMMUNOHISTOCHEMICAL EXPRESSION OF NEUROKININ-A (NKA) AND INTERLEUKIN-8 (IL-8) IN THE BRONCHIAL EPITELIUM OF HORSES WITH RECURRENT AIRWAY OBSTRUCTION (RAO)

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Severe equine asthma (also known as heaves or RAO) is a chronic obstructive disease characterized by exaggerated contraction, inflammation, and structural alteration of the airways, when susceptible horses are stabled and fed hay [1]. Currently, the diagnosis of equine asthma relies on history, clinical signs and on the presence of lower airway inflammation as detected by bronchoalveolar lavage fluid (BALF) cytology [1,2]. However, little is known on the relationship between the degree and type of BALF inflammation, the severity of the disease, and the response to therapy [2]. Literature data concerning endoscopically bioptic samples [3] as well as eventual differences related to the site of biopsy (proximal vs distal bronchus) are limited and almost all focused on biomolecular investigations of gene expression of several inflammatory markers [3,4,5]. In order to investigate the possible correlation between tissue expression of two inflammatory markers (NKA and IL-8) during the acute exacerbation of RAO, a histological and immunohistochemical study was carried out on a series of samples collected by bronchoscopy from five healthy horse and six horses with RAO (in asymptomatic phase) subjected to environmental factors (exacerbation) and then to pharmacological treatment (remission). The results showed in all horses no significant difference between the four different sampling sites, both for histology and IHC evaluation at every experimental point (before and during exacerbation, and at remission). The application of the histological biopsy scoring system [3] revealed only a significantly difference between the control horses and the horses with RAO (P=0.008), but in RAO cases no significant difference was present between exacerbation and remission period. For IHC, only the intensity of NKA positivity increases significantly between healthy horses and the RAO horses at remission (P=0.04). Based on these results we can affirm that: 1) the endoscopic bronchial biopsy generates reliable and homogeneous samples in the same bronchial three; 2) the clinical improvement associated with RAO therapy does not seem to correspond to a decrease in the histological biopsy score and to a significant change of IL-8 immunoreactivity; 3) NKA immunopositivity, on the other hand, appears to increase significantly after treatment with corticosteroids, showing an opposite trend to the expected result. Further studies are necessary to implement both the number of samples and to use other markers of inflammation to characterize the potential role of cytokines in the diagnosis and therapeutic approach of severe equine asthma.

The study was approved by Ethical Committee of the University of Bologna

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P112 - ROLE OF THE CARNITINE SYSTEM AND FATTY ACID OXIDATON IN CANINE MAMMARY TUMORS

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Fatty Acid Oxidation (FAO) sustains malignant phenotype by generating large amounts of ATP necessary for neoplastic cells survival and growth.Carnitine Palmitoyl Transferase System (CPTS) plays a role by facilitating the transport of long chain fatty acids from the cytosol to the mitochondria for oxidation through the combined action of Carnitine-Palmitoyl transferase 1 (CPT1), Carnitine acylcarnitine translocase (CACT), Carnitine-Palmitoyl transferase 2 (CPT2) and Carinitine O-acetyltransferase (CrAT). CPT1 converts Acyl-CoA into acylcarnitine, CACT exchanges acylcarnitine and carnitine between outer and inner mitochondrial membrane, CPT2 converted back acylcarnitine in Acyl-CoAs for oxidation (1). 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.This study investigated the role played by CPTS in canine mammary tumours, and focused on the CACT and CPT2 expression evaluated by immunohistochemistry and Western Blot analysis. It was conducted on spontaneous tumours surgically excised as routine diagnosis and treatment, according to Directives 2010/63/EU and 2010/63/EU. 26 samples of canine mammary neoplasm and 5 normal mammary gland tissues were classified according to Goldschmidt criteria and divided into grade 1 (7), grade 2 (10) and grade 3 (9), applying parameters proposed by Pẽna. Immunoreactivity was scored considering the number of positive cells in 10 HPF (grade 0: no positive cells, 1: <10%; 2:10–30%; 3:31–60%; 4:> 60%) and the intensity of staining graded as weak (1), moderate (2), and strong (3).Then, a combined immunoreactivity score (IRS), ranging from 1 to 12, was calculated for each specimen by multiplying the values of these two categories. One-way ANOVA was used to compare differences between groups. Results with $p< 0.05$ were considered statistically significant. Both CACT and CPT2 expression were found in all tissue types: normal mammary gland (3/5,60% IRS=3 range 0-7) G1 carcinomas (7/7, 100%; IRS=7 range 3-12), G2 carcinomas (100%;IRS=5 range 1-8), G3 carcinomas (4/9,44% IRS=1 range 0-3). Western blot analysis confirmed the cross-reactivity of the anti-human CACT and anti-human CPT2 antibodies in canine mammary gland. Both CACT and CPT2 increased in tumours compared to normal mammary gland ($p<0.001$). G3 carcinomas showed lower IRS compared to G1 carcinomas ($p<0.005$). Our results confirm those obtained in previous evaluations of CPT1A and CRAT suggesting that FAO plays a role in well differentiated tumours in which neoplastic cells can use of neighboring fatty acid to survive and proliferate. On the contrary, in less differentiated tumours, the FAO is attenuated and fatty acids could be shunted from β oxidation to other lipid metabolic pathways, such as lipogenesis. In this way malignant neoplastic cells could use fatty acid as energy substrates necessary for maintaining cell and mitochondrial membranes integrity and for the synthesis of other oncogenic lipids.

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P113 - HYPOXIA-INDUCIBLE FACTOR-1 α (HIF1α) EXPRESSION IN BPV-POSITIVE EQUINE SARCOIDS

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Equine sarcoids are the most common fibroblastic skin tumor in equidae worldwide [1,2], which locally invade and rarely regress [3]. Delta Bovine Papillomaviruses (BPV-1,BPV2, BPV13) are widely recognized as the causative agents of equine sarcoid [3]. We have recently shown that VEGF was strongly expressed in equine sarcoids and that the increased number of vessels was not associated with their complete maturation, possibly leading to a hypoxic condition[4]. Hypoxic tumor cells counteract these low oxygen conditions by activating HIF1α pathway [5]. In this regard the aim of this study was to evaluate, by immunohistochemistry and Western bloting (WB), the expression level of HIF1α in BPV positive equine sarcoids (25) and to compare results with normal skin (5). All normal skins showed weak cytoplasmatic immunostaing for HIF1α in the epidermis, while normal fibroblasts were negative. Fifty-sex percent of equine sarcoids showed strong and finely granular cytoplasmatic staining for HIF1α in neoplastic fibroblasts and keratynocites. Forty-four percent of equine sarcoids showed a moderate cytoplasmatic staining for HIF1α in neoplastic fibroblasts and keratynocites. Results of WB confirmed the specificity of the antibody (~130 kDa), and showed that HIF1α was overexpressed in sarcoid samples compared to normal skin. Concluding, our results showed that HIF1α was overexpressed in BPV positive equine sarcoids, with a prevalent cytoplasmic expression. Abnormal upregulation and accumulation of HIF-1α in the cytoplasm have been described in a broad spectrum of tumors [6]. In normal oxygen condition HIF1α is rapidly degraded by prolyl hydroxylases (PHDs), while in hypoxic condition PHDs are inhibited, leading to HIF1α accumulation and translocation into the nucleus [5]. The shuttling of HIF1α between cytoplasm and nucleus is a complex process involving several members of the nuclear transport receptor family, which can be regulated by numerous factors [5,6]. Among these factors, BPV could plays a relevant role in regulation of HIF1α pathway, contributing to the development of equine sarcoids by promoting HIF1α/VEGF mediated tumor angiogenesis.

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P114 - EFFECTS OF TERT INHIBITOR BIBR1532 IN PRE-CLINICAL MODELS OF FELINE ORAL SQUAMOUS CELL CARCINOMA

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Telomerase reverse transcriptase (TERT) is an enzyme which mantains telomeres length by adding nucleotides hexamers, thus inhibiting cellular senescence, resulting in persistent cell proliferation. Additionally, TERT exerts extra-telomeric functions, such as inhibition of apoptosis and enhancing of cell motility and invasiveness [1]. Expression and activity of TERT is crucial for carcinogenesis, therefore inhibition of TERT is a promising therapeutic tool in several pre-clinical models of cancer, including human oral squamous cell carcinoma (SCC) [2]. However, little is known regarding TERT expression and its possible role in feline oral SCC (FOSCC).

The aim of this study was to assess expression of TERT and the effects of the specific inhibitor BIBR1532 on TERT expression, cell growth, cell viability, expression of apoptosis-related genes and matrix-metalloproteases (MMP)-1, -2 and -9 in two FOSCC cell lines (SCCF2 and SCCF3). Cells were treated for 48h with BIBR1532 at 25, 50, 100uM and DMSO as control, harvested for cell count, RNA and protein extraction and analysed by Real-time qPCR and Western blotting (WB).

WB and qPCR revealed variable expression levels of TERT, MMP-1, -2 and-9 in SCCF2 and SCCF3. BIBR1532 treatment caused a decrease of TERT protein and gene expression in a dose dependent manner, along with cell growth inhibition and decrease in cell viability in both cell lines. Consistently, expression of the cell cycle inhibitor gene p21 as well as the ratio between the pro-apoptotic gene Bax and anti-apoptotic gene Bcl-2 were increased in favour of cell cycle arrest and apoptosis with a dose-dependent trend. Moreover, WB analysis revealed a dose-dependent decrease of MMP-1, -2 and -9 protein expression in treated cells.

FOSCC is the most common oral cancer in cats, for which no 100% efficient therapy is available so far, often leading to euthanasia [3]. FOSCC is considered a spontaneous animal model of human oral SCC, where TERT is a promising target for cancer inhibition [2,3]. BIBR1532 has been employed in pre-clinical studies for different types of tumours, showing potent anti-cancer activities [5]. In this work, we demonstrate that TERT is expressed in FOSCC-derived cell lines and treatment by BIBR1532 affects its expression, cell proliferation, cell survival and might influence the invasive phenotype by down-regulating MMPs, suggesting that TERT inhibition can be considered as a potential therapeutic strategy for FOSCC. Therefore, this study hires relevance in the fields of both veterinary and comparative oncology.

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P 116 - PREVALENCE OF NEOPLASMS IN CAPTIVE WILD FELIDS OF CAMPANIA ZOOS

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Neoplastic diseases are an important cause of morbidity and mortality in several wildlife species [1]. Different types of tumors have been described in captive wild felids, most of which are reported as single cases. However, only few reports documented the tumor prevalence in zoo felids [2]. In this study, we describe the types and the prevalence of tumors in wild felids of Campania zoological gardens died in the period 2008-2018. Data from 19 captive wild felids submitted for necropsy were reviewed retrospectively. The study included 8 tigers (*Panthera tigris*), 6 leopards (*Panthera pardus*), 4 lions (*Panthera leo*), and 1 black jaguar (*Panthera onca*) comprising 7 males and 12 females with age ranging from 7 to 19 years (mean age: 16,6 years). Representative tissue samples were collected, fixed in 10% neutral buffered formalin and routinely processed for histological examination. The species, the sex, the age, the type of neoplasia and the evidence of metastases were documented for each animal and evaluated with t test and binomial test. Tumors were diagnosed in 11 of 19 animals (68.75%), 4/11 males (36,36%) and 7/11 females (63,64%). No sex predisposition was observed. Neoplasms were observed in 5 tigers (45.45%), 3 leopards (27.27%), 2 lions (18.18%) and in one black jaguar (9.09%). The animals with cancer were older than animals without neoplasms (mean age 17.36 vs 13.6 years) ($P<0.05$). Based on histopathological features, tumors were diagnosed as follows: two cholangiocarcinoma, two hepatic hemangiosarcoma, a renal adenocarcinoma, a uterine leiomyoma, an adrenal gland adenoma, an esophageal leiomyosarcoma, a thyroid carcinoma, an oral squamous cell carcinoma and an osteoma spongiosum. The liver was the most common site of primary tumor (4 of 11 cases, 36.36%). No difference of prevalence between epithelial (54.55%) and mesenchymal (45.45%) tumors was found. Eight tumors (72.73%) were malignant and 3 (27.27%) benign. Among the 8 malignant neoplasms, no metastases were observed in 3 cases (37.5%); metastases involved only regional lymph nodes in one case (12.5%) and distant metastases were found in 4 cases (50%). This study provides an overview on spontaneous tumors occurring in captive wild felids held in Campania zoological gardens. Based on our findings, cholangiocarcinoma and hemangiosarcoma were the most common tumors identified and the liver was the most common primary tumor site in captive wild felids. In conclusion, the high rates of malignant and widely metastatic neoplasms observed is in agreement with previous reports [2] and suggest that age may be at least one of the cancer risk factors in these animals. The zoo represents a good epidemiological observatory for neoplastic diseases in captive wild animals, and further studies are required to identify other factors involved in tumors development.

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P117 - APPLICATION OF A NOVEL IMMUNOHISTOCHEMICAL PANEL FOR THE DIFFERENTIAL DIAGNOSIS OF CANINE STERILE GRANULOMAS

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Canine Sterile Granuloma encompasses Cutaneous Sterile Granuloma/Pyogranuloma Syndrome (SGPS) and Reactive Histiocytosis (RH), two uncommon cutaneous disorders of dogs that may show similar clinical and histopathological features [1]. SGPS is characterized by an inflammatory infiltrate consisting mostly of macrophages intermingled with neutrophils, lymphocytes and plasma cells; RH is characterized by nodular to diffuse infiltration of dermal dendritic cells that accumulate in tumor-like configurations. In the last stage of RH, the increased number of macrophages can make a differential diagnosis with SGPS difficult [1]. In these cases, immunohistochemistry (IHC) can help to differentiate the dendritic cells present in RH from the macrophages present in the SGPS. Unfortunately, for this test, commercially available antibodies require fresh-frozen tissue [2]. In order to immunophenotype the inflammatory infiltrate characterizing SGPS and RH in FFPE biopsies, we propose the potential diagnostic use of primary antibodies directed against Ionized calcium-binding adapter molecule 1 (Iba1), a marker that has been demonstrated to be expressed by almost all subpopulations of cells of the monocyte/macrophage lineage and MAC387 described as recognizing blood-derived and infiltrating monocytes/macrophages [3]. For this study, 20 skin biopsies diagnosed as sterile granuloma and 10 canine cutaneous histiocytomas (CCH) were retrospectively collected. In order to further differentiate SGPS and RH, immunohistochemistry was performed using antibodies against Iba1, MAC387, proliferating histiocytic marker CD45, T-cell marker CD3, B-cell marker CD20 and E-cadherin for neoplastic histiocytic proliferation in CCH. A semi-quantitative score ranging from 1 to 4 was applied according to the percentage of positive cells. A final diagnosis of SGPS and RH was made for, respectively, 12 and 8 cases. Our results showed that Iba1 + cells were consistently found in all cases. MAC387+ cells, consisting in macrophages and neutrophils, were found in SGPS, but they were not observed either in RH or CCH. CD3 and CD20 immunolabelling were moderate to strong in all cases. The proliferating histiocytic cells expressed CD45 in RH but they lacked expression of E-cadherin that was conversely consistent in all CCHs. Our preliminary results reveal that an immunohistochemical panel including Iba1 and MAC387 has the potential to be used in the immune-phenotyping of SGPS and RH allowing the differentiation between these conditions also for therapeutic purposes. A panel including Iba1 and MAC387 may confirm the origin of the inflammatory cells of interest, perchance followed by assessment of the expression of other specific histiocytic markers, such as E-cadherin and CD45 that can provide a more precise classification of histiocytic proliferative and neoplastic disorders of dogs.

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P118 - A CASE OF BRONCHIOLOALVEOLAR CARCINOMA - ASSOCIATED SYSTEMIC TOXOPLASMOSIS IN A MOUNTAIN LION (*PUMA CONCOLOR*)

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Spontaneous lung tumors are more frequently in dogs, cats and sheep, but rarely described in wildlife animals. In dogs and cats this neoplasia occurs as a sporadic geriatric disease, not related to any infectious etiology, while a betaretrovirus is responsible for pulmonary adenocarcinoma in sheep [1]. We described, for the first time, a case of lung cancer in a 6 year-old mountain lion (Puma concolor), serologically negative for Feline immunodeficiency virus (FIV) and Feline leukemia virus (FeLV), maintained in captivity in the Falconara Zoo Park, Ancona, Italy. In August 2017, this malnourished adult male, was present with signs of dyspnea, cyanosis and drooling, followed by sudden death. Postmortem examination was performed. Gross necropsy and histopathologic examination revealed nodules of different sized and coalescing whitish areas of lung parenchima consolidation, associated to multifocal atelectasis alternated with discrete emphysema. The toracic cavity was replete of yellowish exudate, citologically characterized by degenerated neutophilic cells and lipids enriched. Additionally a necrotizing, multifocal myocarditis and necrotizing, neutrophilic, and histiocytic interstitial nephritis were observed. In this animal a concurrent systemic toxoplasmosis, with merozoites found also into neoplastic carcinomatous cells was also detected. According to the classification of the main respiratory tumors types observed in dogs and cats, classified as acinar adenocarcinoma, bronchiolo-alveolar carcinoma, adenosquamous and squamous cell tumors [2], the histopathological examination of lung masses in this mountain lion revealed neoplastic epithelial cells compatible with bronchioloalveolar carcinoma (BAC). The immunohistochemical analysis of tumor cells showed positive labeling for pan-Cytokeratin, CK 7, CK 20 and TTF-1. According to macroscopic features, as well as histological and immunohistochemical findings, this tumor was diagnostic as BAC mixed subtype. Histology revealed a large amount of tachyzoites inside different tissues, as well as spleen, lymph nodes and also many neoplastic cells. An anti-Toxoplasma gondii monoclonal antibody stained positively these tachyzoites, and polymerase chain reaction (PCR) analysis targeting the B1 gene, confirmed the presence of Toxoplasma gondii in all the examined organs. The high parasitic burden detected inside neoplastic cells may be related to an opportunistic relationship by the parasite and the cancerous cells that showing an enhanced metabolic rate and scant differentiation.

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P119 - CHARACTERIZATION OF D17: IN VITRO MODELS OF OSTEOSARCOMA

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Osteosarcoma (OSA) is a rare cancer both in humans and dogs; however OSA incidence rates in dogs are 27 times higher than in humans (1). Many studies employed D17 as cell line for *in vitro* test to evaluate conventional anticancer therapies; however, little is known about the expression of genes involved in the innate immune response, in DNA repairs, in cell cycle regulation, and their ability to secrete cytokines and to interact with bacteria that can be used as antitumor therapies (2). The aim of our study was to evaluate the basal level of gene expression of pivotal molecules involved in the innate immune response and cell cycle regulation and to investigate the D17 ability to respond to infectious stressors. In order to evaluate the cell phenotype and the basal level of gene expression we selected a set of 20 immune-related (IL6, IL8, IL10, IL15, IL18, TGF-b, MYD88, NFKb/p65, iNOS, STAT5, TLR4, TLR5, MD2 and CD14) and epithelial gene transcripts, (CD44, CXCR4, RAD51, p53, PTEN, Erb2). D17 cells were grown until confluence at 37°C in 5% CO_2 in BME medium enriched with 10% (v/v) of fetal calf serum and a mixture of antibiotics; cells were tested at 255th and 302th passage. In a second experiment, we evaluated the ability of D17 cells to respond to *S.* Typhimurium (ST). ST was sub-cultured for 2 hrs a 37°C and re-suspended at MOI 100 in BME and used to infect cells; untreated cells were employed as negative control. Bacterial penetration was evaluated as previously described (3). Total RNA extraction and Real Time RT-qPCR reactions were carried out as previously described (4). Differences between data sets were checked for significant differences by *t*-Test or ANOVA (using PRISM graphPad). The significance threshold was set at $P<0.05$. IL-15 was not expressed in D17 cells; IL-10 was expressed in an inconsistent manner among experiments. The other genes under study were expressed in all samples. The expression of CD44 and CXCR4 protein was assessed by immuno-cytochemistry. Moreover, ST showed ability to penetrate D17 cells (titer: log 10 4.14±0.14) causing a pro-inflammatory response. Our results outline the basal expression in D17 cells of important genes involved in the innate immune response. In particular, D17 cells showed high expression of IL-6, IL-8 and NF-kb and low expression of PTEN-like spontaneous tumor gene. Moreover, we demonstrated gene and protein expression of CD44 and CXCR4, a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion, cell migration in osteosarcoma and a receptor involved in cancer invasion, respectivelly. These results provide important data on D17 cells, towards *in vitro* preliminary evaluation of new therapeutic approaches.

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P120 – EVALUATION OF SEROLOGY FOR THE DIAGNOSIS OF TUBERCOLOSIS IN WATER BUFFALO AFTER TUBERCOLIN SKIN TEST: PRELIMINAR RESULTS

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AFFILIATION

Diagnosys of Tuberculosis (TB)in buffaloes is based on the detection of the cell-mediated immunity response(CMI): intradermal tuberculin test (SIT) and interferon-gamma (IFN-γ) assay. As the disease progresses, a shift from Th1 to Th2 is associated with the decrease of CMI and the concomitant increase of humoral responses. Serological assays can show a variable sensitivity and specificity, depending on the antigens enrolled in the test. Therefore, the use of multiple antigens can increase the performances of the test. In this work, we have assessed anELISAassay, which detects antibodies against bovine PPD and four recombinant proteins (MPB70, MPB83, ESAT-6 and CFP10) specific for the Mtb Complex [1]. This assay was applied in combination with the SIT and the in vitro IFN-γ assay. The SIT booster effect on humoral response was evaluated by the analysis of 512 samples collected from naturally *M.bovis*-infected buffaloes herds, prior and 15 days after SIT.The ELISA test was applied in two positive herds, where subsequent samplings were performed during the herd eradication of the disease (N=779).Cut-off values for the positivity threshold were determined by analysis of 522 samples from five TB-free herds,located in the north, central and south of Italy, and 325 samples from one *M.bovis* infected herd located in the south of Italyand positive to at least one of the following tests: SIT, IFN-γ, presence of lesions characteristic of TB in post-mortem examination and microbiological culture. Diagnostic Sp for each antigen was fixed to be at least 99%, and the cut-off value was determined by ROC curve analysis. Consequently, Se range from 8% (CFP10) to 40% (MPB70), while the overall Se was 43.7%. Sensitivity was also estimated using latent class model on 141 heads tested by ELISA, IFN-γ and SIT/post-mortem examination. ELISA sensitivity resulted 64.7%.These results are comparable to those obtained in the bovine species[1].The SIT booster effect was important as 35 versus 80 positive samples were detected prior and post IDT, respectively. In *M.bovis* infected herds, the number of positive animals detected by the ELISA test is lower compared to that detected by SIT and IFN-γ. The concordance between the ELISA and the IFN-γtestwas81% and 77% in the two herds, respectively. However, the group of animals classified positive by the ELISA does not completely coincide with that detected by SIT and y-IFN, in accordancewith the different immune responses revealed. Thus, the ELISA test increases the number of positive animals, if added to those identified bySIT and y-IFN.Therefore, the use of this test allows the detection of a greater number of positive animals compared to the SIT alone, allowing a shorter time for the outbreak closure, with a consequent reduction in the costs of rehabilitation and the risk of spreading the infection within the same breeding orto other farms in the same territory.

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P121 – THE PERFORMANCE OF THE GAMMA-INTERFERON ASSAY FOR DIAGNOSIS OF TUBERCOLOSIS IN WATER BUFFALO: PRELIMINARY RESULTS

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The interferon-gamma (IFN-γ) assay is frequently used for diagnosis of *Mycobacterium bovis* infections in buffaloes [1]. In this study blood was stimulated with a couple of purified protein derivative (PPD) avian tuberculin (PPDA) and bovine tuberculin (PPDB) from Lelystad (Lely), a couple of Italian (IT) PPDA and PPDB, and phosphate buffer saline (PBS = N) as a negative control for cellular stimulation. The IFN-γ test was performed with BOVIGAM® 1G (Thermo-Fisher Scientific). Different interpretative criteria were used: 1) if Lely and IT PPDB≥2N (PBS) and PPDB/PPDA≥1.1 then POSITIVE else NEGATIVE if PPDB/PPDA≤0,9 and NO DISCRIMINATING (ND) in the case of intermediate results; 2) if Lely or IT PPDB≥2N and PPDB-PPDA≥0.050 then POSITIVE else NEGATIVE; 3) if Lely or IT PPDB-N≥0.1 and PPDB-PPDA≥0.1 then POSITIVE else NEGATIVE. Specificity (Sp) was estimated on 466 buffalos from Tuberculosis (TB) Official Free herd while Sensitivity (Se) was estimated on 71 animals, from a TB outbreak, positive at SIT test and/or with TB pathological lesions as well as latent class model using bayesian technics. Criteria 1 showed a Sp of 99.1% (CI95% 97.38-99.8%) and a Se of 91.5% (CI95% 82.5-96.8%), while without ND results the Se decreased to 81.25%. Criteria 2 resulted 96.8% (CI95% 94.5-98.3%) for Sp and 86.3% (CI95% 76.3-96.7%) for Se, while criteria 3 showed 97.3% (CI95% 95.1-98.7%) for Sp and 98.1% (CI95% 89.7-99.9%) for Se. Criteria 1 is the most conservative and in terms of accuracy obtains better performance, but leaves more ND results. The interpretation based on the ratio (PPDB/PPDA) tends to standardize the outcomes and decrease the variability, hence it is advisable when the biological variability is higher and a lower number of false positives is preferred. The criteria based on the difference (PPDB-PBS;PPDB-PPDA) optimize the performances in the realities where the probability to obtain a false positive results due to physiological variability is low. Latent class model showed a sensitivity of the IFN-γ assay of 75%, higher than SIT (Se 45%). Also the evaluations carried out on the relative sensitivity with respect to the post-mortem inspection indicate a greater sensitivity of the IFN-γ compared to the SIT. All the animals with lesions (n=11) were positive to IFN-γ, while only one was positive for the SIT test. However, these data should be interpreted with great caution due to small sample size.

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P122 - DEVELOPMENT OF PORCINE RETINA ORGANOTYPIC CULTURE AS AN *IN VITRO* MODEL FOR EVALUATION OF AAV VECTORS IN OPHTHALMOLOGY

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The retina is a delicate, organized tissue consisting of layers of different cells whose interactions are critical for visual signal transduction. Retinal organotypic cultures, capable of maintaining tissue architecture and cellular connections, can narrow the gap between cell line studies and *in vivo* modelling and are flexible enough for sophisticated experimental procedures. According to the "3Rs" principles they may also represent a mean for partial Replacement and Reduction [1,2]. Due to the anatomic and physiologic similarities between the porcine and human eye and retina, swine is considered the most important animal model for ophthalmology research [3]. The aim of this research was to develop a porcine retina organotypic culture for *in vitro* target-tissue validation of different rod-specific adeno-associated virus (AAV) vectors encoding GFP. Porcine eyes were obtained either from a local slaughterhouse or from control animals used for other scientific purposes. Upon arrival, the anterior parts were removed, and the eye was opened with 4 radial cuts. The retinal tissue was carefully separated from the vitreous to prepare retinal fragments that were subsequently placed on a polycarbonate transwell with 1-mm pore size and cultured in Neurobasal-A medium supplemented with 2mM l-glutamine and 2% B27 supplement (NBA medium). Retinal explants were maintained in culture for a maximum of 3 weeks in a 5% CO_2 atmosphere at 38.5 °C. In order to assess AAV transduction, vectors were immediately added directly on each explant at the beginning of the culture [4]. At different time points, the retina explants were examined by histology and immunofluorescence (IF) staining. Transduction was analysed by live cell imaging, IF staining and cell sorting cytofluorimetry. After *in vitro* culture, the tissue architecture closely resembled that of intact *in vivo* retina, confirming the adequacy of the culturing conditions. Transduction with AAV encoding GFP displayed protein expression at 14 days mainly in rods, confirming the specific tropism of the used vectors. In conclusion, the organotypic culture of porcine retina is a suitable model for pharmacological screening, in particular for the evaluation of efficacy and cellular tropism of AAV vectors, contributing to a reduction in the use of animals in ophthalmology research.

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P123 - GENETIC DETERMINANTS ASSOCIATED WITH BIOFILM CAPACITY OF *LISTERIA MONOCYTOGENES* RECOVERED FROM DAIRY INDUSTRY

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Biofilm is one of the major mechanisms by which *Listeria monocytogenes* (*L.m*) persists within food processing plants [1]. Several outbreaks associated with consumption of cheese have occurred worldwide with a high mortality rate [2].
The identification of the underlying genetic factors contributing to biofilm formation of *L.m* may provide information to improve our understanding on the persistence of *L.m* in the environment. Therefore, the aims of this study were: i) to assess the biofilm capacity of *L.m* isolates from dairy sector; ii) to analyze the correlation between biofilm formation phenotypes and biofilm associated genes. A total of 29 whole genome sequenced isolates from dairy products (n=18) and dairy processing environment (n=11) [3] were tested for biofilm formation according to a previously described method [4], with slight modifications. To determine the presence or absence of a previously described set of biofilm associated genes, a nucleotide BLAST of each gene against each genome assembly was performed. Significant hits were defined as those with coverage of at least 80% and a percent identity greater than or equal to 80%. Truncations were defined as present if a sequence was missing at least ten amino acids from the end of the sequence as compared to the EGD-e reference sequence. Sequences were translated to amino acids, aligned with MUSCLE, and manually inspected for truncations. Among *L.m* isolates, 69% (20/29) and 31% (9/29) exhibited weak or moderate biofilm- forming ability, respectively. No strain was found strong biofilm producer. The percentage of dairy isolates (78%) that were weak biofilm producers was higher than the percentage obtained for environmental isolates (56%). *L.m* strains, possessing the five genes cluster *lmo0444-lmo0448* (Stress Survival Islet 1) and a truncated *inlA* protein, formed increased levels of biofilm. Associating WGS- genotypes and specific phenotypes could contribute to improve prediction of microbial behaviors. The implementation of this information in hazard identification and exposure assessment processes new possibilities to feed Quantitative microbial risk assessment models (QMRA-models). Further studies will provide a better understanding of the process of biofilm formation by *L.m*, which may give interesting information that can be used to develop new strategies for avoiding biofilm formation in dairy plants.

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P124 - RESISTANCE PATTERN OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) ISOLATED FROM PIG PRODUCTION CHAIN IN NORTHERN ITALY

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Pig herds are an important reservoir for methicillin resistant *Staphylococcus aureus* (MRSA), i.e. MRSA CC398, one of the most commonly identified antimicrobial-resistant (AMR) pathogens worldwide [1]. In this study, MRSA isolated from swine chain in Northern Italy were screened for their phenotypic and genotypic AMR pattern against a panel of antibiotics used in veterinary medicine, including critically important antimicrobials (CIA) for human health [2]. A convenience sample of 50 fattening units located in Lombardy was selected. Three environmental sites at each farm (150), and five animals per farm at slaughterhouse (250) were sampled. MRSA were identified by phenotypic analysis and a quadruplex- PCR. Phenotypic AMR was screened by disk diffusion test according to CLSI recommendations [3]. Eleven prototype molecules, belonging to 8 drug classes, were tested. The presence of 20 AMR genes was screened using multiplex PCR. Isolates were also genotyped by multilocus sequence typing (MLST). Statistical analysis was conducted using Graphpad Prism 7. A total of 37 MRSA strains was isolated from 400 samples (9.25%), from 21/50 (42%) fattening units. The majority of the strains (89%) showed multi-resistant phenotypes, and 43% of them were resistant to at least 5 different classes. Isolates were resistant to the following CIAs: penicillins (90%), macrolides (43%), quinolones (23%) and aminoglycosides (13%). The most common AMR genes identified were: *tetM* (70%), *blaZ* and *dfrA* (57%). Four AMR genes (*dfrD*, *aacA-aphD*, *ermA*, *ermB*) were identified in less than 3% of the isolates, while other four (*vga*C, *vga*E, *aphA3*, *ermT)* were not identified in any of the MRSA isolates. The agreement between resistance, as described by phenotype and genotype, ranged from a *k* of 0.978 (tiamulin) to 0.194 (tetracycline). With the exception of one, every antimicrobial outcome had some isolates with resistant phenotype but no genetic explanation. Also reverse situation occurred, in fact AMR genes were identified in at least one susceptible isolate. Although the presence of such inconsistencies, each set of AMR genes was a significant predictor of the resistance phenotype, with the exception of tetracycline. MLST profiles were also analyzed, with the majority of the isolates belonging to ST398 (CC398), accordingly to other findings. Nonetheless, three isolates were identified as ST97 (CC5) and one as ST30 (CC30), one of the worldwide community acquired-MRSA, that was found to be resistant to all the classes tested. In conclusion, both genotypic and phenotypic multidrug resistance was observed in many isolates, with concern for farmers, veterinarians, and slaughterhouse employees.

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